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The impact of clinical and environmental toxicological exposures and eye health

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The impact of clinical and environmental toxicological exposures and eye health

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Editorial: The impact of clinical and environmental toxicological exposures and eye health

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Editorial on the Research Topic
The impact of clinical and environmental toxicological exposures and eye health

Increasing focus has been placed on understanding how our environment may influence our health, with ample research being conducted towards understanding how factors such as weather, air pollution, chemicals, and medications may affect the human body. Through these efforts, we have gained a more robust understanding of how these factors may have harmful, albeit variable, effects on ocular health, depending on the exposure in question. Galor et al. (2020), Villani et al. (2020) Research has linked environmental exposures to eye disorders, such as dry eye disease (DED) (Galor et al., 2014), Sjögren's disease (Xin et al., 2022), and allergic conjunctivitis (Patel et al., 2021), among others. In this Research Topic, we highlight environmental exposures that relate to ocular disease, with an aim of improving understanding and promoting precision-based management. A range of exposures were examined, including weather, air pollution, chemicals, and medications. Furthermore, study methodologies varied, including reviews of existing literature (de Los Santos et al.; Graca et al., 2023; Huang et al.; Ma et al.; Menke et al.; Patel et al.; Quiroga-Garza et al.; Ruiz-Lozano et al.), analyses of health outcomes (Shanbag et al.), and exposure assessment *in vivo* (Chen et al.; Tyszkiewicz et al.) and *in vitro* models. Fukuda et al..

First, Patel et al. focused on environmental exposures, such as airborne pollutants (particulate matter (PM) and reactive gases like ozone or nitrogen dioxide), meteorological conditions (temperature, relative humidity (RH)), and behavioral factors (smoking, contact lenses) and summarized how these factors relate to ocular surface disease. This article centered around risk for DED, a common source of ocular morbidity across the world. The authors concluded that these toxicological exposures related to DED risk in variable ways, depending on the exposure. DED was consistently linked to air pollution, with studies reporting positive linear relationships with various aspects of DED risk, including its diagnosis, symptoms, and clinical signs. Relationships for temperature and RH were on the other hand 'U-shaped,' suggesting that extremes in either direction increased the risk for these same aspects of DED.

Other authors focused on chemical exposures. Menke et al. reviewed the toxic effects of chemical agents used in threat control and civilian conflicts, like sulfur mustard, sarin, and caustic hydrofluoric acid. Ocular complications ranged from self-limiting conjunctival irritation to more

severe corneal disease (neovascularization, opacification, perforation), and in some cases resulting in blindness. Graca et al. summarized the molecular and anatomic mechanisms that linked chemical injury to ocular pathology. For example, the authors described how acidic exposures are limited damage the cornea, while alkali agents can penetrate deeper layers of the eye via saponification and lysis of epithelial membranes. Furthermore, the authors explained how chemical exposures can lead to the development of chronic ocular pain even after healing at the ocular surface. This included a discussion of the ocular pain pathway, starting from nociceptive responses at the ocular surface to eventual development of central sensitization. Finally, in a review by Quiroga-Garza et al., riot control agents such as oleoresin capsicum and chloroacetophenone (components of Mace and pepper spray) were examined in relationship to the eye. While use of such agents is intended to incapacitate through transient neurogenic inflammation secondary to activation of corneal surface afferents, the authors highlighted the possibility of more severe corneal complications (e.g., necrosis and neovascularization).

A few articles also examined how non-prescription drugs may affect the eye. Huang et al. reviewed the ocular effects of methamphetamine over-use, including the risk of self-limited (e.g., conjunctivitis and keratitis) and permanent (e.g., corneal melting, amaurosis fugax, non-arteritic anterior optic ischemic neuropathy (NAION), orbital cellulitis) sequela. The authors also examined on molecular mechanisms by which methamphetamine can affect the eye, from the ocular surface to the optic nerve. In another review by de Los Santos et al., the toxic effects of mercury were examined, beyond its known deleterious effects on the central nervous system. The article highlighted the potential for damage in not only the cornea (decreased subbasal nerve density), but also within the retina (direct toxicity to photoreceptors) and optic nerve (direct toxicity to optic nerve glial cells).

Other papers examined the impact of prescription drugs on the eye. Ruiz-Lozano et al. reviewed the effects of glaucoma therapies, including outflow enhancers (e.g., prostaglandin analogs and cholinergic agents), aqueous humor blockers (e.g., beta blockers), and others (e.g., nitric-oxide modulating agents), in relation to ocular disease. The authors summarized that these medications could induce ocular pathology via lacrimal and meibomian gland dysfunction as well as direct ocular surface toxicity (contact dermatitis, drug-induced conjunctivitis). In another review by Ma et al., the impact of biologic agents used to treat cancer were examined. Epidermal growth factor receptor inhibitors and immune checkpoint inhibitors were two categories that were closely linked to eye manifestations, including ulcerative keratitis and Stevens-Johnson syndrome (SJS). A review of hydroxychloroquine-associated eye manifestations was undertaken by Yusuf et al. These authors took a step further and summarized the current diagnostic decision-making process, and postulated how we can improve this process, including with the integration of artificial intelligence, to aid in earlier diagnosis of retinopathy. Finally Shanbag et al. examined vision, pain, and quality of life in 15 individuals with chronic SJS/TENS who were treated during the acute phase of their disease using their respective hospital's protocol (some individuals received amniotic membrane transplant). The authors examined outcomes of these 15 individuals with the National Eye Institute Visual Function Questionnaire (NEI-VFQ-25, which tests 11 subscales regarding visual acuity, social functioning, daily quality of life, ocular pain, and mental health) and found that apart from ocular pain and mental health status, all other subscale scores were comparable to those collected from 122 controls. The authors concluded that early treatment of SJS/TENS can lead to improved long-term outcomes.

This Research Topic also included experimental studies. Chen et al. utilized a mouse model to examine the impact of bisphenol A, an organic compound used in plastics and resins, on ocular health. Two groups of mice were examined—a control group, and a group that received 100 mg/kg BPA administered daily for 14 days by intraperitoneal injection. After exposure, the authors observed upregulated expression of scleral endoplasmic reticulum stress proteins associated with matrix remodeling and fibrosis (Activating transcription factor 6 [ATF6] and Protein kinase RNA (PKR)-like ER kinase [PERK]) with resultant axial lengthening and scleral remodeling in exposed eyes, suggesting that exposure may lead to an increased risk for myopia. Tyszkiewicz et al. instead examined retinal function in healthy Wistar Han rat eyes. The authors assessed male and female retinal structure and function via electroretinography (ERG, to test scotopic and photopic luminance responses of retinal cells), optokinetic responses (to assess visual acuity and tracking responses), and histology (to assess retinal, brainstem, and visual/auditory area cell structure), among others. The study found that a significant fraction of male rats (13%–19%) had abnormal ERG signals and decreased visual tracking responses, without notable changes in retinal or brain cell morphology. The authors thus cautioned that baseline sex-related differences must be considered when examining retinal function in these animals, including during toxicological exposure testing.

Finally Fukuda et al. used an *in vitro* model to study ocular toxicity. These authors examined the utility of a human corneal epithelial-derived cell line with enhanced proliferation. This cell line was developed through co-expression of a mutant cyclin-dependent-kinase 4 (CD-K4), Cyclin-D1, and telomerase reverse transcriptase (TERT) which allowed for increased proliferation. The authors challenged the cell line with glycolic acid and benzalkonium chloride, chemical preservatives often found in topical eye therapies, and noted a dose-dependent decrease in viability post-exposure. The authors concluded that this new cell line could be used to evaluate the impact of additional chemicals in future studies.

In summary, this special edition highlights that toxicological exposures, whether environmental, chemical, or drug-associated, can induce ocular disease. Pathology can range from the ocular surface to the optic nerve, and these disorders are driven by a variety of underlying mechanisms. It is important for providers to be aware of these associations as this knowledge can be incorporated into practice by discussing exposure avoidance or mitigation for susceptible patients. Rozanova et al. (2009).

Author contributions

SP: Formal Analysis, Investigation, Writing–original draft, Writing–review and editing. AG: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing–review and editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Human-Derived Corneal Epithelial Cells Expressing Cell Cycle Regulators as a New Resource for *in vitro* Ocular Toxicity Testing

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The Draize test has been used on rabbits since the 1960s to evaluate the irritation caused by commercial chemicals in products such as cosmetics or hairdressing products. However, since 2003, such tests, including the Draize test for cosmetics, have been prohibited in European countries because they are considered problematic to animal welfare. For this reason, replacement of *in vivo* methods with the alternative *in vitro* methods has become an important goal. In this study, we established a corneal epithelial cell line co-expressing a mutant cyclin-dependent kinase 4 (*CDK4*), Cyclin D1, and telomerase reverse transcriptase (*TERT*). The established cell line maintained its original morphology and had an enhanced proliferation rate. Furthermore, the cells showed a significant, dose-dependent decrease in viability in an irritation test using glycolic acid and Benzalkonium chloride. These cells can now be shared with toxicology scientists and should contribute to increasing the reproducibility of chemical testing *in vitro*.

Keywords: corneal epithelial cells, immortalization, cell cycle regulators, cyclin-dependent kinase 4, cyclin D1, telomerase reverse transcriptase

INTRODUCTION

The safety of chemical compounds, especially the evaluation of their potential as irritants, is important for commercial chemicals that are used daily, such as cosmetics or hairdressing products. Since the 1960s, various types of animal experiments have been performed to evaluate the potential side effects of commercial chemicals (Buehler and Newman, 1964). As one representative animal experiment, the Draize test using rabbits has been a practical standard. However, animal experiments, including the Draize test for cosmetics, have been prohibited in European countries since 2003 because this method is currently considered problematic from the viewpoint of animal welfare (Sharpe, 1985). For this reason, the development of alternative *in vitro* ocular toxicity tests is worth further investigation.

Corneal epithelial cells exist at the outermost layer of the cornea and play a role in protecting the eyes from invasion by foreign materials. Corneal epithelial cells could, therefore, be useful for *in vitro* ocular toxicity testing. In this regard, an irritation test using rabbit corneal epithelial cells,

referred to as the short time exposure (STE) test, has been previously established (Takahashi et al., 2008). However, since there is a species difference between humans and rabbits, the results of an irritation test that uses rabbit cells are likely to be different from what is actually experienced by the human eye. For this reason, we considered the use of human corneal epithelial cells as a new approach to *in vitro* ocular testing. However, primary corneal epithelial cells are limited in their usefulness because these cells stop growing after only a few passages. The halt of cell proliferation can mainly be attributed to cell culture stress and Hayflick limitation (Hayflick and Moorahead, 1961).

To overcome the limitations of cellular senescence, several standard methods to immortalize cells have been established. Simian vacuolating virus 40 (SV40) and E6/E7 human papillomavirus-derived oncoproteins are well known to inactivate p53 and retinoblastoma protein (RB) (Tsao et al., 2002) and are effective for standard cellular immortalization. However, immortalized cells using SV40 or E6/E7 are reported to have abnormalities in their chromosomes (Ray et al., 1990; Duensing et al., 2000).

In 2011, a newly developed method for cell immortalization was reported. Briefly, Shiomi et al. (2011) achieved the immortalization of primary cells by co-expressing a mutant (R24C) cyclin-dependent kinase 4 (*CDK4*), cyclin D1, and telomerase reverse transcriptase (*TERT*). The immortalized cells maintained the karyotype and differentiation ability of the original cells (Shiomi et al., 2011). Based on the names of the expressed cells, this established method was referred to as the K4DT method (mutant CDK4, Cyclin D, and TERT). Furthermore, our group reported that this newly established K4DT method could be used in a variety of species of animals, including bovine, swine, monkey, prairie vole, and midget buffalo, which could possibly be explained by the highly conserved amino acid sequence of these cell cycle regulators in these animals (Donai et al., 2014; Kuroda et al., 2015; Katayama et al., 2017). Based on these data, we formed the hypothesis that co-expression of mutant *CDK4*, *Cyclin D1*, and *TERT* might allow us to establish a new corneal epithelial cell line, which retains the original nature of the primary cells better than the traditional oncogenic method. In this study, we report the establishment of human corneal epithelium-derived cells and its biological characterization for toxicity evaluation. These cells should contribute to the evaluation of chemical toxicity with high reproducibility. Furthermore, these cells can now be shared with toxicology scientists, which should promote the replacement of animal models for experimentation and contribute to animal welfare.

MATERIALS AND METHODS

Cell Culture

Corneal epithelial cells were commercially obtained from Lifeline Cell Technology (Frederick, MD, USA; cat. no. FC-0029) through Kurabo (Osaka, Japan). The cells were cultured in six-well dishes with OcuLife basal medium (Lifeline Cell Technology; cat. no. LM-0012) containing OcuLife LifeFactors (Lifeline Cell Technology; cat. no. LS-1057) with 6 mM L-glutamine, 0.4% v/v bovine pituitary extract, 1.0 μM epinephrine, 100 ng/ml hydrocortisone hemisuccinate, 5 μg/ml recombinant human insulin, and 5 μg/ml apo-transferrin at 37°C in an atmosphere of 5% CO_2. Before cell passage, the cells were washed with 1 × D-PBS (phosphate buffered saline) (−) (Nacalai Tesque, Kyoto, Japan; cat. no. 11482-15) and dispersed with StemPro Accutase (Life Technologies, Waltham, MA, USA; cat. no. A11105-01) for 5 min at 37°C. The dispersed cells were then centrifuged at 800 × *g* for 5 min, and the pelleted cells were resuspended in fresh medium.

Preparation of Recombinant Lentiviruses and Infection to the Cells

To establish the new corneal epithelial cell line, the primary cells were infected with recombinant lentiviruses. The basic backbone of the recombinant lentiviruses was derived from the CSII vector, which was kindly provided by Dr. Miyoshi (Keio University, Tokyo, Japan). CSII-CMV-CDK4R24CF2A-CyclinD-IRES (internal ribosomal entry site)–EGFP (enhanced green fluorescent protein) is a polycistronic vector that expresses both CDK4R24C and Cyclin D. In order to monitor transfection efficiency, the CSII-CMV-CDK4R24CF2A-CyclinD-IRES–EGFP was constructed such that the expression cassette was linked with EGFP through an IRES. Corneal epithelial cells were also infected with a mixture of three monocistronic lentiviruses, CSII-CMV-TERT, CSII-CMV-CyclinD, and CSII-CMV-hCDK4R24C. We named the corneal epithelial cells infected with polycistronic virus as K4D (CDK4R24C + Cyclin D) cells and the corneal epithelial cells infected with a mixture of monocistronic lentiviruses as K4DT cells (CDK4R24C + Cyclin D + TERT). Details of the production of these recombinant viruses and their infection have been described in our previous report (Fukuda et al., 2016). The titer of the TERT lentivirus was usually lower than that of the mutant CDK4 and Cyclin D lentiviruses, due to it having the relatively longer cDNA insert size of around 4 kb (Fukuda et al., 2018). Therefore, K4DT cells were additionally infected with a retrovirus, which expresses TERT and a hygromycin selection marker. We refer to these hygromycin-resistant cells as K4DT + T cells.

Western Blotting

To extract proteins from primary, K4D, and K4DT + T cells, we lysed cells in a buffer containing 50 mM Tris–HCl (pH 7.4), 0.15 M NaCl, 1% Triton X-100, 2.5 mg/ml sodium deoxycholate (Wako, Osaka, Japan; cat. no. 194-08311), and a protease inhibitor cocktail (1/200 dilution, Nacalai Tesque; cat. no. 25955-11). The protein expression levels of CDK4 and Cyclin D were detected by Western blotting using an anti-CDK4 antibody (1/2,500 dilution, MBL, Nagoya, Japan; cat. no. 25955-11), an anti-cyclin D antibody (1/5,000 dilution, MB; cat. no. 553), and an anti-alpha-tubulin antibody (1/1,000 dilution, Santa Cruz Biotechnology, Dallas, TX, USA; cat. no. sc-32,293). Anti-mouse IgG (1/2,000 dilution, GE Healthcare, Buckinghamshire, UK; cat. no. NA931) or anti-rabbit IgG (1/2,000 dilution; GE Healthcare; cat. no.

NA934-1ML) was used as secondary antibodies. The detailed Western blot procedure has been reported previously (Fukuda et al., 2005, 2008). The intensity of signals was measured by the Image J software.

Population Doubling Assay

To measure the proliferative capacity of primary, K4D, and K4DT + T cells, we sequentially passaged the cells. Each cell line was initially seeded into six-well plates at a density of 5.0×10^4 cells in triplicate. When the cell line reached confluency, the cells were dispersed using StemPro Accutase (Life Technologies). We recorded the total number of cells in each dish using an automatic cell counter (Thermo Fisher Scientific, Waltham, MA, USA). Cells (1.0×10^5) were then seeded into a new dish to evaluate their growth rate by determining the population doubling level (PDL). PDL was calculated using the following equation: $PDL = \log_2 (A/B)$, where A is the number of cells harvested at each passage and B is the number of seeded cells.

PCR Analysis

We extracted genomic DNA from the primary, K4D, and K4DT + T cells using NucleoSpin Tissue (Takara Bio, Shiga, Japan; cat. no. 740952) following the manufacturer's protocol. To monitor insertion of the transgene, TERT and Tuberous Sclerosis Type II (TSC2; internal control) were detected using a PCR analysis. TSC2 was chosen as the internal control, since the TSC2 gene is a unique gene in the human genome, and furthermore there are no TSC2 pseudogenes. The cDNAs were amplified in a reaction solution containing KOD FX Neo (Toyobo, Osaka, Japan; cat. no. KFX-201) dNTPs and specific primers. After amplification, the PCR reaction was mixed with loading dye, and the PCR products were separated by electrophoresis on 1% agarose gels followed by ethidium bromide staining.

Fluorescent Staining of F-Actin

We seeded the primary, K4D, and K4DT + T cells into a Lab-Tek chamber Slide (Thermo Fisher Scientific) to determine the morphology of each cell line. The cells were exposed to 500 µl of 0.2% Triton X-100 to permeabilize them, after which rhodamine-labeled phalloidin (1/40 dilution, Wako, Osaka, Japan; cat. no. 165-21641) was used to stain F-actin, and the nuclei were counterstained with DAPI (4′,6-diamidino-2-phenylindole) (1/300 dilution, Dojindo, Kumamoto, Japan; cat. no. 28718-90-3). We captured fluorescence images with a benchtop microscope (Keyence, Osaka, Japan; cat. no. BZ-9000).

Cell Cycle Analysis

For cell cycle analysis, approximately 4×10^5 primary, K4D, and K4DT + T cells were fixed with 70% ethanol. Six replicates within each experimental group were stained with the cell cycle assay kit (Merck Millipore, Darmstadt, Germany; cat. no. MCH100106). The stained cells were then analyzed using a Muse cell analyzer (Merck Millipore). The results were statistically evaluated using a non-parametric multiple comparison test by the steel method.

Immunostaining

For the immunostaining, we used Lab-Tek Chamber slide with Permanox Slide for the cell culture of corneal epithelial cells (Thermo Scientific Nunc, Waltham, MA USA). The cells were washed with 1× PBS and fixed with 4% paraformaldehyde solution (Nacalai Tesque). The cells were permeabilized with Triton X-100 solution, and blocking reaction was carried out with 1% bovine serum albumin in PBS. Primary antibody (anti-Cytokeratin 3/2p, Santa Cruz, sc-80,000) was exposed at 1:40 dilution with blocking buffer. The secondary antibody Alexa 568-labeled goat anti-mouse IgG was used for the detection with the counterstaining by DAPI. The staining images were obtained by a fluorescence microscope (BZ-9000, Keyence, Tokyo, Japan).

Ocular Toxicity Test

We evaluated irritation based on the protocol for the STE test, as previously described (Takahashi et al., 2008). We selected glycolic acid (Wako; cat. no 071-01512) as a positive chemical for the irritant test. Briefly, primary cells or K4DT + T cells in six-well plates were exposed to either a 0.5% or 5% glycolic acid solution for 5 min; PBS was used as a negative control. Furthermore, we also tested the toxicity of Benzalkonium chloride. The cells in each well were washed twice with PBS and then treated with Accutase for 5 min. The total number of cells and the cell viability were determined using trypan blue staining. To avoid bias in cell counting, an automatic cell counter was used for all measurements. Cell viability was calculated based on the ratio of the number of living cells relative to the total cell number.

MTS Assay

We detected cell toxicity of Benzalkonium chloride with MTS assay. MTS assay is a colorimetric method for determining the number of viable cells in proliferation, cytotoxicity, or chemosensitivity. The corneal epithelial cell immortalized with K4DT expression was seeded around 80% density in a 96-well plate with 100 µl of medium. We exposed the cells to 5, 0.5, 0.05, 0.005, and 0.0005% Benzalkonium chloride solution in PBS. After the 5-min exposure to the cells, the medium was changed into basal medium and incubated at 37°C and 5% CO_2 for 1 h. After the incubation, 25 µl of CellTiter 96, AQueous One Solution Cell Proliferation Assay (MTS) (Promega, Madison, WI, USA) was added to the cell culture medium, and incubated at 37°C for 1 h. The absorbance of wells at 490 nm was measured with a microplate reader (SpectraMax M, Molecular Device, San Jose, CA, USA).

Statistical Analysis

The data on cell cycle analysis (n = 6, **Figure 1**), absolute cell number after the STE method (n = 6, **Figure 2**, n = 8, **Figure 3**), and absorbance of MTS1 assay (n = 16, **Figure 4**) were analyzed with non-parametric Steel method using Statcell 3 (OMS Publishing, Tokyo, Japan).

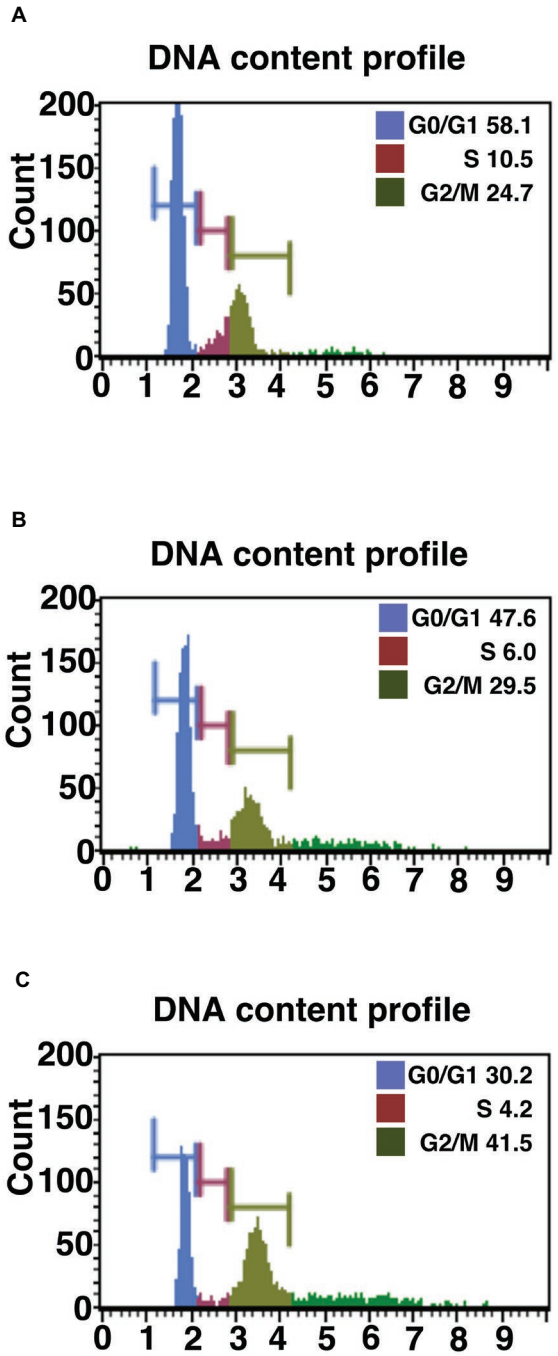

FIGURE 1 | Cell cycle analysis of primary, K4D, and K4DT cells. **(A)** Cell cycle analysis of primary cells. **(B)** Cell cycle analysis of K4D cells. **(C)** Cell cycle analysis of K4DT + T cells.

RESULTS

Morphological Changes After Gene Transduction

To establish a corneal epithelial-derived cell line, we used the polycistronic lentivirus (CDK4R24CF2A-CyclinD) or a combination of the monocistronic lentiviruses expressing the R24 mutant of *CDK4*, *Cyclin D1*, and *TERT* (**Figure 5A**) to infect these cells. To evaluate the potential toxicity of these genes in the corneal epithelial cells, we compared the cell morphologies of the recombinant cells with that of primary cells. The recombinant cells had a similar morphology to primary cells (**Figure 6A**). Furthermore, the staining patterns of F-actin, stained with rhodamine-labeled phalloidin, in the recombinant K4D and K4DT + T cells were almost identical to that in the primary cells (**Figure 6B**), indicating that the expression of mutant CDK4, Cyclin D, and TERT do not affect F-actin distribution. From these data, we conclude that the exogenous expression of mutant CDK4, Cyclin D, and TERT do not change the cell morphology of corneal epithelial cells. Although the cell size was relatively smaller in K4D and K4DT cells, the smaller cell size was explained by the increased cell proliferation and constant cytoplastic replication speed (Cooper, 2004).

The Detection of Transgenes by PCR and Western Blotting

To monitor genomic insertion of exogenous genes, we performed PCR using genomic DNA extracted from primary, K4D, and K4DT + T cells (**Figure 5B**). The TERT gene was only detected in the genomic DNA isolated from K4DT + T cells, but not from genomic DNA isolated from primary or K4D cells. Furthermore, we confirmed the protein expression of Cyclin D and CDK4 by Western blotting (**Figure 7**). It should be noted that the Western blot data for the K4D recombinant cells showed a mobility shift for CDK4 to a higher molecular mass compared to CDK4 present in primary cells (**Figure 7A**). This observation is reasonable since the 2A peptide was inserted between the mutant CDK4 and Cyclin D, after which cleavage of the 2A peptide occurs at its carboxyl-terminal side, resulting in a shift in the mobility of the transgenic CDK4 (**Figure 7A**, top panel). From these results, we conclude that the genomic integration of mutant CDK4, Cyclin D, and TERT had been successfully achieved in corneal epithelial cells. Furthermore, from the band intensity of the Western blots, we concluded that the protein expression of CDK4 and Cyclin D was elevated in K4D and K4DT + T cells (**Figure 7B**).

The Sequential Culture of Recombinant and Primary Corneal Epithelial Cells

We carried out sequential cell passaging to evaluate the cell proliferation of primary, K4D, and K4DT + T cells. At passage 1, the K4D and K4DT+T cells continued to proliferate, whereas the growth of the primary cells was completely arrested (**Figure 8A**). This cell cycle arrest in primary cells was reasonable since their cell proliferation was only guaranteed for five passages after thawing of the primary cells (based on the protocol provided by the supplier, the primary cells were already at passage 4 at the beginning of the study). The cell growth data were evaluated using the PDL, which showed the cumulative cell division number over sequential passages (**Figure 8A**). The data showed that cell proliferation in the K4D and K4DT + T cells was accelerated compared to primary cells (**Figure 8B**). Notably, the cell proliferation was continued more than 200 days in case of K4DT + T cell (**Figure 8A**).

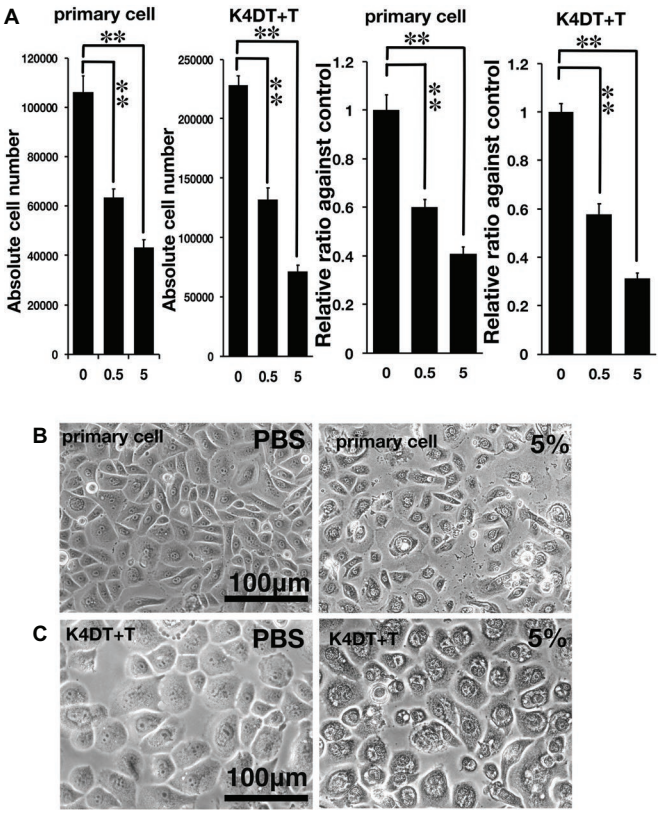

FIGURE 2 | Assessment of glycolic acid (0.5 and 5%) as an irritant in primary and K4DT + T cells using the STE method. **(A)** The number of primary and K4DT cells. The data are expressed as the mean, with the error bars representing the standard error. Two stars indicate a statistical significance of more than 1%. **(B)** Cell morphology of PBS-treated (control) primary cells (left panel) and 5% glycolic acid-treated primary cells (right panel). **(C)** Cell morphology of PBS-treated K4DT + T cells (left panel) and 5% glycolic acid-treated K4DT + T cells (right panel).

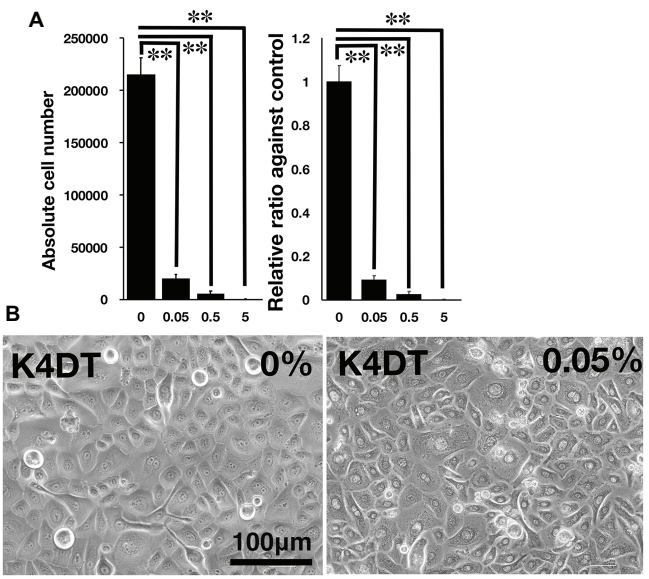

FIGURE 3 | Detection of toxicity of Benzalkonium chloride using the STE method. **(A)** The number of K4DT cells. The data are expressed as the mean, with the error bars representing the standard error. Two stars indicate a statistical significance of more than 1%. **(B)** Cell morphology of PBS-treated (control) K4DT cells (left panel) and 0.05% Benzalkonium chloride-treated K4DT cells (right panel).

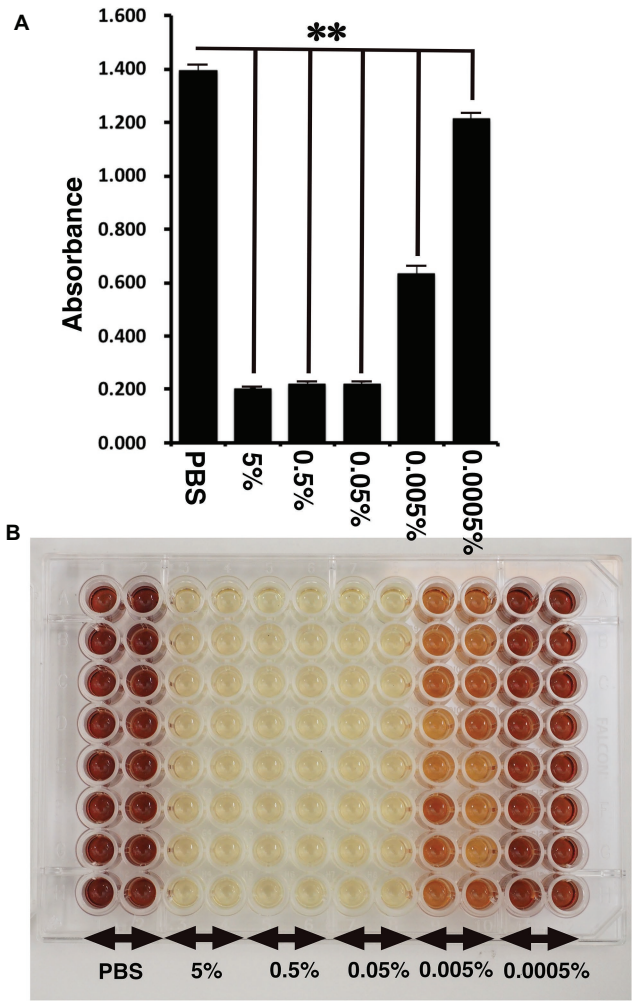

FIGURE 4 | Detection of toxicity of Benzalkonium chloride using MTS assay. **(A)** The absorbance of K4DT cells. The data are expressed as the mean, with the error bars representing the standard error (n = 16). Two stars indicate a statistical significance of more than 1%. **(B)** The appearance of 96 microplates after 1-h incubation at 37°C after the addition of MTS reagent.

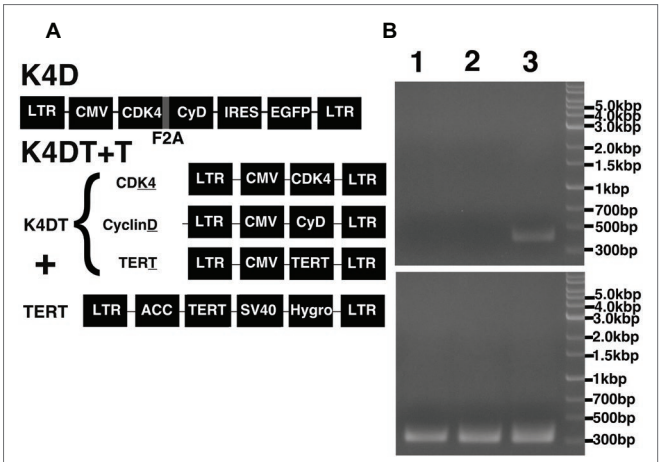

FIGURE 5 | Schematic representation of the different expression vectors used in this study and confirmation of genomic integration and protein expression. **(A)** Structure of the expression vectors used to establish the K4D and K4DT + T cell lines. LTR, long terminal repeat; CMV, cytomegalovirus promoter; Hygro, Hygromycin-resistant gene. **(B)** Confirmation of the genomic integration of the TERT expression cassette by PCR: (1) primary cells; (2) K4D cells; (3) K4DT + T cells. The upper panel shows the specific 500-bp PCR product confirming the presence of the TERT expression cassette, whereas the lower panel shows the 400-bp PCR product derived from the human Tuberous Sclerosis Type II gene.

Furthermore, we also observed that the K4D-expressing corneal epithelial cells had a morphology change around passage 3 that caused them to resemble fibroblasts (**Figures 8C,D**, left side). This morphological change was quite reproducible, being observed in four independent experiments. The ratio of fibroblast-like cells also gradually increased with increasing passage number.

Accelerated Cell Growth in K4DT + T Corneal Epithelium Cells

We evaluated the effect of the expression of mutant CDK4, Cyclin D, and TERT on the cell cycle at passage 1. **Figures 1A–C** show representative results from this cell cycle analysis. The ratio of cells at each stage of the cell cycle is shown in different colors. In both the K4D and K4DT + T cells, there was a significant increase in the ratio of cells at G2/M and a decrease in the ratio of cells at G0/G1 (**Table 1**). From these data, we conclude that cell proliferation is accelerated after the expression of mutant CDK4 and Cyclin D.

Lower Transgene Expression Levels in the K4D Fibroblast-Like Cells

We wondered why the fibroblast-like cells appeared in culture of the K4D cells from around passage 3 and onwards. To obtain a clue to explain this phenomenon, we examined the cells by fluorescence microscopy, as shown in **Figures 9A,B**. Interestingly, the fluorescence intensity in the fibroblast-like cells was much lower than that in cells with an epidermal-like shape. The expression cassette for CDK4-F2A-CyclinD is linked with EGFP *via* an IRES sequence. Therefore, the protein levels of EGFP should be correlated with that of CDK4-F2A-Cyclin D. Since the percentage of fibroblast-like cells increased after several passages, we conclude that the K4D corneal epithelial cells without TERT generated using the polycistronic expression vector are not suitable for use as a cell line for *in vitro* irritant testing. To evaluate the biological status of the fibroblast-like cells, we carried out the immunostaining of Cytokeratin 3/2p, which is the marker gene of the corneal epithelial cells. As shown in **Figure 10**, fibroblast-like cells (indicated with arrows in **Figure 10**) showed negative neither for EGFP nor for Cytokeratin 3/2p. From these data, the fibroblast-like cells have lost the nature of the epithelial origin cells.

Irritation Test Using the STE Method of Glycolic Acid in Human Corneal Epithelial Cells *in vitro*

As described in the section "Introduction," chemical irritants can be detected using the well-established STE method in

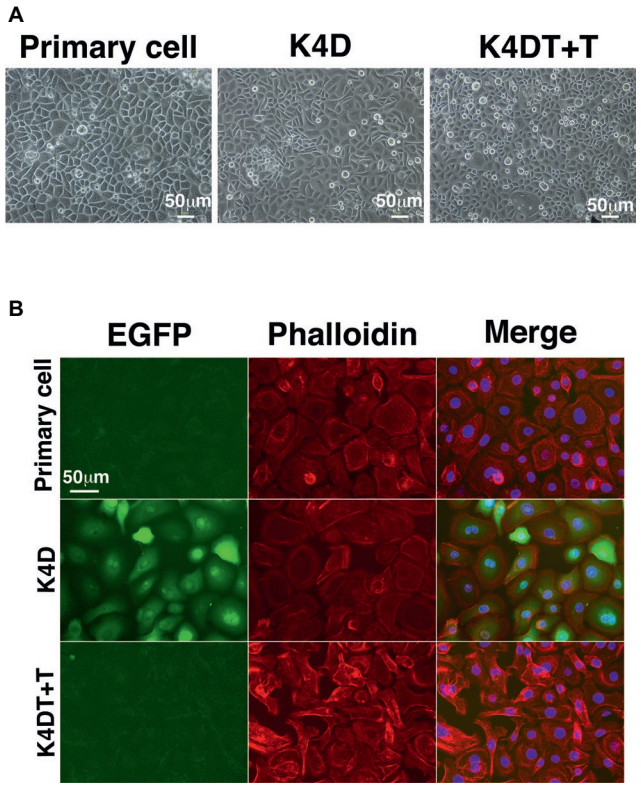

FIGURE 6 | Cell morphology of primary, K4D, and K4DT + T cells. **(A)** Cell morphology using phase contrast microscopy at passage 0. **(B)** Morphological observation of the cytoskeleton (F-actin) and the nucleus, following staining with rhodamine-labeled phalloidin and DAPI, respectively. Left panel: EGFP fluorescence. Middle panel: phalloidin staining. Right panel: merged images of the phalloidin, DAPI, and EGFP stains.

rabbit-derived corneal epithelial cells (Takahashi et al., 2008). Accordingly, we evaluated the effect of a positive control irritant, glycolic acid, in human-derived corneal epithelial cells. We exposed either primary or K4DT + T human corneal epithelial cells to 0.5 and 5% glycolic acid. As shown in **Figure 2A**, the number of primary cells exposed to 0.5 and 5% glycolic acid solution was significantly decreased (**Figure 2A**, left side, primary cell) compared with the PBS control. Exposure of the K4DT + T cells to 0.5 and 5% glycolic acid also resulted in a statistically lower number of cells (**Figure 2A**). Decreased cell viability following exposure to 0.5 and 5% glycolic acid solution was reproduced in K4DT + T cells. Furthermore, we also compared the cell morphology between control and 5% glycolic acid-treated primary and K4DT + T cells before Accutase digestion. As shown in **Figure 2B** (right panel), cytoplasmic vacuolization and degeneration of the cell nucleus were evident in cells treated with 5% glycolic acid, indicating that the glycolic acid irritant severely damaged the cells. From these data, we conclude that our established K4DT + T human corneal epithelial cells have a potential use in evaluating chemical irritants.

Irritation Test of Benzalkonium Chloride in Human Corneal Epithelial Cells *in vitro*

We furthermore tested the toxicity of Benzalkonium chloride with the STE method using our established immortalized human corneal epithelial cells. The toxicity of Benzalkonium chloride to corneal epithelial cells is well recognized since Benzalkonium chloride is the primary preservative chemical additive for eye drops. As shown in **Figures 3A,B**, the STE method showed a significant reduction in cell number in all doses of Benzalkonium chloride. Furthermore, the cell morphology treated by Benzalkonium chloride showed the nature of the cellular membrane, which possibly resulted in the cell death of epithelial cells (**Figure 3B**).

Cell Toxicity of Benzalkonium Chloride Detected by MTS Assay

CellTiter 96 AQueous One Solution Cell Proliferation Assay is a colorimetric method for determining the number of viable cells in proliferation, cytotoxicity, or chemosensitivity assays, which was provided by Promega. We treated the immortalized cells with PBS, 5, 0.5, 0.05, 0.005, and 0.0005% Benzalkonium chloride in PBS. As shown in **Figure 4A**, the living cell number can be detected as the absorbance of microplate detector at 490 nm. Interestingly, treatment of Benzalkonium chloride even at 0.0005% showed statistically significant reduction of the cell viability. The appearance of the treated plate is shown in **Figure 4B**. From these data, we concluded that our established corneal epithelial cell is a useful tool to detect eye toxicity.

DISCUSSION

In this study, we established a new human corneal epithelial-derived cell line through the co-expression of a mutant CDK4, Cyclin D, and TERT. An analysis of cell proliferation over sequential passages showed that primary cells ceased proliferating when grown at low cell densities. However, the K4DT + T cell lines showed enhanced cell growth even at low cell densities. This property should provide an advantage to our K4DT + T cell line since it increases the ease by which the cells can be handled and should increase the reproducibility of the toxicity test. In contrast, in primary cells, there were significant differences between different cultures. With respect to the K4DT + T cells, although we have confirmed cell proliferation up to a PD of 200 days, we believe that the cell line is sufficiently well established that it can be shared with toxicology scientists.

When cells are exposed to cellular stresses, such as low cell density, the stressed cells accumulate the p16 protein, which is a negative regulator of the cell cycle (Meerson et al., 2004). The p16 protein binds to CDK4, resulting in the inactivation of the CDK4-Cyclin D enzymatic complex and a halt in cell proliferation. In cells expressing the R24C mutant CDK4, the p16 protein cannot bind to the mutant CDK4 protein, since the R24C mutation changes the protein structure of the binding pocket for p16. Co-expression of Cyclin D, therefore, induces constitutive activation of the mutant CDK4-Cyclin D complex, and this mutant CDK4-Cyclin D complex induces the phosphorylation

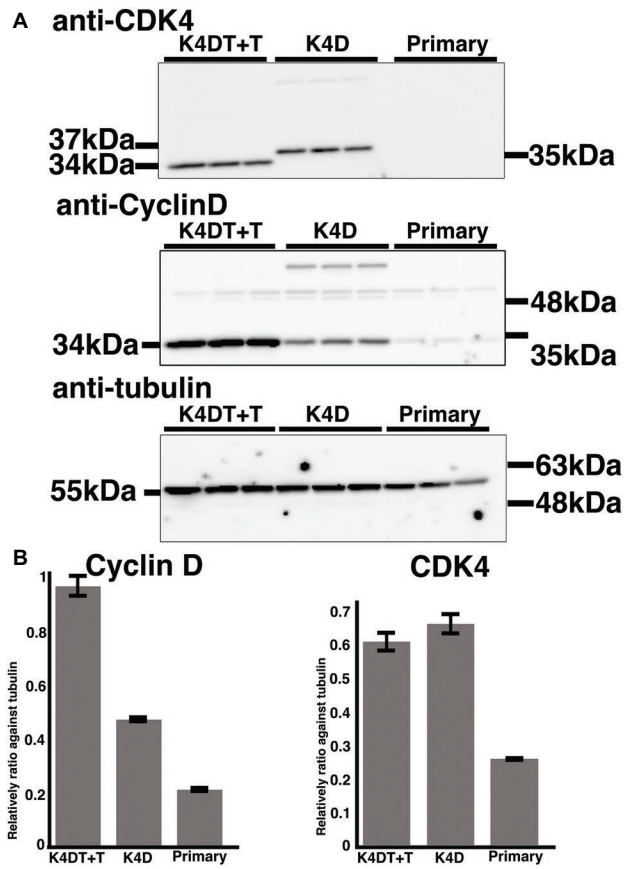

FIGURE 7 | Detection of introduced proteins with western blotting. **(A)** Primary, K4D, and K4DT+T cells were applied into the western blotting using anti-CDK4, anti-Cyclin D1, anti-tubulin antibodies. The triplicated technical replicates were examined. **(B)** Quantitation results of the signal intensity with Image J software. The signal intensity of primary, K4D, and K4DT cells were shown in the graphs.

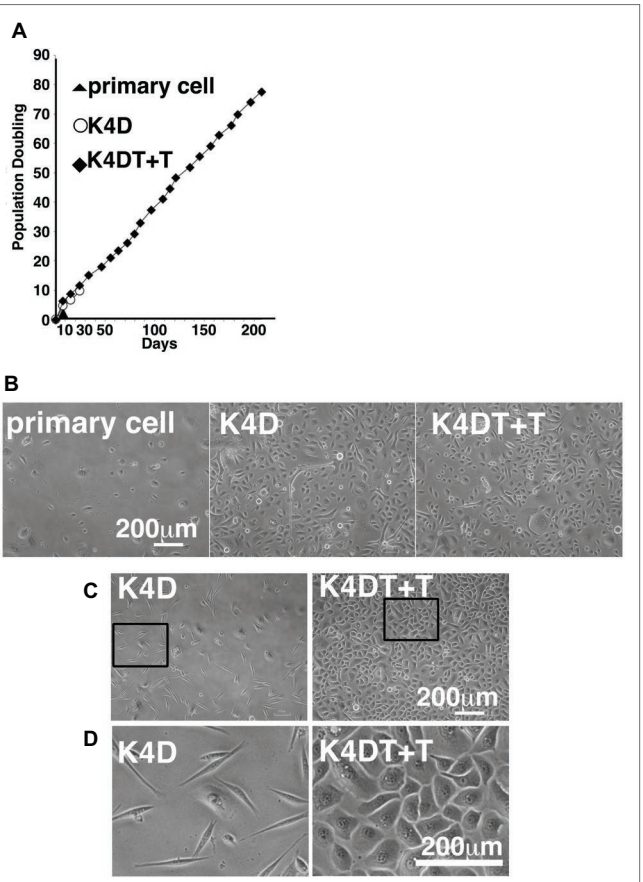

FIGURE 8 | Cell growth status and morphological observation of primary, K4D, and K4DT + T cells. **(A)** Cell growth and sequential passaging of primary, K4D, and K4DT + T cell. Cell growth is represented by the cumulative population doubling value. **(B)** Morphological observation at passage 1 in the population doubling assay. **(C)** Cell morphology of K4D and K4DT + T cells at passage 3. Lower magnification of K4D (left panels) and K4DT + T cells (right panels). **(D)** Higher magnification of K4D cells (left panels) and K4DT + T cell (right panels).

of the tumor suppressor protein, retinoblastoma protein (RB), resulting in increased cell proliferation (Fukuda et al., 2018). Since this inactivation is limited to the RB pathway, the function of p53, which can be viewed as the guardian angel of the genome, remains intact. Although the expression of mutant CDK4 and Cyclin D accelerates cell proliferation, cells co-expressing these two proteins cannot escape from the shortening of the telomere sequence found at the end of chromosomes. To overcome this limitation, the additional co-expression of TERT allows for an extension of the telomere sequence, essentially creating immortalized cells. Since we confirmed the integration of the TERT cassette in the KD4T + T cells (**Figure 5B**), these cells have the potential to proliferate indefinitely.

Interestingly, corneal epithelial cells only expressing the mutant CDK4 and cyclin D showed evidence of a cellular morphological change from an epidermal shape to a fibroblast-like shape (**Figures 9A,B**). Two possibilities could explain this morphological change in these cells. The first possibility is the lack of the TERT gene and shortening of telomere repeat. Due to the lack of TERT, the epithelial might limit the cell proliferation due to the shortening of the telomere repeat sequence at the end of the chromosome. The second possibility is the decreased transcriptional activity of the cytomegalovirus (CMV) promoter due to a lack of TERT expression. Although the detailed molecular mechanism is unknown, we had observed that the protein levels of mutant CDK4 and Cyclin D, driven from the CMV promoter, are significantly elevated when we co-expressed TERT in megabat-derived cells (Fukuda et al., unpublished

TABLE 1 | Cell cycle analysis of wild-type, K4D, and K4DT + T human-derived corneal epithelial cells.

	Cell cycle phase			
	G0/G1	**S**	**G2/M**	**Debris**
WT	58.2 ± 3.1	10.9 ± 1.0	22.5 ± 2.0	53.4 ± 4.7
K4D	48.8 ± 0.9*	6.6 ± 0.5*	27.5 ± 0.8*	56.4 ± 24.9
K4DT + T	29.0 ± 0.6**	4.9 ± 0.3**	40.0 ± 0.7**	51.1 ± 2.7

**Significance at 5% level between WT and K4D, WT and K4DT + T.*
***Statistical significance at 1%.*

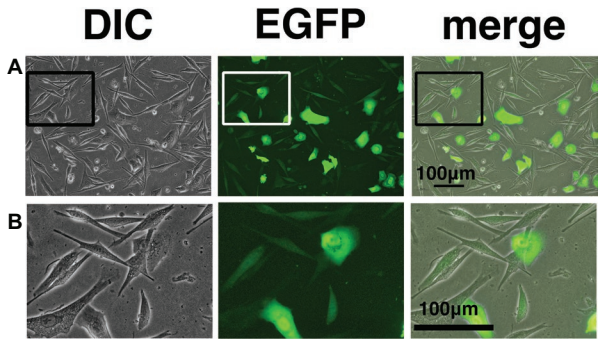

FIGURE 9 | Cell morphology and EGFP expression of K4D cells at passage 3. **(A)** K4D cells at passage 3. Cell morphology by differential interference contrast microscopy (DIC, left panel), EGFP fluorescence (EGFP, middle panel), and merged images (merge, right panel). **(B)** High-magnification view of K4D cells at passage 3 by DIC (left panel), EGFP fluorescence (EGFP, middle panel), and merged images (right panel).

data). As supportive evidence for this notion, we detected that fibroblast-like cells were negative neither for EGFP nor for Cytokeratin 3, which is the marker gene for corneal epithelial cells (**Figure 10**). These data indicate that fibroblast-like cells already lost the nature of corneal epithelial cells.

After establishing the new human corneal epithelial cell line, we carried out an irritation toxicity test, comparing both primary and K4DT + T human corneal epithelial cells. As a first step, we evaluated glycolic acid as an irritant, assessing its cell toxicity *in vitro*. Exposure to glycolic acid resulted in a significant, dose-dependent decrease in cell viability. The K4DT + T cells showed a significant decrease in cell viability at both doses of glycolic acid (0.5 and 5%). Our data suggest that the human corneal epithelial K4DT + T cells have the potential to detect chemical irritants. Although the original STE method was developed using rabbit-derived corneal epithelial cells, evaluations using human-derived cells should increase the precision of the toxicology test.

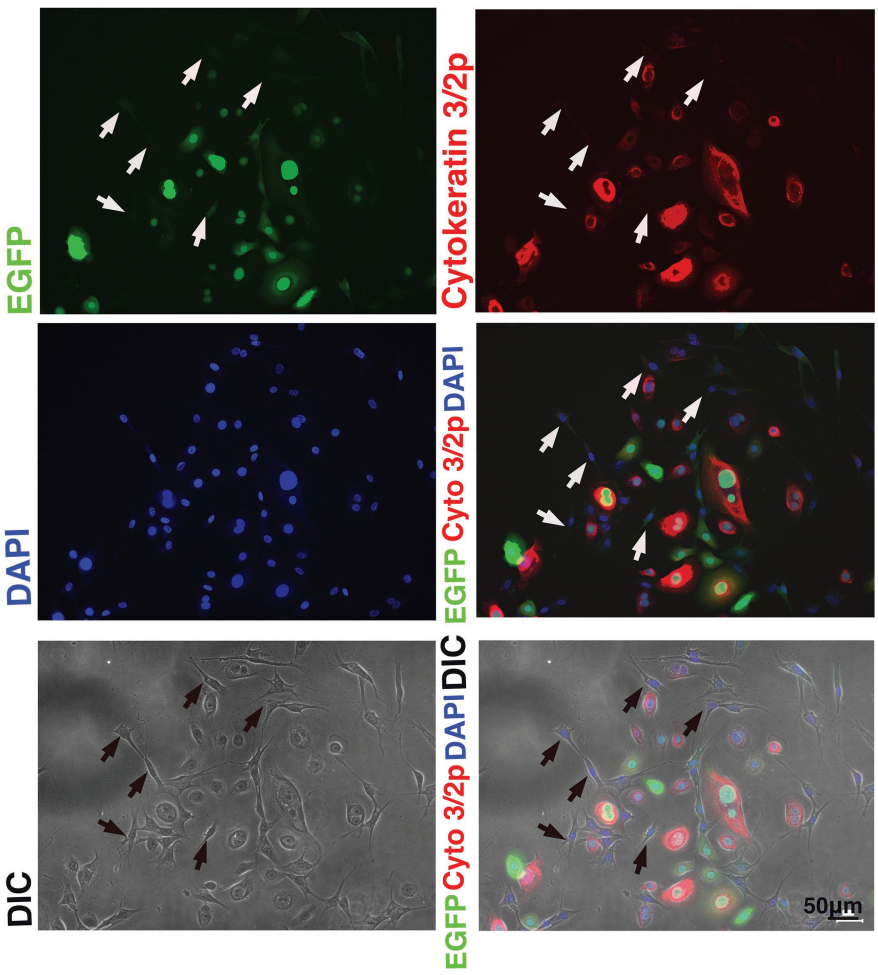

FIGURE 10 | Immunostaining of K4D cells expressing EGFP. (Upper left) EGFP expression in K4D cells at passage 3. (Upper right) Immunostaining of cytokeratin 3/2p in K4D cells detected with Alexa 568. (Middle left) Nuclear counterstaining with DAPI. (Middle right) The merged picture among EGFP, cytokeratin 3/2p, and DAPI channels. (Lower left) Cell morphology of difference in contrast (DIC). (Lower right) The merged picture among EGFP, cytokeratin 3/2p, DAPI, and DIC channels. Note that fibroblast-like cells that are indicated by arrows were neither negative for EGFP and cytokeratin 3/2p.

Furthermore, we evaluated the toxicity of Benzalkonium chloride with the STE method. As shown in **Figure 2**, Benzalkonium chloride showed strong toxicity against our immortalized corneal epithelial cell. Based on the results of STE method, we further tested the toxicity of Benzalkonium chloride at PBS, 5, 0.5, 0.05, 0.005, and 0.0005% with MTS assay. In principle, MTS assay is a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Therefore, cell viability can be detected by the absorbance at 490 nm. As shown in **Figure 4**, notably, we detected the cytotoxicity even at the 0.0005% Benzalkonium chloride solution. These results indicate that our established cells are quite sensitive to the toxicity of the chemicals.

Recently, we have also succeeded in generating corneal epithelial-derived cells that express a secreted form of luciferase (Goko et al., unpublished data). Since the sensitivity of luciferase detection is quite high, we can use this to estimate the number of surviving cells after chemical treatment. The use of a secreted type of luciferase will allow us to monitor surviving cells in the culture medium, which should allow for use in high-throughput screening. Our established cells should contribute to accuracy in the evaluation of chemical toxicity and reduce the sacrifice of animals in experiments, which will be required for next-generation science.

AUTHOR CONTRIBUTIONS

TF, RG, TE, KT, and RS did the experiments. KN, ES, HT, and TF designed the experiments. TK contributed to essential experimental materials.

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Visual function and quality of life in patients with Stevens-Johnson syndrome who received acute protocol-based ocular care

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Purpose: To report visual function and quality of life (VF/QOL) using the National Eye Institute Visual Function Questionnaire (NEI-VFQ-25) and the ocular surface disease index (OSDI) in patients in the chronic phase of Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN).

Methods: The NEI-VFQ-25 questionnaire was administered to 15 patients who received protocol-based care in the form of topical medications with or without amniotic membrane transplantation (AMT) for acute SJS/TEN. The scores obtained were compared with scores from a healthy population. The associations between the NEI-VFQ-25 and dry eye symptoms as measured by OSDI questionnaire were also studied.

Results: Patients were surveyed at a mean of 4.47 $\pm$ 2.22 years after acute SJS/TEN. Eleven patients received AMT in the acute phase. The median best corrected visual acuity at the time of administration of the questionnaire was 20/20. The mean composite NEI-VFQ-25 score was 86.48 $\pm$ 12. Patients who received protocol-based treatment in the acute phase of SJS/TEN had comparable NEI-VFQ-25 scores with healthy subjects on all subscales except ocular pain (p = 0.027) and mental health (p = 0.014), which were significantly reduced. The NEI-VFQ-25 composite scores significantly correlated with OSDI (R = -0.75, p = 0.001).

Conclusion: A protocol-based management strategy composed of early ophthalmic evaluation, grading based on severity, the use of topical corticosteroids and AMT in the acute phase of SJS/TEN in patients with ocular complications helped preserve the VF/QOL. This study highlights the impact of appropriate management of the ocular complications in the acute phase of SJS/TEN.

KEYWORDS

stevens-johnson syndrome (SJS), toxic epidermal necrolysis (TEN), visual function, national eye institute visual function questionnaire (NEI-VFQ-25), ocular surface disease index (OSDI)

Introduction

Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) are a spectrum of disease acutely affecting the skin and the mucous membranes (Kohanim et al., 2016). These conditions cause significant necrolysis and desquamation of the skin and mucosae with involvement of the oral, conjunctival, and genital mucosa being most frequent (Shanbhag et al., 2020a). In the acute phase, SJS/TEN affects the ocular surface in the form of denudation of the epithelium of the lid margin, conjunctiva, and cornea (Gregory, 2011). If these complications are not addressed quickly and appropriately, they can progress to long-term sequelae such as lid margin keratinization, symblepharon, dry eye disease (DED), corneal vascularization, and limbal stem cell deficiency (LSCD), which could culminate in bilateral corneal blindness in the chronic phase (Di Pascuale et al., 2005; Gueudry et al., 2009; Basu et al., 2018). Ocular sequelae are reported to be one of the most common and most disabling long-term sequelae in patients with SJS/TEN (Yang et al., 2016; Lee et al., 2017).

For patients who present with ocular involvement in the acute phase of SJS/TEN, a protocol-based management strategy including early ophthalmology consult, grading based on severity of ocular findings, usage of topical corticosteroids, and amniotic membrane transplantation (AMT), when indicated, has been reported to reduce the incidence of vision-threatening complications in the chronic phase (Shanbhag et al., 2019b). Reports on the long-term outcomes of AMT in SJS/TEN have been recently published to show that AMT, when done within the window of opportunity (5–7 days of symptom onset), can mitigate vision-threatening complications in the chronic phase (Shanbhag et al., 2020b; Yang et al., 2020). However, lid-related complications and DED may persist. Outcomes in cases that have been managed in a standardized fashion in the acute phase of SJS/TEN have not yet been reported from the patient's perspective. The 25-item National Eye Institute Visual Function Questionnaire (NEI VFQ-25) was developed in 1995 to assess visual health and the influence of visual impairment on health-related quality of life (Mangione et al., 2001). This study was undertaken to report the vision-related quality of life (VF/QOL) and the symptoms of ocular surface disease utilizing the NEI-VFQ-25 questionnaire and the ocular surface disease index (OSDI) scoring system, respectively, in patients who received protocol-based management for ocular involvement in the acute phase of SJS/TEN.

Materials and methods

Approval

This study was approved by the institutional review board of the Massachusetts Eye and Ear (MEE). The study was conducted under Health Insurance Portability and Accountability Act (HIPAA) compliance and adhered to the tenets of the Declaration of Helsinki.

Patient selection

All patients who received protocol-based management for ocular disease in the acute phase of SJS/TEN and visited the clinic for follow-up between November 2017 and June 2018 were interviewed in this prospective study. This included a subset of SJS/TEN patients who had onset of disease between January 2011 and July 2017. The SJS/TEN protocol that was followed for the care of these patients in the acute phase has been previously described and is shown in Figure 1 (Shanbhag et al., 2019b). Patients who received AMT underwent the procedure as previously described. (Ma et al., 2016; Shanbhag et al., 2019a). Data regarding demographic information, employment status, the etiology of the SJS/TEN, the time interval from AMT to the day the questionnaire was administered, the best corrected visual acuity (BCVA) on the day of the interview, the status of DED, ocular findings of trichiasis and distichiasis, and the history of usage of scleral contact lenses, were collected before the questionnaire was administered. Only patients who spoke English and were above the age of 18 years were interviewed.

Outcome measures

The NEI VFQ-25 data were administered in an interviewer based-format and collected prospectively from each patient, as described in Version 2000 of the NEI-VFQ-25 [12–13]. The NEI VFQ-25 questionnaire consists of 25 questions with 11 subscales that include questions specific for general health, general vision, ocular pain, difficulty with activities related to distant vision, difficulty with activities related to near vision, limitations in social functioning due to vision, mental health and problems with mental well-being due to vision, role limitations due to vision, dependency due to vision, difficulties with the activity of driving, problems with color vision, and limitations with peripheral vision (Mangione et al., 2001). The NEI-VFQ-25 composite and subscale scores were calculated according to the manual [13]. The primary outcome for this study was the composite NEI-VFQ-25 score. The NEI-VFQ-25 scores range from 0 to 100, with lower scores indicating more ocular symptoms resulting in a poorer quality of life. The mean NEI-VFQ-25 subscale scores from patients who were administered the questionnaire in this study were compared with the mean NEI-VFQ-25 subscale scores of 122 healthy subjects with no ocular disease (except refractive errors with BCVA ≥20/25 in the worse eye) from the original NEI-VFQ-25 study (Mangione et al., 2001). The mean age of the

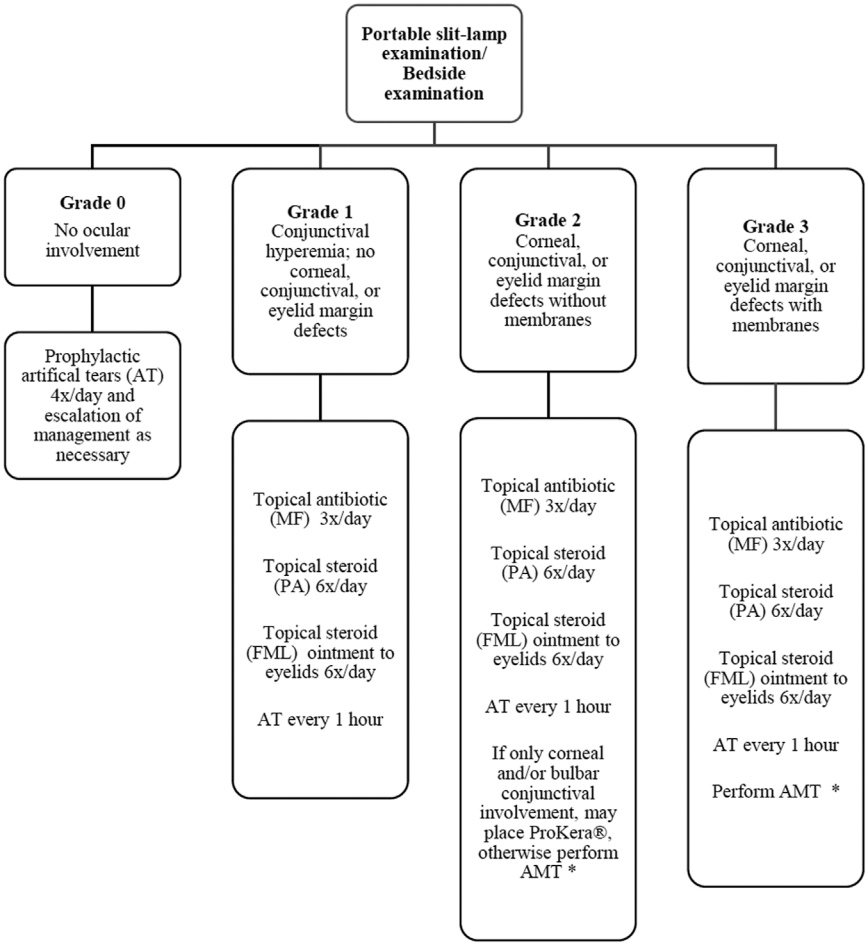

FIGURE 1
Flow diagram outlining protocol for management of ocular manifestations in acute Stevens-Johnson syndrome/toxic epidermal necrolysis. (MF = moxifloxacin 0.5%; PA = prednisolone acetate 1%; FML = fluorometholone 0.1%; AT = artificial tears; AMT = amniotic membrane transplantation). Decision to perform AMT based on feasibility (intubation status, cooperation, *etc.*). ProKera is acceptable if only bulbar conjunctival or corneal involvement is present or when AMT is not feasible. Reprinted with permission from Shanbhag SS, Rashad R, Chodosh J et al. Long-Term Effect of a Treatment Protocol for Acute Ocular Involvement in Stevens Johnson Syndrome/Toxic Epidermal Necrolysis. Am J Ophthalmol, 208, 331–341.

reference population was 59 ± 14 years with all subjects above 21 years. The median visual acuity in the better eye was 20/20.

The OSDI questionnaire was administered to each patient in an interview-based format. This questionnaire consists of three subscales with questions on ocular discomfort, how these symptoms limit activities such as reading, and how environmental triggers can impact dry eye symptoms (Schiffman et al., 2000). Each question is graded in accordance with the frequency of the symptoms, with a score of "0" correlating with symptoms occurring "none of the time" and a score of "4" correlating with symptoms occurring "all the time." The OSDI scores can range from 0 to 100, with higher scores indicating greater disability due to symptoms related to DED.

Data and statistical analysis

The statistical analysis was performed using Stata statistical software 15 (StataCorp, College Station, Texas). Normality of the data were evaluated with the Shapiro-Wilk test. Quantitative variables were expressed as mean ± standard deviation (SD), and qualitative variables were expressed as percentages. Visual acuities were measured with a standardized Snellen chart and converted to logMAR values for analysis. Comparison of NEI-VFQ-25 subscale scores with the subscale scores of the reference population was performed with an unpaired *t*-test. The Spearman correlation coefficient was used to test the associations between the NEI-VFQ-25 subscale scores and

TABLE 1 Characteristics of patients who underwent protocol-based management in the acute phase of Stevens-Johnson syndrome/toxic epidermal necrolysis.

Characteristics	
Number of patients//eyes	15//30
Gender, Male: Female	3:12
Age, mean ± SD	37.3 ± 14
Interval since acute SJS/TEN in years, mean ± SD	4.47 ± 2.22
Etiology of SJS/TEN	
Drug-induced	
Cotrimoxazole	9
NSAIDs	2
Lamotrigine	1
Others	2
Unknown	1
Employment status	
Employed	12
Student	3
Unemployed	0
Grade of ocular involvement in the acute phase* (median)	2
Number of eyes that received AMT in the acute phase	22
BCVA at the time of administration of questionnaire, Median (IQR)	0 (0–0.1) [LogMAR] 20/20 (20/20–20/25) [Snellen equivalent]
Number of eyes with dry eye disease in the chronic phase[a]	
Grade 2	6
Grade 3	4
Number of eyes that use scleral lenses in the chronic phase	8
Number of eyes with trichiasis and distichiasis in the chronic phase	12
Number of eyes that underwent additional surgery post AMT	1

SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis; IQR, Inter-quartile range; SD, standard deviation; BCVA, best corrected visual acuity.
*Grading as per Sotozono classification (Sotozono C, et al.; Japanese Research Committee on Severe Cutaneous Adverse Reaction. Predictive Factors Associated With Acute Ocular Involvement in Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis. Am J Ophthalmol. 2015; 160:228–237. e2. doi: 10.1016/j.ajo. 2015.05.002).
[a]Grading of dry eye disease as per DEWS, criteria.

OSDI scores, between the NEI-VFQ-25 composite scores and OSDI scores, and between the NEI-VFQ-25 composite scores and patient-related parameters such as patient age, BCVA in the worse eye, and the duration of time since the acute phase of SJS/TEN. A two-sided p value of <0.05 was considered statistically significant.

Results

The mean age of the patients to whom the NEI-VFQ-25 questionnaires were administered was 37.3 ± 14 years (range: 20–59). The mean time interval between the acute episode of SJS/TEN and the time at which the questionnaires were administered was 4.47 ± 2.22 years (range: 0.85–9.1). The characteristics of all the patients included in this study are mentioned in Table 1.

The median OSDI score for patients who underwent protocol based-management for acute SJS/TEN was 18.75 (interquartile range: 10.41–45.83). The mean NEI-VFQ-25 composite score was 86.48 ± 12 (range: 56–100). The mean NEI-VFQ-25 composite and subscale scores and the comparison to the scores in the reference population are shown in Table 2. The subscale scores for ocular pain ($p = 0.027$) and mental health ($p = 0.014$) were significantly reduced in the patients with SJS/TEN as compared to the healthy population. The scores were comparable for all other subscales.

The correlation between the NEI-VFQ-25 scales and OSDI scores is shown in Table 3. The correlation between the NEI-VFQ-25 composite score and OSDI was statistically

TABLE 2 25-item National Eye Institute Visual Function Questionnaire scores in patients who received protocol-based management for ocular manifestations of Stevens-Johnson syndrome/toxic epidermal necrolysis.

NEI-VFQ-25 Scales	Mean ± SD (range) in SJS/TEN patients (n = 15)	Mean ± SD (range) in reference patients (n = 122)	*p* Value
General health	63.33 ± 21 (25–100)	69 ± 24	0.34
General vision	80 ± 13 (60–100)	83 ± 15	0.42
Ocular pain	73.33 ± 26 (25–100)	90 ± 15	0.027
Near activities	88.34 ± 13 (67–100)	92 ± 13	0.32
Distance activities	88.34 ± 16 (42–100)	93 ± 11	0.29
Driving	77.22 ± 27 (42–100)	87 ± 18	0.19
Color vision	100 ± 0 (100)	98 ± 8	0.006
Peripheral vision	98.33 ± 6 (75–100)	97 ± 10	0.46
Vision specific			
Role difficulties	89.16 ± 21 (25–100)	93 ± 13	0.5
Dependency	91.67 ± 18 (42–100)	99 ± 6	0.14
Social functioning	93.33 ± 11 (62–100)	99 ± 3	0.91
Mental health	70.91 ± 29 (6–100)	92 ± 12	0.014
Overall composite score	86.48 ± 12 (56–100)		

SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis; NEI-VFQ-25, 25-item National Eye Institute Visual Function Questionnaire; SD, standard deviation.

TABLE 3 Correlations of NEI-VFQ-25 subscale scores with OSDI scores in patients who received protocol-based management for ocular manifestations of Stevens-Johnson syndrome/toxic epidermal necrolysis.

NEI-VFQ-25 subscales	R Based on OSDI score	*p* Value
General health	-0.6	0.017
General vision	-0.56	0.03
Ocular pain	-0.69	0.004
Near activities	-0.6	0.017
Distance activities	-0.5	0.03
Social functioning	-0.5	0.029
Mental health	-0.8	0.0003
Role difficulties	-0.6	0.02
Dependency	-0.56	0.03
Driving	-0.32	0.2
Color vision	-	-
Peripheral vision	-0.31	0.26
Overall composite score	-0.75	0.001

SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis; NEI-VFQ-25, 25-item National Eye Institute Visual Function Questionnaire; R = correlation coefficient.

significant (R = -0.75; $p = 0.001$). The correlation between all the NEI-VFQ-25 subscales and OSDI was statistically significant except for the subscales of driving, color vision, and peripheral vision.

There was no correlation between the composite NEI-VFQ-25 score and the duration of time from acute SJS/TEN (R = 0.09; $p = 0.73$) and the BCVA in the worse eye (R = -0.46; $p = 0.08$). However, there was a statistically significant correlation between the composite NEI-VFQ-25 score and the age of the patient (R = 0.64; $p = 0.009$). There was no correlation between the OSDI score and the duration of time from acute SJS/TEN (R = -0.09; $p = 0.75$), the BCVA in the worse eye (R = 0.36; $p = 0.19$) and the age of the patient (R = -0.4; $p = 0.14$).

Discussion

SJS/TEN is a life-threatening condition, and survivors of SJS/TEN can suffer from a multitude of complications affecting various organ systems in the chronic phase many years after the initial acute episode (Shanbhag et al., 2020a). Ocular disease in particular is common in SJS/TEN patients and can be debilitating. In one study, 77% of survivors had ocular complications in the chronic phase, and all patients had ocular involvement in the acute phase (Haber et al., 2005). Patients who suffer from chronic ocular complications such as DED and chronic photophobia in the chronic phase of SJS/TEN have significantly lower overall health-related quality of life compared to the normal population (Haber et al., 2005). In a recent questionnaire-based study, out of 57 survivors of SJS/TEN, 70% patients had chronic ocular complications; DED was the most common complication affecting 87% of patients (Ingen–Housz-Oro et al., 2020). Between one-third and two-thirds of patients in this study with chronic ocular complications had difficulty using a computer or cellular phone, watching television, or driving a car. Another study

of 17 survivors of SJS/TEN conducted at a mean follow-up of 51.6 months after the acute phase showed that only 29% were employed (Dodiuk–Gad et al., 2016). A significant number of patients require multiple surgical interventions in the chronic phase for ocular complications along with life-long topical medications which contribute to increased financial burden (Ingen–Housz-Oro et al., 2020).

Our study shows that patients who receive an early ophthalmology consult, have acute severity graded based on ocular findings, and are treated with a protocol comprising of topical corticosteroids, topical antibiotics, and AMT have NEI-VFQ-25 scores comparable to the general population for most subscales. We have shown previously that patients treated with this protocol had significantly better visual outcomes and fewer vision-threatening complications over a median follow-up period of 2.6 years after acute SJS/TEN (Shanbhag et al., 2019b).

Previous studies have demonstrated higher OSDI scores that correlate with higher functional impairment and severity of DED in patients with chronic ocular complications post SJS/TEN. In a study by Gueudry et al., at a mean duration of 82 months from discharge for acute SJS/TEN, the median OSDI score for 31 patients with ocular sequelae was 41.6 (range: 0–97.5), corresponding to severe functional impairment (Gueudry et al., 2009). In our study, the OSDI score was 18.75, which corresponds to normal to mild functional impairment due to DED. In the former study, 89% of patients received topical antibiotics, 63% of patients received topical lubricants, only one out of 31 patients received topical corticosteroids, and no patients received AMT in the acute phase of SJS/TEN despite 12 and 13 eyes being described as moderate or severe, respectively. In comparison, in our study, all 30 eyes of 15 patients received topical antibiotics, topical lubricants, and topical steroids, while 22 eyes of 11 patients received AMT in the acute phase. While the baseline patient characteristics were likely different between these two studies, it does not appear that a standardized protocol was followed in the former study. In another study by Tougeron-Brousseau et al., the mean OSDI in 36 patients with chronic SJS/TEN was found to have improved significantly after scleral lens placement (Tougeron–Brousseau et al., 2009). It is unclear what kind of treatment these patients received in the acute phase. Compared to the severe functional impairment demonstrated by the OSDI scores in the study by Tougeron-Brousseau et al., the OSDI score in our study was lower and only four patients in our cohort required scleral lenses in the chronic phase.

Previous studies have also demonstrated that lower NEI-VFQ-25 scores correlate to poorer VF/QOL in patients with chronic ocular complications post SJS/TEN. In the study by Tougeron-Brousseau et al., the mean composite NEI-VFQ-25 score in 32 patients with chronic SJS/TEN was 25.1 ± 16.8 and 67.4 ± 22.1 at presentation and 6 months after scleral lens placement, respectively (Tougeron–Brousseau et al., 2009). Other studies have also shown low NEI-VFQ-25 scores in patients with chronic SJS/TEN. Again, details of acute phase management are not discussed in these studies and it does not appear that standardized treatment protocols were followed. (Kaido et al., 2004) (Papakostas et al., 2015). In our study, the mean composite NEI-VFQ-25 score was 86.48 ± 12 in the chronic phase of SJS/TEN, higher than in all previously reported studies on SJS/TEN, and not significantly different than that of a healthy reference population.

In our study, the subscale scores for ocular pain and mental health were significantly reduced in patients with SJS/TEN as compared to the healthy population, despite a median BCVA of 20/20. These findings suggest that it is not just visual acuity that affects vision-related quality of life. It has been shown in various studies that survivors of SJS/TEN have higher incidences of depression, anxiety, and post-traumatic stress disorder and decreased health-related quality of life (Dodiuk–Gad et al., 2016; Hefez et al., 2019; Hoffman et al., 2021). Patients with SJS/TEN, even with good vision, may suffer from ocular discomfort and pain, affecting their daily activities. The etiologies in these specific cases is not known but may be due to DED and coexisting pathologies such as trichiasis and distichiasis (Kaido et al., 2006). Color vision was also found to be significantly different between the two groups, with the reference population scoring worse. We believe this is due to several factors. The question specific to color vision on the VFQ is “Because of your eyesight, how much difficulty do you have picking out and matching your own clothes?“. The answer to this question may reflect mobility issues in addition to color vision. Combined with the older age of the otherwise healthy reference population vs. our SJS/TEN population, this result is likely confounded by age. Additionally, the difference between a mean score of 100 vs. 98 is not clinically significant.

The most common etiology for SJS/TEN in our study was sulfonamide drugs, specifically cotrimoxazole. A recent study demonstrated that patients with SJS/TEN secondary to cotrimoxazole had significantly less severe chronic stage ocular complications and better long-term visual acuity as compared to patients who had SJS/TEN secondary to lamotrigine (Rashad et al., 2021). This could be a contributing factor to the better NEI-VFQ-25 OSDI scores in our cohort, thus reflecting a better VF/QOL and lesser severity of DED. However, we believe that a more significant contribution to the NEI-VFQ-25 and OSDI scores in our cohort is that all patients received an early ophthalmology consult after the diagnosis of SJS/TEN, received timely and appropriate protocol-based treatment, and were asked to follow-up frequently in the chronic phase. This ensured that any issues in the acute, sub-acute, and chronic phases were addressed expediently.

This study highlights the role of timely and appropriate acute care for ocular involvement in patients with SJS/TEN. Recent studies have shown that only 66% of burn centers in the United States routinely get an ophthalmology consult for their SJS/TEN patients (Le et al., 2016). Another study noted that although 67% of their patients admitted for SJS/TEN had ophthalmologic complications in the acute phase, only 6% were followed by an ophthalmologist in the chronic phase after discharge (Olteanu et al., 2018). We believe that if every patient with SJS/TEN receives an ophthalmology consult in the acute phase, is graded for severity, treated and managed in a standardized and appropriate fashion, and has the necessary follow-up in the chronic phase for any new potential issues, the VF/QOL of these patients can be optimized. Indeed, we have previously shown that a standardized protocol-based approach to acute ocular care can reduce chronic complications, and it follows that VF/QOL would also be improved. Barriers to implementing such care may include the time and coordination that an AMT procedure can take. However, we have previously reported a technique which significantly reduces the time and resources necessary for the procedure (Shanbhag 2019a).

The limitations of this study include its small sample size and the absence of the NEI-VFQ-25 and OSDI scores in a control group that did not receive protocol-based care in the acute phase of SJS/TEN. However, patients who did not receive protocol-based care in the acute phase of the disease were often patients who received no or poor acute care and went on to undergo surgeries such as keratoprosthesis that would confound the NEI-VFQ-25 scores. Another limitation is that all patients seen in the acute phase were not seen in the chronic phase, resulting in selection bias. However, it is likely that those who did follow up had more severe disease and so the OSDI and NEI-VFQ-25 scores may actually be skewed towards worse symptomatology. Despite this, the scores on both were better than those reported in previous studies. Lastly, Schirmer's test scores may have been a useful adjunct in assessing dry eye and, if available, could have been correlated with OSDI and NEI-VFQ25 scores; however, Schirmer's scores were not consistently recorded and were not analyzed in this study.

SJS/TEN is a rare condition, and patient-reported outcomes several years after acute SJS/TEN, as reported in this study, add to our knowledge about the impact that treatment in the acute phase can have on the quality of life of these patients. Hence, we conclude that efforts should be directed at spreading awareness among physicians about the need for an ophthalmology consult in the acute phase immediately after SJS/TEN is suspected, and emphasizing the need to follow an evidence-based treatment protocol as we have described.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Massachusetts Eye and Ear Internal Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SS was responsible for administering the NEI-VFQ-25 questionnaire to the patients, completing the statistical analysis, interpreting the results, and writing the paper. MT was responsible for writing, editing, and formatting the paper. HS was responsible for interpreting the results and editing, writing, and providing feedback on the paper. JC was responsible for editing and providing feedback on the paper.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Noxious effects of riot control agents on the ocular surface: Pathogenic mechanisms and management

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Riot Control Agents (RCAs) are chemical compounds used by law enforcement agencies to quell violent demonstrations as an alternative to lethal force and as part of police/military training. They are also known as tear gases because of the hallmark ocular irritation and lacrimation they cause. The most common RCAs include oleoresin capsicum (contained in Mace and pepper spray), chlorobenzylidene malononitrile, dibenzoxazepine, and chloroacetophenone (previously the main content of Mace); some of which have been in use for decades. Their immediate incapacitating effects are mediated through polymodal afferent fibers innervating the corneal surface, inducing the release of peptides that cause neurogenic inflammation. Although previously thought to have only transient effects on exposed patients more severe complications such as corneal stromal opacities, corneal neovascularization, neurotrophic keratopathy, conjunctival necrosis, and pseudopterygium can occur. Concerningly, the lack of research and specific therapies restrict the current management to decontamination and symptom-tailored support. This manuscript will provide an overview of the toxic mechanisms of RCAs, their clinical manifestations, and current therapy after exposure to tear gases.

KEYWORDS

riot control agents, tear gas, toxicity, oleoresin capsicum, chlorobenzylidene malononitrile, neurogenic inflammation, ocular surface

1 Introduction

Riot control agents (RCAs), also known as chemical crowd control agents, are chemical agents that cause temporary disability, usually a little longer than the exposure period (Menezes et al., 2016). They represent a non-lethal and non-confrontational alternative for authorities to pacify large crowds causing a civilian disturbance or curtail advancing enemy military forces (Toprak et al., 2015). To manage violent crowds, the ideal RCA has a rapid onset of action, a brief duration of effects, and a good safety profile to avoid permanent damage (Kim et al., 2016). In contrast, to hinder the advancement of a military force, the chemical should ideally remain in the environment for weeks to months (Menezes et al., 2016). Due to their ease of use and immediate onset of action, aerosolized chemicals, the so-called tear gases, are the most frequently used RCAs, including chloroacetophenone (CN), oleoresin capsicum (OC), dibenzoxazepine (CR), and chlorobenzylidene malononitrile (CS) (Brown et al., 2000;

Zollman et al., 2000; Yeung and Tang, 2015). These chemicals are the main constituents of pepper sprays, and CN was the active compound in the original formula of the product marketed as "Mace" for self-defense use or as an animal deterrent (bear mace). However, OC and CS alone or in combination have replaced CN in modern formulations due to less toxic effect profiles (Smith and Greaves, 2002; Kearney et al., 2014). To this day, exposure to these agents is part of the training regime used in some law enforcement academies.

Tear gases rapidly disable the victim by inflicting damage to the eye's ocular surface, the outermost part (Krishnatreyya et al., 2018b). The extent of the damage varies depending on the form of delivery. In the acute setting, aerosolized agents may cause lacrimation, erythema, conjunctival edema, blurred vision, and eye pain (Dimitroglou et al., 2015). In contrast, explosive weapons may cause thermal, chemical, and physical damage imposed by the blast (MacLeod, 1969; Tidwell and Wills, 2019). If left untreated, tear gases may lead to permanent vision loss due to conjunctival scarring and loss of corneal sensation leading to neovascularization, stromal thinning, ulceration, infection, and perforation (Levine and Stahl, 1968). Although rare, blindness could also result from secondary glaucoma, cataract formation, vitreous hemorrhage, and traumatic optic neuropathy (Kim et al., 2016). Thus, acute management and careful follow-up are necessary after eye exposure to RCAs to avoid sight-threatening complications.

The ramifications that arise from the use of these substances are not only limited to the eyes. The respiratory system is the other main target of RCAs, but dermatological, gastrointestinal, and even neurologic symptomatology can be observed (Hu et al., 1989; Dimitroglou et al., 2015). Depending on the concentration used and the length of exposure, manifestations range from copious rhinorrhea, sneezing, salivation, and skin erythema to more severe complications like laryngeal edema, pulmonary edema, chemical burns, and panic attacks (Vaca et al., 1996; Varma and Holt, 2001). Some of these exposures have proven to be lethal (Haar et al., 2017). Considering that the eyes are one of the main targets of RCAs, it is crucial for clinicians to possess knowledge of how these patients could present. This review aims to provide an up-to-date overview of the clinical presentation, pathogenic mechanisms, and treatment of ocular surface toxicity induced by RCAs.

2 Historical background and epidemiological data

The use of poisonous gases was reported as early as 430, 431 BC when Spartans released irritating gases of coal, burning wax, and pitch to the environment during the Peloponnesian War against the Athenians (Sanford, 1976). During World War I (1914–1918 AC), the German army was the first to use chemical agents that caused temporal disability by producing excessive blepharospasm and lacrimation, including chloropicrin, benzyl bromide, and acrolein, among others. By the early 1920s, civilians could purchase pocket-size tear gas devices containing CN to carry for self-defense purposes (Sanford, 1976; Frey et al., 2022). In 1925, in Geneva, the Protocol for the Prohibition of the use in War of Asphyxiating, Poisonous or Other Gases, and of Bacteriological Methods of Warfare was signed under the auspices of the (World Health Organization, 1970). Despite the latter, the United States signed an executive order in 1975 that allowed using RCAs in certain situations, including control of war prisoners and convoy protection outside combat zone; thus, they do not consider RCAs as warfare agents (Frey et al., 2022).

During 1998–2002, the Texas poison centers reported 1,531 human pepper spray (OC) exposures (Forrester and Stanley, 2003). Of those, 84% were unintentional, 68% occurred in the house, 64% involved children and teenagers, and 56% occurred in men (Forrester and Stanley, 2003). In 2017, the National Poison Data System (NPDS) reported 4,007 total exposures to tear gases, including OC (83%), CN (12%), CS (0.2%), and others (4%) (Gummin et al., 2018). Although 25% of the cases were treated in a health care facility, only 0.12% of victims suffered major adverse outcomes (Gummin et al., 2018).

3 Delivery systems of riot control agents (RCAs)

RCAs are usually referred to as "tear gases." However, rather than a gas, they are compounded as an aerosol of solid particles (Rothenberg et al., 2016). They may be projected from solutions or as airborne dispersions. While the former includes personal defense sprays and gas cartridges, canisters, and grenades employed by law enforcement (Ilgaz et al., 2019), the latter contains dispersions generated as smokes, aerosol mists, or powder clouds (Ballantyne, 2006). Hand-held devices contain liquid formulations released through narrow or wide-angle pressurized sprays to incapacitate one person (Schep et al., 2015). On the other hand, canisters and grenades are a pyrotechnic mixture blended with a powder form that is aerosolized for dispersion as smokes (Olajos and Stopford, 2004; Rothenberg et al., 2016). These tear gas pyrotechnic devices can engage targets as far as 300 m^2, ideal for crowd control in riots (Rothenberg et al., 2016). Aircraft, vehicle, and drone-guided technologies are also used as delivery systems. Additionally, non-lethal projectile weapons have a high risk of inducing severe traumatic injuries when fired at a person (Ifantides et al., 2020).

4 Anatomical features

The cornea is the most densely innervated tissue in the body. The ophthalmic branch (V_1) of the trigeminal nerve oversees the nociceptive functions of the eye, including the blinking reflex, tear production, and wound healing (Ruiz-Lozano et al., 2021). The nasociliary nerve, a V_1 branch, enters the orbit to cover the ocular surface. Nasociliary nerve branches decussate, pierce the sclera, and travel anteriorly to innervate the corneoscleral limbus and the corneal stroma (Marfurt et al., 2010). Subsequently, they form the subepithelial plexus and cross the Bowman's membrane to form the subbasal nerve plexus, which innervates the corneal epithelium (Marfurt et al., 2010; Ruiz-Lozano et al., 2021). There are three types of sensory corneal nerves, all of which evoke pain. They are classified into polymodal nociceptor neurons, pure mechanoreceptors, and cold thermoreceptors based on the activating noxious stimuli (Belmonte et al., 2015; Belmonte et al., 2017). The mechanism of action of tear gases occurs due to the activation of transient receptor potential (TRP) ion channels, a group of sensitizing chemosensory receptors located in peripheral nerve endings (Rothenberg et al., 2016; Frey et al., 2022). The TRP vanilloid (TRPV1), an agonist of OC, also known as capsaicin, and ankyrin (TRPA1) agonist of CS, CN, and CR, are

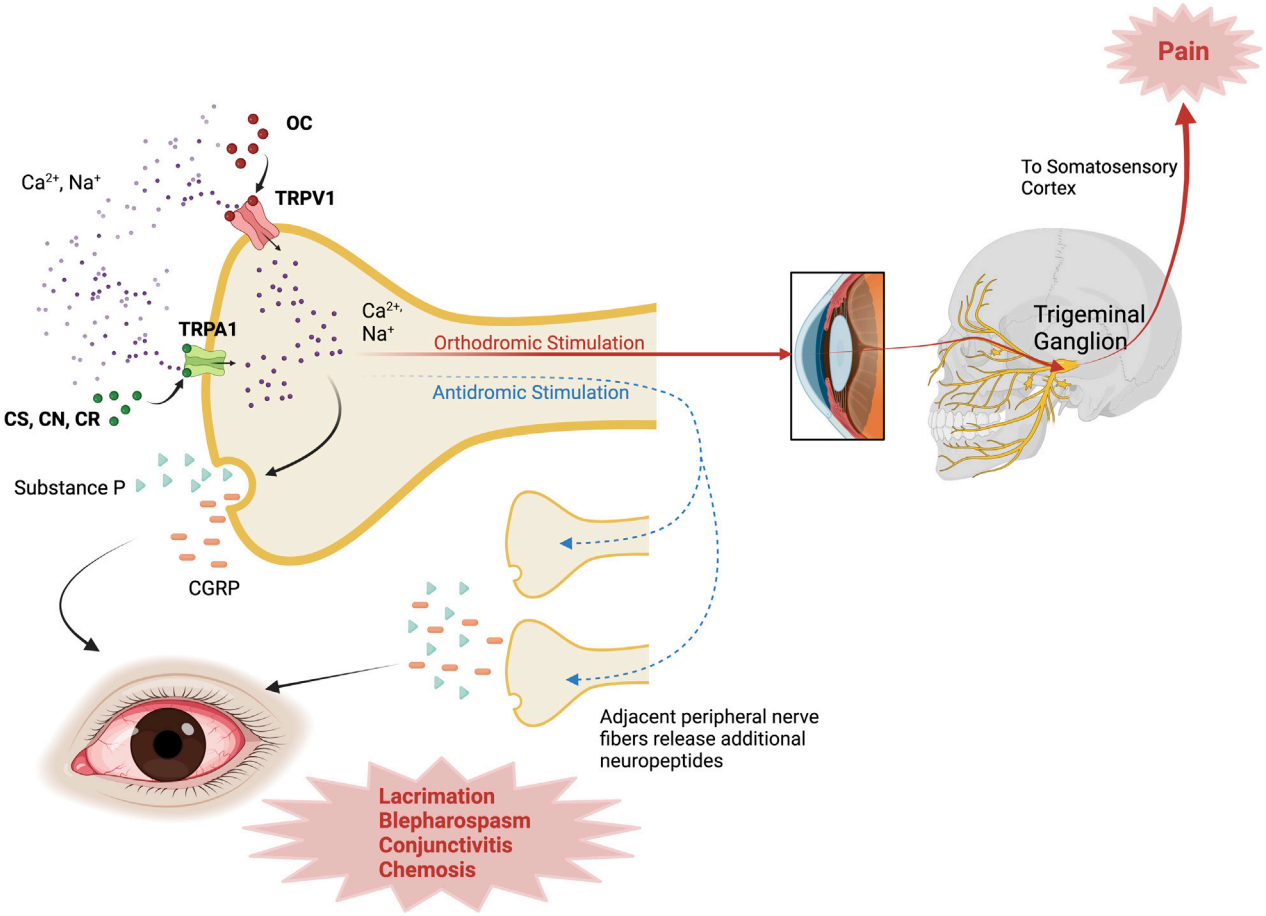

FIGURE 1
Mechanism of action of Riot Control Agents. OC activates TRPV1 channels; while CS, CN, CR activate TRPA1 channels in corneal sensory neurons, inducing Ca^{2+} influx. With orthodromic stimulation, the impulse travels through the trigeminal pathway to the somatosensory cortex and elicits the pain sensation. In antidromic stimulation, TRP channel activation triggers an impulse that travels to adjacent nerve fibers, inducing the release of additional neuropeptides (i.e., SP, CGRP) to propagate inflammation. CGRP, Calcitonin-Gene Related Peptide; CN, chloroacetophenone; CR, Dibenzoxazepine; CS, chlorobenzylidene malononitrile; TRPA1, Transient Receptor Potential Ankyrin 1; TRPV1, Transient Receptor Potential Vanilloid 1. Created with Biorender.com.

two subfamilies of TRP ion channels (Schep et al., 2015). They are both expressed in the peripheral pain-sensing nociceptive nerves of the skin, the mucous membranes of the lung and upper and lower airways, and the ocular surface (cornea and conjunctiva) (Rothenberg et al., 2016).

5 Ocular surface toxicity of specific chemicals used as riot control agents (RCAs)

5.1 Oleoresin capsicum (OC)

5.1.1 Chemical properties

OC is a mixture of fat-soluble phenols (capsaicinoids) obtained from the pepper plants *Capsicum frutescens* and *Capsicum annuum* (Ballantyne, 2006). Capsaicin ($C_{18}H_{27}NO_3$), the main component of OC, has a melting and boiling point of 64°C and 210–220°C, respectively, and a molecular weight of 305.41. The threshold for ocular irritation is 0.002 mg/m^3 (Schep et al., 2015; Kim et al., 2016). The concentration of OC in pepper sprays varies between manufacturers (1.2%–12.6%) (Ballantyne, 2006).

5.1.2 Mechanism of toxicity

Capsaicin has agonistic activity at TRPVI, a non-selective channel permeable to calcium and sodium in corneal sensory neurons (Belmonte et al., 2017; Alamri et al., 2018). Upon painful stimuli with OC, the TRPV1 channel opens, allowing calcium entry with subsequent channel inactivation and resulting analgesia (Bates et al., 2010). Besides pain, OC stimuli also trigger an inflammatory response, the so-called neurogenic inflammation. This process also involves membrane depolarization through non-selective channel opening, thus increasing intracellular calcium and sodium, allowing the release of neuropeptides by polymodal nociceptive neurons such as substance P and calcitonin-gene-related peptide (Kumar et al., 2018). The neurogenic inflammation model involves the axon-reflex hypothesis, where depolarization of the afferent fiber triggers an action potential traveling in one direction to the CNS to elicit the pain sensation (orthodromic stimulation); additionally, at axonal branch-points, an opposite-direction nervous impulse induces the

release of neuropeptides from nearby afferent nerve endings to potentiate inflammation (antidromic stimulation) (Yeung and Tang, 2015; Sorkin et al., 2018). A schematic representation of these mechanisms is found in Figure 1.

In a rabbit model, Gallar et al. demonstrated delayed corneal wound healing after topical and retrobulbar capsaicin application, suggesting damage to trigeminal nerve fiber endings and neuropeptide depletion (Gallar et al., 1990). In a murine model, Lambiase et al. observed a significant decrease in corneal innervation, peripheral sensitivity, corneal healing rate, and tear secretion after subcutaneous injection of capsaicin (Lambiase et al., 2012). After epithelial debridement, the authors report a significant decrease in nerve growth factor (NGF), a crucial factor that oversees the proliferation and survival of sensory neurons (Lambiase et al., 2012).

5.1.3 Ocular manifestations

Ocular irritation can occur with small capsaicin particles (2 μm), whereas severe and prolonged irritation occurs with more extensive (50 μm) particles. Although the lipid-soluble properties of capsaicin confer the ability to penetrate the corneal epithelium easily, its poor water solubility avoids damage to deeper corneal layers (Krishnatreyya et al., 2018a). In 47 cadets, Zollman et al. reported conjunctival injection, variable pain, and blepharospasm in all cases, punctate epithelial erosions (PEE, 21%), and a significant reduction in corneal sensitivity, measured with the Cochet-Bonnet esthesiometer 10 min after exposure to pepper spray (5.7 ± 0.4 cm vs. 0.6 ± 1.0 cm) during a training exercise (Zollman et al., 2000). After 1-week, the PEE was healed, and the corneal sensation was restored (Zollman et al., 2000). Vesaluoma et al. also found decreased corneal sensitivity and transient *in vivo* confocal microscopy (IVCM) changes, including corneal epithelial swelling, in ten police officers exposed to OC in a controlled setting (Vesaluoma et al., 2000).

Although results from the previous studies suggest that OC is harmless to the ocular surface, in all of them, exposure occurred in a controlled setting. Sustained corneal abrasions occurred in 7% of subjects in a jail's emergency department exposed to pepper spray at a 10% concentration (Brown et al., 2000). Holopainen et al. reported deep conjunctival and corneal damage that partially resolved after weeks to months in four cases exposed to pepper sprays, three containing OC. One case was only exposed to the solvent, suggesting the latter also causes ocular surface toxicity caused by OC sprays (Holopainen et al., 2003). IVCM findings revealed keratocyte activation in the deep corneal stroma of one case (Holopainen et al., 2003). Another study reported a significant reduction in tear production, measured with the Schirmer test, and dry eye symptoms 2 weeks after exposure to pepper spray in patients during a public protest in Turkey (Rasier et al., 2015). A 75-year-old man developed severe conjunctival chemosis with necrosis, symblepharon formation, and a subtotal corneal epithelial defect after exposure to topical capsaicin (Das et al., 2005).

5.2 Chloroacetophenone (CN)

5.2.1 Chemical properties

CN, also known as phenylacyl chloride and α-CN, was developed after World War I and has been used for riot control and self-defense. However, severe adverse effects are reported with its use, including death due to pulmonary asphyxia (Chapman and White, 1978; Ballantyne, 2006). Thus, countries like the United Kingdom no longer use CN for peacekeeping operations. It is still used in the United States (Ballantyne, 2006). CN (C_8H_7ClO) has a melting and boiling point of 58°C–59°C and 244°C–245°C, respectively, and a molecular weight of 154.59. It is soluble in ether, ethanol, and benzene and insoluble in water (Schep et al., 2015). The threshold for ocular irritation is 1.0 mg/m^3. CN is sold as MACE®, a 1% CN solution in a solvent of 5% 1,1,1-trichloroethane, 4% kerosene, and Freon 113 (Blain, 2003). It is a micro-pulverized powder that can cause thermal and mechanical due to the force of the blast and chemical damage to the eye (Levine and Stahl, 1968). The half-maximal activation of TRPA1 induced by CN is EC_{50} CN = 91 ± 12 nM (Bessac et al., 2009).

5.2.2 Mechanism of toxicity

CN and CS (See Section 5.3) are SN_2-alkylating agents that react with nucleophilic sites, the former tenfold more potent. The studies performed by Ballantyne and Swanston in 1978 determined that the toxicity induced by CN is caused by the inactivation thiol and sulphydryl-containing enzymes, including pyruvic decarboxylase and glutamic dehydrogenase (Blain, 2003; Ballantyne, 2006). Additionally, CS can also reversibly inhibit lactate dehydrogenase, while CN cannot (Sanford, 1976). Therefore, some of the toxic effects caused by these RCAs are caused of the disruption of intracellular metabolic pathways including glycolysis and the tricarboxylic acid cycle (Mackworth, 1948; Castro, 1968). Studies on animal models showed that CN was the more toxic compound in comparison to CS, demonstrated by the higher rate of lethal tissue damage in small mammals (Ballantyne and Swanston, 1978). This reaction causes the degradation of enzymes related to sensory nerve activity (Levine and Stahl, 1968). The TRPA1 receptor, another cationic channel permeable to calcium, sodium, and potassium, is also present in polymodal nociceptor neurons and, thus, can be activated by chemical stimuli (Kaneko and Szallasi, 2014; Ruiz-Lozano et al., 2021). TRPA1 receptors contain nucleophilic groups (i.e., cysteine thiols) that form covalent interactions with CN, CS, and CR, potent agonists of these receptors (Bautista et al., 2006; Brône et al., 2008). The transcription of the TRPA1 gene has been found in the trigeminal neurons, dorsal root ganglion neurons, and corneal nerves of mice, rats, and humans (Brône et al., 2008; Canner et al., 2014). Bessac et al. reported absent or minimal response to pain in mice with genetic ablation or pharmacological blockade of TRPA1, confirming the role of TRPA1 in pain detection (Bessac et al., 2009). Corneal expression of TRPA1 is also related to transforming growth factor (TGF)-β1 fibrotic responses, as TRPA−/−mice corneas remained more transparent after alkali-burned injury (Okada et al., 2015). Furthermore, TRPA1 activation also leads to increased corneal levels of substance P, which facilitates a lower neuronal threshold of activation that sensitizes the cornea to further stimuli, including non-noxious ones (Zhang et al., 2007).

5.2.3 Ocular manifestations

In a rabbit model, Ballantyne et al. reported lacrimation, purulent discharge, blepharitis, conjunctival chemosis, increased intraocular pressure (IOP), hyperemia, iritis, keratitis, and corneal neovascularization after conjunctival sac instillation of 0.1 mL of CN dissolved in polyethylene glycol 300 (PEG300) at concentrations ranging from 1% to 10% (Ballantyne et al., 1975). The severity and duration of the ocular manifestations were

concentration-dependent, with 10% CN causing moderate iritis, keratitis, corneal scarring, and neovascularization with minimal resolution (Ballantyne et al., 1975). These results were supported by Gaskins et al., who found that >4% CN dissolved in 1,1,1-trichloroethane caused permanent corneal damage in rabbits (Gaskins et al., 1972).

Oksala et al. described five cases of eye injuries caused by aerosol irritant projections and one by tear-gas pistol (Oksala and Salminen, 1975). In all cases, patients were under the influence of alcohol when the damage occurred. Ocular manifestations were lid and conjunctival erythema, corneal epithelial erosions, stromal edema, Descemet membrane folds, pseudo-pterygium formation, and anterior chamber inflammation. The vision was only partially restored at the last visit since most cases developed corneal opacifications (Oksala and Salminen, 1975). The authors suggest that permanent corneal damage could have resulted from an impaired blinking reflex in drunk patients leading to increased ocular surface exposure and time of contact with the chemical (Oksala and Salminen, 1975). Gerber et al. managed a 2.5-year-old-boy who was accidentally exposed to OC from approximately 30 cm. At presentation, the slit-lamp exam was normal. However, 3 weeks after the incident, the proliferation of conjunctival tissue at the superior and temporal limbus developed and was subsequently removed surgically. Histopathological examination showed mixed acute and chronic inflammation between collagen fibers. The authors hypothesize that the impact on the limbal stem cell niches may have stimulated cellular proliferation and that special vigilance for limbal stem cell deficiency will be required in this case (Gerber et al., 2011).

In animals, milder ocular surface lesions were observed when they were not anesthetized or restrained; thus, their ability to blink was not affected (MacLeod, 1969). Moreover, in all cases, the firing distance was less than 1 m, and immediate management was not given, hindering adequate ocular surface healing (Oksala and Salminen, 1975). Levine and Stahl evaluated 14 human enucleated eyes after tear-gas explosions at close distance (Levine and Stahl, 1968). Five eyes were enucleated 2 months or less after injury due to necrotizing keratitis and suppurative iridocyclitis. The remaining nine eyes, enucleated up to 15 years after insult, exhibited neurotrophic keratopathy (NK), leading to corneal neovascularization, ulceration, and chronic perforation (Levine and Stahl, 1968). Histological analysis revealed epineurium thickening resulting in an impaired sensory activity. The latter is probably due to CN reaction with sulfhydryl protein groups, irreversible enzyme inhibition, and denaturation (Levine and Stahl, 1968). NK results in absent corneal sensation leading to impaired trophic function, corneal epithelial regeneration, and increased risk of infection, ulceration, and perforation (Ruiz-Lozano et al., 2021).

5.3 Chlorobenzylidene malononitrile (CS)

5.3.1 Chemical properties

CS is an electrophilic molecule developed in 1928 by the American scientists Corson and Stoughton, hence the abbreviation using the first letters of their last names (Olajos and Salem, 2001). However, it was not used as an RCA until 1958 by the British army in Cyprus, when it replaced CN as a more potent but less toxic alternative for non-lethal crowd control. This crystalline-white powder with a cyanocarbon structure has a melting point of 93°C and a boiling point of 310°C. It is slowly hydrolyzed into *o*-chlorobenzaldehyde and malononitrile in water (O'Neil et al., 2006). The half-maximal activation concentration of CS for the TRPA1 channel is EC_{50} CS = 7 ± 1 nM (Bessac et al., 2009).

5.3.2 Mechanism of toxicity

Previously, researchers hypothesized that CS reacted with glutathione, mercapto group-containing enzymes, cysteine thiol groups (present in TRPA1 channels), proteins, and nucleic acids (Olajos and Salem, 2001; Committee on Acute Exposure Guideline Levels et al., 2014). However, it is now known that CS is an agonist of the TRPA1 channel, which facilitates the nerve-ending release of Substance P, CGRP, and other substances after activation (Brône et al., 2008). This elicits neurogenic inflammation and hypersensitivity to mechanical and thermal stimuli as part of the physiological function of these fibers to protect the cornea from noxious cold (Bautista et al., 2006).

5.3.3 Ocular manifestations

The most common manifestations of CS exposure include lacrimation, blepharospasm, irritation, and conjunctivitis, all of which have immediate onset (Kiel, 1997; Davey and Moppett, 2004). Conjunctivitis and tearing can occur even with indirect exposure to the gas, especially if this happens in enclosed spaces (Karaman et al., 2009). Interestingly, some police officers have developed clinical features when handling items contaminated with CS after entering rooms previously occupied by detainees exposed to tear gas. Some cases of contact allergic reactions have been reported where the patients develop dramatic eyelid edema (Watson and Rycroft, 2005). Hill presented a case report of a man directly sprayed with CS on his face, chest, and arms. This patient only developed periorbital edema and conjunctival injection, but he did have more severe respiratory symptoms (Hill et al., 2000). Kiel describes six patients who were affected inside a public house, where all of them only had conjunctival injection and decreased tear break-up time (Kiel, 1997). It seems that CS has less severe clinical manifestations compared to the other tear gases.

5.4 Dibenzoxazepine (CR)

5.4.1 Chemical properties

CR is a pale yellow crystalline solid with a melting point of 73°C. It is not hydrolyzed when in aqueous solutions and has a pepper-like odor. This compound has irritant properties in concentrations of 0.0025% or lower. It has fewer respiratory effects than CS but more pronounced dermatologic consequences. Additionally, it remains longer in the air and on clothing than the other tear gases. Finally, it has a higher lethal median dose than CS. For CR, the half-maximal activation dose is EC50 CR = 308 ± 150 nM (Bessac et al., 2009).

5.4.2 Mechanism of toxicity

Like CS and CN, CR is a potent, selective agonist of the TRPA1 cation channels (Brône et al., 2008). The discovery of the mechanism of actions of the other tear gases was dependent on the study of CR and the structurally similar morphanthridine tricyclic moieties (Gijsen et al., 2010).

TABLE 1 Mechanism of action and ocular manifestations of Riot Control Agents.

RCA	Toxic mechanisms	Acute ocular manifestations	Complications	References
Oleoresin capsicum (OC)	TRPV1 agonism	Blepharospasm, ocular pain, conjunctival injection, PEE	Symblepharon, chemosis, pseudopterygium, persistent corneal conjunctivalization	Brown et al. (2000), Zollman et al. (2000), Epstein and Majmudar, (2001), Holopainen et al. (2003), Das et al. (2005), Kniestedt et al. (2005), Voegeli and Baenninger, (2014), Rasier et al. (2015)
		Corneal hypoesthesia and decreased tear production	DED, Neurotrophic Keratitis, irregular astigmatism, and corneal opacification	
Chloroacetophenone (CN)	TRPA1 agonism	Periocular erythema, PEE, corneal stromal edema, Descemet's folds, and anterior chamber inflammation	Pseudopterygium formation, conjunctival proliferation	Uhde, (1948), Levine and Stahl, (1968), Rose, (1969), Oksala and Salminen, (1975), Gerber et al. (2011), Dimitroglou et al. (2015)
			Iridocyclitis	
			Secondary glaucoma	
		Neurotrophic keratitis with corneal neovascularization, ulceration, and perforation	Blindness	
Chlorobenzylidene malononitrile (CS)	TRPA1 agonism	Lacrimation, blepharospasm, conjunctivitis, allergic reactions, eyelid edema	Not associated with chronic ocular manifestations or complications	Gaskins et al. (1972), Kiel, (1997), Hill et al. (2000), Davey and Moppett, (2004), Watson and Rycroft, (2005)
Dibenzoxazepine (CR)	TRPA1 agonism	Most potent lacrimator of the RCAs, blepharospasm with lower concentrations	Corneal edema, necrotizing keratitis, iridocyclitis	Levine and Stahl, (1968), Leopold and Lieberman, (1971), Ballantyne and Swanston, (1974), Blain, (2003)
		Corneal edema, necrotizing keratitis, iridocyclitis	Anterior chamber angle deformation	

DED, Dry eye disease; PEE, Punctate epithelial erosions; RCA, Riot control agent; TRPA1, Transient receptor potential ankyrin 1; TRPV1, Transient receptor potential vanilloid 1.

5.4.3 Ocular manifestations

The experiment conducted by Ballantyne et al. (1975) in rabbits determined that a solution >5% CR induced transient keratitis in the animals Ballantyne et al., 1975. On the other hand, Rengstorff et al. used 5% CR in propylene glycol 5 days per week for 4 weeks and found only moderate transient conjunctivitis, but no anatomical alterations in the *post mortem* examination of corneal and palpebral structures (Rengstorff et al., 1975).

In humans, CR causes intense blepharospasm, conjunctival irritation and lacrimation when it meets the ocular surface. It is the most potent lacrimator of the RCAs described in this review and has the least systemic toxicity. To this point, Ballantyne and Swanston determined that the concentration required to elicit blepharospasm in humans is lower for CR than it is for CS (Ballantyne and Swanston, 1974). The blepharospasm impedes eye opening, but visual acuity (VA) frequently remains unaffected if patients manage to open their eyes. In cases were sprayed from close range and with highly concentrated preparations, corneal edema, necrotizing keratitis, iridocyclitis, and anterior chamber angle deformities can occur (Leopold and Lieberman, 1971; Blain, 2003).

6 Complications

RCAs are associated with ocular surface complications including slow-healing corneal defects, opacification, neovascularization, hypoesthesia, decreased VA, and dry eye disease (DED) (Hoffmann, 1967; Oksala and Salminen, 1975; Epstein and Majmudar, 2001; Holopainen et al., 2003). VA alterations range from transient blurred vision to permanent irregular astigmatism depending on chemical concentration and distance of impact, with some patients recovering almost fully while others do not (Hoffmann, 1967; Kim et al., 2016). In the case of CN, studies in rabbits and monkeys show that directly inoculated animals develop corneal scarring and neovascularization that can persist for months (MacLeod, 1969). In a human study by Rose et al., nine out of 12 cases exposed to CN had epithelial defects that resolved within 3 days, yet three out of 12 patients had confluent corneal punctate staining that remained for 3 weeks, one of which had stromal opacification that persisted for as long as 5 months (Rose, 1969). Uhde describes military cases from World War I. In this report, a patient developed permanent blindness secondary to close-range explosion of a CN grenade detonation, while another who was shot by a tear gas pistol developed corneal edema, hypopyon, and was also left blinded (Uhde, 1948). Some of the more chronic findings in Oksala's evaluated patients included persistent corneal opacifications, Descemet's folds, and even a pseudopterygium that reduced VA in one of the patients (Oksala and Salminen, 1975).

Other severe complications can be found in the literature (Midtbo, 1964; Blain, 2003). The report by Levine discusses findings from cases of the Armed Forces Institute of Pathology related to 14 eyes that were enucleated following injury from tear gas weapons (Levine and Stahl, 1968). Half of the cases involved soldiers who accidentally self-inflicted their wounds while examining gas canisters and other devices, while the other half was wounded by a second person (law enforcement officer) who fired with the intent to disable. Five eyes were enucleated within 2 months of the injury, while nine eyes were enucleated between 8 months and 15 years after the inciting event. Medical records indicated that the patients' corneas were opaque, vascularized, or ulcerated. Notably, anterior chambers of four eyes contained debris, pus, and fibrin was also found, as well as hypopyon. Secondary glaucoma was present in three eyes. Microscopic examination of all eyes revealed intense necrotizing keratitis with deep coagulative necrosis. Iridocyclitis was commonly found along with inflammatory debris, shallowing of the anterior chamber, and retrocorneal membranes (Levine and Stahl, 1968).

It is important to consider that CN is more toxic than CS, as shown in the testing done by Gaskin et al. In this experiment, CN and CS were administered at comparable concentrations (1%–4% and 10%) to unanesthetized rabbit corneas and skin. The rabbits who received CN developed corneal opacities in addition to iritis and conjunctivitis, while those who were received CS developed no enduring corneal injuries (Gaskins et al., 1972). A systematic review identified symptoms like lacrimation, blepharospasm, conjunctivitis, and decreased vision. However, all of these toxic effects were transient and no chronic manifestations were reported (Dimitroglou et al., 2015). Although CS does not produce severe ocular manifestations like the other RCAs commented in this review, it is in fact associated with serious respiratory complications that may necessitate intensive care (Hill et al., 2000).

OC is also associated with the previously mentioned complications as well as conjunctival chemosis, pseudopterygium, and neurotrophic keratitis (Brown et al., 2000; Kniestedt et al., 2005; Voegeli and Baenninger, 2014). One specific case of OC with tardive irrigation led to permanent VA deterioration related to irregular astigmatism and corneal opacification (Epstein and Majmudar, 2001). A report of close-range exposure describes a severe ocular chemical burn that resulted in a pseudopterygium with persistent corneal peripheral conjunctivalization 6 months post-exposure. This patient presented with a corneal erosion and microhyphema which were treated topical corticosteroids, antibiotics, and autologous serum tears. However, the erosion persisted in subsequent consultations, and after 4 weeks, slit lamp examination revealed the pseudopterygium with corneal neovascularization suggestive of limbal necrosis. Conjunctivalization was still present at 6-months post initial evaluation (Voegeli and Baenninger, 2014). DED is also a significant long-term complication, as demonstrated in a study evaluating the decrease in aqueous tear production following pepper spray exposure. In this report, 96 patients who were exposed to OC during the Gezi Parks protests in Turkey evaluated for DED using Schirmer's test and the Dry Eye Questionnaire (DEQ). All patients were treated by irrigation with alkaline substances (milk and antacid solutions). Additionally, 82 individuals reported using protective goggles during the episode. The authors determined statistically significant differences between Schirmer's I and II between those who used goggles and those who did not (3.21 ± 1.55 to 8.24 ± 1.24 mm $p < 0.001$; and 5.15 + 1.5 to 13.2 ± 1.66 mm $p < 0.001$, respectively). Additionally, 24.4% and 35.7% of those who did and did not wear goggles reported symptoms in the DEQ (Rasier et al., 2015).

Other studies mention non-ocular surface manifestations like with cataracts, glaucoma, vitreous hemorrhage, and optic nerve damage (Hoffmann, 1967). However, traumatic injury is most likely the culprit of these complications, because patients from these reports were exposed in the context of explosive devices that cause blasts, shock-wave damage or direct impact from tear gas cannisters. A summary of the mechanism of action, ocular manifestations, and complications caused by RCA exposure is presented in Table 1 of this review.

7 Management of exposure to RCAs

7.1 Decontamination

The management of patients exposed to tear gas should begin immediately with field decontamination. First and foremost, it is vital that physicians avoid their own contamination and that of their equipment. This can be done by wearing protective eyewear, surgical masks, and gowns. Patients should be lifted off the ground and be treated in well ventilated spaces, as tear gas particles can accumulate easily (Carron and Yersin, 2009; Schep et al., 2015). Contact lenses should be removed if appropriate. The most important step is irrigation with water or normal saline for 15–20 min to remove tear gas particles from the ocular surface (Breakell and Bodiwala, 1998; Blain, 2003; Carron and Yersin, 2009). In those with pronounced blepharospasm, topical anesthetics can facilitate eye-opening for irrigation of the superior and inferior *cul-de-sacs*, where the chemicals may accumulate. Historically, some authors recommended blowing air into the patient's eyes, but it has been determined that this technique may worsen the symptomatology by dispersing tear gas to unaffected areas (Breakell and Bodiwala, 1998; Gray, 2000).

7.2 Ophthalmological evaluation

Patients with moderate-severe ocular symptoms warrant referral to an ophthalmologist for comprehensive evaluation (Kearney et al., 2014). The initial assessment by the ophthalmologist must include an account of the exposure, to determine its duration, whether it was direct or indirect, and distance from which the substance was fired. VA should be obtained using a Snellen chart at 20 feet inside a dim room to establish a baseline measurement. Patients with reduced VA after exposure warrant a thorough ophthalmic evaluation.

The slit lamp exam to search for conjunctival hyperemia, chemosis, and skin inflammation. Lissamine green staining should preferentially be used to determine the extent of conjunctival epithelium damage. A corneal exam should include an evaluation of its epithelial integrity, with fluorescein staining helping to detect epithelial erosions (Brown et al., 2000). Fluorescein can also aid to find corneal ulcers, especially in patients presenting with severe pain and hyperemia (Shimada et al., 2012). The anterior chamber should be carefully evaluated for signs of an inflammatory response, such as cells and flare. Visibly embedded RCA particles can be removed under the biomicroscope with a cotton swab or a needle (Blain, 2003).

It is important to evaluate corneal sensation in follow-up visits, after the acute symptoms have subsided. Patients with hypoesthesia are predisposed to develop corneal ulcers, especially if they engage in eye rubbing (Holopainen et al., 2003; Shimada et al., 2012). The Cochet Bonnet Esthesiometer is an instrument used to measure the corneal sensitivity threshold and can help clinicians detect nerve damage. It consists of a nylon monofilament with variable length that is used to touch the central and peripheral corneal, where the patient's blinking is considered a positive response. The test should begin using the full length of the filament (60 mm) and continue with 5-mm decreases until a positive response is obtained. Additionally, another objective measurement to evaluate corneal nerve integrity is *in vivo* confocal microscopy, which allows for the direct visualization of the fibers (Holopainen et al., 2003). The combination of both techniques provides a thorough examination of corneal sensory function.

7.3 TRP inhibitors

The TRPA1 channel is the primary driver of the tissue response after CS, CN, and CR exposure and its concomitant release of inflammation-

inducing neuropeptides. Therefore, some studies investigating potential drugs to block the TRPA1 channels show promise as a treatment for the hazardous health effects of RCAs. Although none of these compounds are approved for the treatment of RCA exposure in humans, previous *in vitro* and animal studies have had success blocking their effects.

For example, the study by Bessac et al. proved that TRPA1-mediated Ca^{2+} influx mediates the toxic effects of CS, CN, and CR; and that genetic ablation or pharmacological inhibition of the TRPA1 channel deterred CS or CN-induced nocifensive behavior. The *in vitro* results from this experiment determined the half-maximal activation concentrations for CS, CN, and CR, which were mentioned previously in each agent's subsection. For the *in vivo* section, one group of genetically ablated TRPA1−/−mice and another group of wild-type animals receiving the first-generation TRPA1 antagonist *HC-030031* were exposed to CS and CN (100 mM dosage) *via* ocular or dermal routes. The genetically ablated mice failed to perceive the tear gases as noxious agents, demonstrated by a total abolishment of response after their administration. On the other hand, the wild-type, pharmacologically treated mice had reduced nocifensive responses after applying the TRPA1 antagonist (Bessac et al., 2009). Based on these findings, the authors suggested that *HC-030031* reduces the acute sensory irritation induced tear-gas mediated TRPA1 pathway activation.

In humans, biopharmaceutical companies have performed early-phase clinical trials investigating the effectiveness of TRPA1 antagonism in other clinical conditions, such as neuropathic pain and allergic asthma. A phase two randomized, controlled, double-blinded clinical trial evaluating the TRPA1 blocker ISC-17536 (Glenmark Pharmaceuticals) as monotherapy for painful diabetic neuropathy was published recently (Agarwal et al., 2014). *ISC-17536* did not show significant efficacy in treating diabetic peripheral neuropathy. Still, the authors hypothesize that since the pharmacological site of action is on small peripheral nerve fibers, patients who have lost these neurons are unlikely to respond to TRPA1 inhibition. However, an effect could be seen in those who have preserved small nerve fibers (Jain et al., 2022). Clinical trials studying other molecules, such as *HX-100*, *GDC-0334,* and *ODM-108,* for allergic asthma and neuropathic pain have halted due to unfavorable pharmacokinetics (Chen and Terrett, 2020; Souza Monteiro De Araujo et al., 2020).

TRPV 1 blockade may also effectively eliminate ocular symptoms of pain. Although TRPV 1 blockade in RCA exposure has not been studied, it has reduced ocular pain and inflammation in other clinical contexts. For example, in a murine model developed by Fakih et al., DED mice who received topical TRPV1 antagonist capsazepine twice daily for 2 weeks showed inhibition of commonly upregulated genes involved in inflammatory and neuropathic pain. This experiment also demonstrated a reduced sensation of ocular pain (Fakih et al., 2021). In another model for allergic keratoconjunctivitis, pretreatment of mice with TRPV1 antagonists reduced the inflammatory reaction and prevented sensitization of nociceptors, resulting in decreased ocular pain (Callejo et al., 2015).

7.4 Chelating agents

Diphoterine® solution (Prevor Laboratory, Valmondois, France) is a hypertonic, amphoteric, and chelating substance recommended for dermal or ocular exposure to various chemicals (Gerard et al., 2002; Rihawi et al., 2006; Dohlman et al., 2011). This compound has six binding sites that allow clearance of different substances, including acids, bases, and alkylating agents, among others (Hall et al., 2002). Gerard et al. successfully managed a severe ocular chemical burn patient after rinsing the eye with 1 L of Diphoterine and prevented the development of sequelae (Gerard et al., 2002). Importantly, Diphoterine rinsing reduced the patient's stromal edema, a risk factor that has been correlated with the severity of subsequent leucomas (Kubota and Fagerholm, 1991). Viala et al. conducted an experiment in which five French Gendarmes voluntarily entered a chamber with CS concentrations of 3,000 mg/m^3. Four of them quickly developed incapacitating symptoms, which resolved in 4 min after exiting the chamber and being decontaminated with 250 mL of Diphoterine. One of the Gendarmes applied the solution before entering the chamber, and his only symptom was mild cough that also resolved after a few minutes (Viala et al., 2005). The results that Brvar obtained years later further support the use of Diphoterine, in an experiment where Slovenian police officers in training ran for 20 s through a cloud generated from CS grenades in an open field. Officers who sprayed Diphoterine on themselves before CS exposure had lower levels of facial pain and were more rapidly able to return to duty. Those treated with the solution after the exercise also recovered from their symptoms, albeit not as quickly; finally, those who did not receive treatment reported the highest levels of pain and were incapacitated the longest (Brvar, 2016).

8 Conclusion

RCAs can be more harmful than initially thought. Although, in many cases, the classic manifestations of conjunctivitis, lacrimation, and blepharospasm are transient, this review reveals that there can be much more severe sequelae that necessitate ophthalmological referral and follow-up. Much of the available literature comes from case reports and case series. These documents have a low level of epidemiological significance. Still, the complications described in them should alert the medical community about the need for more in-depth knowledge of the effects of tear gases. Additionally, the basis of care currently rests upon decontamination through irrigation. Some efforts to develop specific antidotes have been taken, but none have yet been successfully released into the public. To completely understand the long-term sequelae, more thorough research efforts are needed and to develop target-specific treatments. Formal therapeutic guidelines should be implemented to standardize the treatment of exposed patients and protect the medical team from contamination.

Author contributions

Design of the work: MQ-G and RR-L. Conceptualization: MQ-G, RR-L, and VP. Literature investigation and selection: MQ-G, RR-L, and JS-L. Main writing: MQ-G and NA. Manuscript reviewing: MQ-G, RR-L, and HM. Figure editing: MQ-G, NA, and SK. Critical reviewing: VP. Project Coordination: VP. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Vision health perspectives on *Breaking Bad*: Ophthalmic sequelae of methamphetamine use disorder

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Methamphetamine use has become a rampant public health issue that not only causes devastating consequences to the user but also poses a burden to surrounding communities. A spectrum of ophthalmic sequelae is associated with methamphetamine use and includes episcleritis, scleritis, corneal ulceration, panophthalmitis, endophthalmitis, retinal vasculitis, and retinopathy. In many instances, prompt recognition of the condition and associated infectious process and early initiation of antimicrobial therapy are crucial steps to preventing vision loss. In this review, we summarize the reported ocular complications that may result from methamphetamine use in addition to several postulated mechanisms regarding the ocular toxicity of methamphetamine. The increasing prevalence of methamphetamine use as a public health threat highlights the need for continued investigation of this ophthalmologic issue.

KEYWORDS

methamphetamine, vision loss, ocular injury, keratitis, neurotoxicity, retinopathy

1 Introduction

Methamphetamine, a stimulant with the chemical formula $C_{10}H_{15}N$, was initially produced as an amphetamine derivative in the 1890s and was widely used in the 1940–1950s until people became aware of its adverse effects. The U.S. government passed legislation in 1970 labeling amphetamine-type stimulants as controlled substances, limiting medical methamphetamine use, and ensuring close monitoring of the manufacture, prescription, and sale of amphetamine-type stimulants (Vearrier et al., 2012). While this was initially effective in decreasing its use, illegal manufacturing soon emerged in response to the restriction in legal distribution. Since then, steady growth in illicit methamphetamine production and consumption has given rise to a drug use epidemic, a topic which has been depicted in the popular drama television series Breaking Bad (Breaking Bad, 2008). Based on data from the National Survey on Drug Use and Health, the scope of the affected population continues to expand, with approximately 2.5 million people reporting methamphetamine use in 2020 (Administration, 2021). From 2015–2019, there have been upward trends in overdose mortality, risk patterns of methamphetamine use, and increased diversity in populations at risk for methamphetamine use disorder (Han et al., 2021).

Following COVID-19 shelter-in-place orders, poison control centers reported an increased call rate for exposure to controlled substances, including opioids and methamphetamine (Maeng et al., 2022). These alarming statistics highlight a growing public health concern and warrant attention from healthcare providers of all disciplines.

Methamphetamine misuse causes various short-term and long-term damages to one's health. Immediate effects include heightened alertness, euphoria, quickened heartbeat, and increased respiration (NIDA, 2021b). Long-term abuse is deleterious by contributing to development of tolerance with chronic drug use, fueling craving during withdrawal periods. Users may generally require more frequent and higher doses to achieve the original desired effect (Courtney and Ray, 2014). Consequences of long-term methamphetamine use include psychosis, changes in brain structure, deficits in cognitive functioning, memory loss, dental problems ("Meth mouth"), and malnutrition (NIDA, 2022). Increased transmission of hepatitis, HIV, and other infectious diseases is also seen among methamphetamine users (NIDA). Additionally, harmful effects of methamphetamine extend beyond the health of the individual and create a devastating impact on families and communities in the form of increased violence, crime, and corruption (Watt et al., 2014).

This mini-review discusses broader implications of the methamphetamine use epidemic, including the medical, psychosocial, financial, and environmental impacts. We also provide a comprehensive summary of the ophthalmic complications associated with methamphetamine use. The pathophysiology of methamphetamine-related ocular complications has not yet been fully elucidated and is a topic of ongoing investigation.

2 Harms of methamphetamine use

2.1 Pathophysiology of complications from methamphetamine use disorder

Like other drugs, the mode of methamphetamine consumption can be variable. Smoking is the most common route, followed by injection and inhalation or snorting (Pro et al., 2022). Regardless of how it is consumed, many harmful effects of methamphetamine can be attributed to its sympathomimetic effects, which leads to diffuse vasoconstriction (Hazin et al., 2009). This can present as toxicity of multiple organ systems, some of which are described below.

2.2 Medical harm

Methamphetamine elicits a harmful vasoconstrictive effect that can directly cause cardiovascular, cerebrovascular, hepatic, renal, neurologic, and ocular complications. Cardiovascular injuries include arrhythmias or ischemia with or without infarction (Ahmad et al., 2017). Narrowing or frank occlusion of cerebral vessels leads to cerebral ischemia with stroke or hemorrhagic stroke arising from intracerebral hemorrhage (Schep et al., 2010) and may also cause transient cortical blindness (Gospe, 1995; Edinoff et al., 2022). Intoxicated patients who are agitated and hyperthermic are at risk for rhabdomyolysis, which could result in liver damage and renal failure with resultant electrolyte abnormalities (Richards et al., 1999). Individuals may exhibit psychological and neurologic manifestations such as anxiety, depression, psychosis, and deficits in memory and executive functioning (Rusyniak, 2013).

Indirectly, methamphetamine use may contribute to behavioral and immunologic factors that harm the health of an individual. Methamphetamine users engage more frequently in high-risk sex behaviors, increasing avenues for transmission of infectious diseases such as hepatitis and HIV (Hittner, 2016). Behaviors such as unprotected sex, anal sex, use of commercial sex venues, and having multiple concurrent partners are more prevalent in methamphetamine users of the men-who-have-sex-with-men (MSM) population and are associated with a high incidence of sexually transmitted infections (STIs) such as *Chlamydia*, gonorrhea, and syphilis (Taylor et al., 2007; Reback and Fletcher, 2018). Needle or syringe-sharing behavior among users who inject contributes to a higher prevalence of infections (Wada et al., 1999). Methamphetamine may play a role in the immunopathogenesis of HIV and HCV infectivity as well. *In vitro* studies with human cells and animal models have shown that methamphetamine use is associated with higher viral loads, immune dysfunction, antiretroviral resistance, and accelerated progression to AIDS (Marcondes et al., 2010; Harms et al., 2012; Passaro et al., 2015; Skowronska et al., 2018; Liu et al., 2021). Methamphetamine may also damage the integrity of the blood brain barrier, thereby increasing likelihood of CNS involvement during HIV infection (Silverstein et al., 2011). It was also found to enhance replication of HCV in human hepatocytes *in vitro* (Ye et al., 2008).

2.3 Social and environmental harm

The methamphetamine epidemic has had significant socioeconomic repercussions, costing the nation an estimated $23.4 billion in 2005 (The RAND Corporation, 2005). This amount was comprised of costs associated with morbidity and mortality, criminal justice and social welfare services, environmental clean-up from methamphetamine production, and lost productivity and quality of life burden due to drug dependence (Nicosia et al., 2009). Methamphetamine users are more likely to have unstable housing, low income, and residence in rural areas (Shearer et al., 2020) with populations of lower socioeconomic status being disproportionately affected. Multiple barriers may prevent these individuals from receiving medical care, as evidenced by higher rates of missed appointments, decreased compliance, and factors that interfere with effective substance use disorder treatment (Marquez et al., 2009; Lai et al., 2020). Additionally, high frequency of co-occurring addiction and mental health problems may lead to a higher risk of treatment non-adherence and missed appointments (Morasco et al., 2014). Children of methamphetamine users are exposed to a myriad of risk factors, including maltreatment, exposure to violence, and criminal behaviors. This environment negatively impacts a child's psychological development and perpetuates a cycle of neglect and abuse (Gonzales et al., 2010; Messina et al., 2014).

TABLE 1 Spectrum of ophthalmic complications associated with methamphetamine use.

Anatomical structure	Ophthalmic complication
Conjunctiva	Conjunctivitis
Sclera	Episcleritis Scleritis
Cornea	Keratitis Keratolysis or corneal melting
Iris, lens, anterior chamber	Mydriasis Decreased accommodation or convergence Higher risk of angle closure glaucoma
Retina	Amaurosis fugax Retinal vasculitis Retinal vascular occlusions Intraretinal hemorrhages Crystalline retinopathy Endophthalmitis
Optic nerve	Non-arteritic anterior ischemic optic neuropathy (NAION)
Orbit	Panophthalmitis Orbital cellulitis

3 Relevance to ophthalmology

3.1 Overview of ocular signs, symptoms, and complications

Immediately following methamphetamine use, the user may experience blurred vision, mydriasis, disturbances in perception, or visual hallucinations. (Grant and Thomas, 1987; Srisurapanont et al., 2003). Following the acute phase of intoxication, additional ocular complaints may arise if corneal damage occurs. Patients with keratitis may experience days to weeks of decreased vision, eye pain, photophobia, and redness (Franco et al., 2022). They may also describe irritation or a foreign body sensation. Due to hyperstimulation from the drug, the user may fixate on the ocular discomfort and repeatedly rub or pick at the eye, increasing risk for corneal epithelial defects (Poulsen et al., 1996). On exam, focal opacification of the cornea often indicates a corneal ulcer, and severe cases of infection may present with a hypopyon or accumulation of white blood cells that settles in the anterior chamber.

Less frequently reported ocular symptoms arising from the vasoconstrictive effects of methamphetamine include amaurosis fugax, which manifests as short episodes of transient vision loss spontaneously resolving without treatment (Shaw et al., 1985). Similarly, sudden onset of painless vision loss hours after intranasal methamphetamine use may suggest a central retinal artery occlusion (CRAO) or non-arteritic ischemic optic neuropathy (NAION) (Wallace et al., 1992; Wijaya et al., 1999). The presence of blurred vision or visual field defect such as a scotoma may suggest intraretinal hemorrhages (Wallace et al., 1992). Less frequently described ocular findings include painful lid edema, proptosis, chemosis, and corneal melt, which may indicate panophthalmitis (Reed et al., 2021).

3.2 Ocular complications associated with methamphetamine use

A spectrum of ophthalmic manifestations associated with methamphetamine use is reported in the literature (Table 1). These infectious or inflammatory processes affect various parts of the ocular anatomy, such as the conjunctiva, sclera, cornea, retina, optic nerve, or orbit.

3.2.1 Conjunctiva and sclera

The vasoconstrictive effect of methamphetamine interferes with ocular perfusion, causing vasculitis, which may manifest as conjunctivitis, episcleritis, or scleritis (Isaak and Liesegang, 1983; Hazin et al., 2009).

3.2.2 Cornea

Postulated mechanisms regarding development of corneal injury among methamphetamine users can largely be grouped into two categories: 1) direct effects and 2) route-related effects. Factors related to methamphetamine production, such as addition of diluting agents or manufacture-related effects, may also contribute to harm caused to the cornea. While each explanation seems plausible, further study is required to determine the exact pathogenesis.

Methamphetamine is a sympathomimetic agent that elevates the pain threshold and disrupts the normal blink mechanism, increasing risk for corneal epithelial insults. With repeated use, damage to dopamine and serotonin receptors could lead to neurotrophic keratitis and microulceration, subsequently presenting as a corneal ulcer (Kroll et al., 2004; Huang et al., 2022) (Figure 1). Methamphetamine is usually sold in the form of hydrochloride salt, which may cause a chemical burn progressing to epithelial defect, a nidus for infection. Toxic damage to the corneal surface may

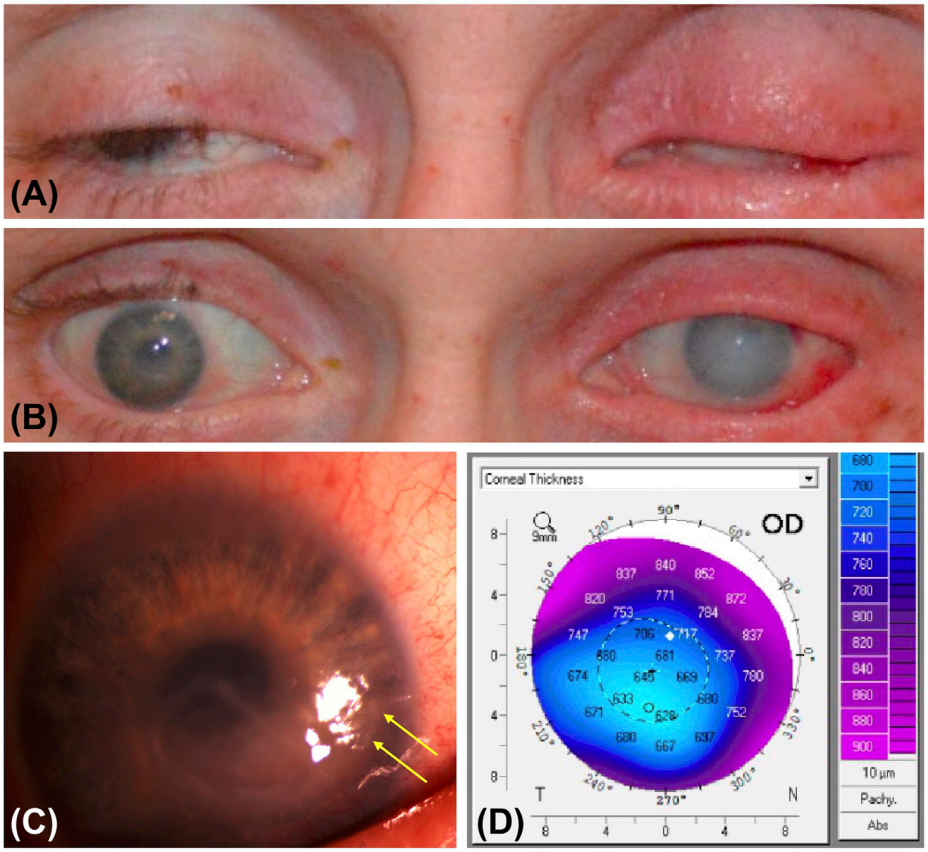

FIGURE 1
Photographic features and imaging findings of a patient with methamphetamine associated keratitis. **(A)** External photograph of eyes closed show bilateral lagophthalmos, erythema, and lid thickening. There is hypotrichosis of the right eyelid and madarosis of the left eyelid. **(B)** External photograph of eyes open show conjunctival hemorrhage and diffuse corneal edema greater in the left than in the right eye, perilimbal injection in the right eye, and subconjunctival hemorrhage in the left eye. **(C)** Slit lamp photograph of the right eye shows residual corneal ulceration with thickened margins and area of desiccation (yellow arrows). **(D)** Pentacam image of the right eye shows central thinning in the region of ulceration compared to peripheral cornea.

predispose patients to exposure keratopathy and secondary infection (Franco et al., 2022). A case report describes repeated corneal ulcers in a patient recurring concomitantly with periods of heavy methamphetamine smoking (Chuck et al., 1996). Aerosolized and inhaled stimulant use is also associated with keratolysis, a progressive dissolution of the corneal stroma, although the mechanism by which this occurs is unknown (Heer et al., 2020). Interestingly, methamphetamine concentrates in saliva at a tenfold greater concentration than in plasma (Cook et al., 1992). Structural and functional similarities in the lacrimal and salivary glands suggest the possibility for a similar elevation of methamphetamine concentration in the tear film, which could indicate direct corneal toxicity. Risk for drug-to-hand-to-eye exposure is present regardless of the route of methamphetamine consumption. In particular, the fumes from smoking methamphetamine may directly irritate ocular tissues, causing increased eye rubbing and risk of damage to the corneal epithelium.

Addition of diluting agents to methamphetamine samples is meant to increase profits of sale and compounds the likelihood of corneal damage. Anesthetics (lidocaine, procaine) may predispose to ulcer formation, and bases (e.g., bicarbonate, strychnine) can result in alkaline chemical burns. Other sympathomimetics (e.g., caffeine, ephedrine) may augment the vasoconstrictive effect (Poulsen et al., 1996). Hazardous methamphetamine production technique may expose manufacturers and users to harmful contaminants such as metals and solvents, many of which are known corneal toxins, such as mercury (Grant and Thomas, 1987). Manufacturers may also have exposure to corrosives such as sodium hydroxide and sulfuric acid (Burton, 1991; Irvine and Chin, 1991).

3.2.3 Iris, lens, and anterior chamber

Methamphetamine, like other stimulants, commonly causes mydriasis with decreased pupillary reaction (Grant and Thomas, 1987). Decreased accommodation and convergence may also occur, perceived as blurred near vision. Chronic users showed more shallow anterior chamber depth and reduced volume with a higher crystalline lens rise. The combination of these factors with the mydriatic effect of methamphetamine may precipitate risk of angle closure glaucoma (Mahjoob and Heydarian, 2022a). Also, a case of bilateral congenital triangular cataracts in a newborn may have been associated with prenatal methamphetamine exposure from maternal use (Clarke et al., 2009).

3.2.4 Retina and optic nerve

Retinal complications associated with methamphetamine use have also been observed, albeit less frequently than corneal findings. These may include vascular, neural, and infectious implications.

Amaurosis fugax manifesting as episodes of transient vision loss may occur from vasospasm as a direct result of drug use (Shaw et al., 1985). Retinal vasculitis has been observed in association with retinal arteriolar attenuation, optic disc edema, cotton wool spots, and vascular leakage on fluorescein angiogram (Shaw et al., 1985). Retinal emboli are hypothesized to occur from direct intranasal injection *via* retrograde flow of emboli through anastomoses of anterior and posterior ethmoidal arteries and the ophthalmic artery (Byers, 1979; Mabry, 1981). Interestingly, crystalline retinopathy has been reported once as an ocular complication of intranasal methamphetamine use. Postulated causes include drug absorption through vasculature of nasal mucosa or absorption of small particles into pulmonary capillaries (Kumar et al., 2006). Intraretinal hemorrhages and bilateral simultaneous retinal artery and vein occlusions have been reported following intranasal methamphetamine use as well (Wallace et al., 1992; Hazin et al., 2009; Guo et al., 2019). Two potential mechanisms leading to these manifestations may include vasospasm or sudden severe transient hypertension, which can lead to rupture of smaller retinal vessels (Wallace et al., 1992). A case of non-arteritic anterior ischemic optic neuropathy (NAION) was attributed to acute ischemia of short posterior ciliary arteries occurring through vasoconstriction and platelet aggregation contributing to vascular occlusion (Wijaya et al., 1999).

Evidence also suggests methamphetamine has a neurotoxic effect on the retina and may affect retinal morphology. Animal models have demonstrated loss of retinal neurons associated with methamphetamine administration (Yang et al., 2018; Lee et al., 2020). In humans, a statistically significant association was observed between chronic methamphetamine use and retinal nerve fiber layer (RNFL) thickness (Mahjoob et al., 2022) and Bruch's membrane opening minimum rim width (MRW) (Talebnejad et al., 2020). This phenomenon may result from inflammation and oxidative stress, ultimately leading to visual function disturbances (Yang et al., 2018). Microvascular damage arising from chronic methamphetamine use may also lead to progressive neuronal loss (Guo et al., 2019). Measurement of visual evoked potentials (VEP) is a sensitive tool to assess the functional integrity of the visual pathway, specifically optic nerve activity. Delay in VEP of methamphetamine users has confirmed the detrimental effect of methamphetamine on afferent pathways (Mahjoob and Heydarian, 2022b).

3.2.5 Orbit

Injection drug use increases risk for endophthalmitis, predominantly caused by mycotic and bacterial microorganisms (Poulsen et al., 1996; Kim et al., 2002; Keyashian and Malani, 2007). One case of endophthalmitis secondary to presumed intravenous methamphetamine use required enucleation. Another case of rapidly progressive *Bacillus cereus* panophthalmitis and concomitant orbital cellulitis in an intravenous user required enucleation (Reed et al., 2021).

3.3 Ophthalmic implications of methamphetamine production laboratories

In addition to consumption-related smoking or thermal injury, production-related causes of ocular involvement include direct injury, exposure to caustic chemicals during production, or exposure to toxic impurities used to dilute the methamphetamine (Heer et al., 2020). This is especially relevant amidst the rise of illicit methamphetamine production, which uses low-cost ingredients, some of which are dangerous and caustic (Movahedan et al., 2015). A "shake and bake" methamphetamine lab explosion resulted in combined thermal and alkali ocular injury (Chan et al., 2011). While "shake and bake," also known as the one-pot method for cooking meth, simplifies the cooking process, this particularly dangerous method confers a high risk of fire and explosions with resultant chemical burns, and poisoning (Village, 2022).

Three individuals suffered ocular injuries after using a technique involving combination of ephedrine or pseudoephedrine, sodium or lithium, and anhydrous ammonia (Lee et al., 2003). Ocular complications resulting from an explosion accident include ocular surface failure, symblepharon, ankyloblepharon, and foreshortening of fornices (Movahedan et al., 2015). Nearby individuals such as children and first responders have also been reported to suffer from injury (Watanabe-Galloway et al., 2009; Melnikova et al., 2011). Patients who suffer from methamphetamine production-related burns typically have a larger burn size, higher incidence of inhalation injury, and increased morbidity from injuries (Spann et al., 2006). Patients may be dishonest about the cause of injury, which further confounds the diagnosis. Therefore, it is reasonable to consider the likelihood of a methamphetamine production-related accident when constructing a differential for the presentation of chemical and thermal ocular injuries (Charukamnoetkanok and Wagoner, 2004).

4 Future considerations for healthcare providers

The process of diagnosing and effectively caring for methamphetamine-using patients is oftentimes complex. Providers may consider methamphetamine-associated ophthalmic injury as a possibility in patient with uncertain cause, multiple risk factors, and suspicion of patient reluctance to disclose drug use. From an ophthalmologic standpoint, continued methamphetamine use increases predisposition to chronic, recurrent, bilateral corneal ulcers (Chuck et al., 1996). Prompt recognition and initiation of treatment for corneal ulcers and methamphetamine-induced keratitis is crucial to preventing infection progression, which may be unresponsive to aggressive antimicrobial therapy and require more intensive intervention. Continued close monitoring for development or spread of infection is also necessary (Chuck et al., 1996; Poulsen et al., 1996; Hazin et al., 2009).

Providers face a multitude of barriers when treating these patients, including social stigma, limited clinical knowledge, comorbid medical and behavioral health conditions, and a paucity of available treatments (Dunn et al., 2022). Currently, there is no medication approved by the Food and Drug

Administration to treat methamphetamine use disorder, although results of a clinical trial utilizing a combination of naltrexone and bupropion are promising (NIDA, 2021a). Behavioral interventions have been efficacious in treating patients with drug use disorders. Implementation of contingency management, which provides incentives to patients dependent on biological confirmation of substance abstinence, has shown improvement in outcomes (AshaRani et al., 2020; Brown and DeFulio, 2020). Other interventions such as cognitive behavioral therapy have also been effective (AshaRani et al., 2020). The Motivational Incentives for Enhancing Drug Abuse Recovery studies performed by the National Institute on Drug Abuse (NIDA) yielded favorable results using incentive-based methods to promote methamphetamine abstinence (Stitzer et al., 2010).

Furthermore, another consideration in caring for these patients is the importance of continuing care, a long-term management approach to maintain abstinence, address relapse, and connect patients to sources of support (McKay, 2021). Continuing care methods used in treatment of substance use disorders include telephone-based continuing care, mindfulness-based relapse prevention, recovery management checkups, and physical health programs. These modalities use strategies such as active patient outreach, incentives, measurement-based care, and adaptive treatment (McKay, 2021). While outcomes are generally beneficial, further analyses should be performed regarding the most effective components of care in improving outcomes of substance addiction.

5 Conclusion

The worsening methamphetamine epidemic has brought about reports of the numerous health and societal complications associated with its use. Although ocular complications from methamphetamine use disorder are not as closely studied as other systemic effects, they can rapidly progress to devastating vision loss if not adequately recognized and treated. A range of ocular structures have been implicated, and the reported spectrum of findings includes corneal ulcerations, retinal vascular occlusions, intraretinal hemorrhages, and other retinal findings. While the pathogenesis of these ophthalmic sequelae is not entirely understood, processes involving direct toxicity, vasoconstriction, vasospasm, and transient hypertension are hypothesized to be included. Further investigation of the mechanism of methamphetamine's ocular toxicity is needed.

Ethics statement

Written informed consent was obtained from the patient for the publication of any potentially identifiable images or data included in this article.

Author contributions

SY and YH conceptualized the manuscript and performed literature synthesis. YH, NN, and SY wrote and edited the manuscript. BH, DM, TA, BT, and RK provided guidance and edited the manuscript critically. All authors made direct and intellectual contribution to the work and approved the final version of the manuscript for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Mercury intoxication and ophthalmic involvement: An update review

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Human intoxication after mercury exposure is a rare condition that can cause severe damage to the central nervous, respiratory, cardiovascular, renal, gastrointestinal, skin, and visual systems and represents a major public health concern. Ophthalmic involvement includes impaired function of the extraocular muscles and the eyelids, as well as structural changes in the ocular surface, lens, retina, and optic nerve causing a potential irreversible damage to the visual system. Although, there are many pathways for poisoning depending on the mercury form, it has been suggested that tissue distribution does not differ in experimental animals when administered as mercury vapor, organic mercury, or inorganic mercury. Additionally, visual function alterations regarding central visual acuity, color discrimination, contrast sensitivity, visual field and electroretinogram responses have also been described widely. Nevertheless, there is still controversy about whether visual manifestations occur secondary to brain damage or as a direct affectation, and which ocular structure is primarily affected. Despite the use of some imaging techniques such as *in vivo* confocal microscopy of the cornea, optical coherence tomography (OCT) of the retina and optic nerve, and functional tests such as electroretinography has helped to solve in part this debate, further studies incorporating other imaging modalities such as autofluorescence, OCT angiography or adaptive optics retinal imaging are needed. This review aims to summarize the published structural and functional alterations found in the visual system of patients suffering from mercury intoxication.

KEYWORDS

corneal sensitivity, dry eye disease, mercury intoxication, ocular surface, ophthalmic involvement, optic nerve, retina, visual function alterations

1 Introduction

Mercury is a toxic metal that exists in three forms with different toxicological properties, as elemental or metallic mercury (mercury liquid and mercury vapor) or as inorganic (mercury salts) and organic compounds (methylmercury and ethylmercury) when combined with other elements (Park and Zheng, 2012; Fowler and Zalups, 2022), and it is considered by the World Health Organization (WHO) as one of the top 10 chemicals or groups of chemicals of major public health concern (World Health Organization, 2017). A significant incident occurred in Minamata, Japan, between 1932 and 1968, where a factory dumped waste liquid with high concentrations of methylmercury in the bay which was rich in fish and shellfish, the primary food sources for local and other areas residents, affecting at least

50,000 people and causing neurological symptoms in over 2,000 people (hence the name Minamata disease) (World Health Organization, 2017). Later in 1972, over 6,000 people in Iraq developed methylmercury poisoning from eating baked grain bread treated with methylmercury-based fungicide (Fowler and Zalups, 2022; Posin et al., 2022).

There are many pathways for intoxication after mercury exposure, including ingestion of contaminated seafood, contact with broken mercury-containing devices such as thermometers, barometers and electrical switches, or inhaling mercury vapor from dental amalgam (which are currently less and less likely because their manufacture has been prohibited) (Fisher, 2003; World Health Organization, 2017; Fowler and Zalups, 2022; Posin et al., 2022). However, most human mercury intoxication occurs in occupational settings when workers inhale odorless and colorless elemental mercury vapors, in mercury and artisanal or small-scale gold mining, physics and pharmaceutical laboratories and some industrial processes such as zinc-mercury amalgam, coal-fired power and chloroalkali plants, paint factories, non-ferrous and ferrous metal production, and in fluorescent lamp, batteries and other instruments manufacturing (Fisher, 2003; Rustagi and Singh, 2010; UN Environment, 2019; Fowler and Zalups, 2022). These mercury vapors are absorbed up to 80% through the lungs with rapid diffusion to the blood and later distribution throughout the body. In contrast, inorganic mercury is absorbed mainly in the gastrointestinal tract in about 2%–38%, while methylmercury when ingested is almost 100% absorbed in the duodenum, then in the blood combines with glutathione and other amino acids or peptides (Hong et al., 2012; Fowler and Zalups, 2022; Posin et al., 2022).

Due to the lipophilic nature of elemental and organic mercury, both can cross the blood-brain barrier. Then, they are oxidized by the hydrogen peroxide-catalase pathway to an inorganic divalent form with poor lipid solubility, and therefore, accumulate for several years in the brain, interrupting cellular enzymes and proteins systems, and causing neurotoxicity (Fisher, 2003; Posin et al., 2022).

Peripheral nerve function, renal function, immune and endocrine systems, and muscle function may also be affected by the three forms (Fowler and Zalups, 2022). To reduce these adverse effects, a global agreement named Minamata Convention on Mercury was adopted in 2013 and entered into force in 2017 to take actions to protect human health and environment from anthropogenic release of mercury (UNEP, 2019). Mercury levels in blood are useful after short-term and high-level exposure, whereas mercuric values in urine mercury is the ideal biomarker for long-term exposure to both elemental and inorganic mercury (Park and Zheng, 2012).

Eye and visual pathway damage have been reported given the fact that the retina and the optic nerve are specialized extensions of the central nervous system (CNS) (London et al., 2013). Furthermore, mercury intoxication may also cause damage to the corneal nerves as the cornea is the most densely innervated tissue in the body. In this review we focus on ophthalmic involvement due to mercury intoxication and summarize the clinical experience of our center about this topic based on 29 workers suffering acute and subacute exposure to mercury vapor in an aluminum manufacturing industry that were studied and followed by the Institute of Applied Ophthalmobiology (IOBA), University of Valladolid, Spain (Cañadas et al., 2021; Pastor-Idoate et al., 2021).

2 Materials and methods

Articles were sourced using PubMed database with the following terms: “color vision”, “contrast sensitivity”, “cornea”, “exposure”, “eye”, “glaucoma”, “heavy metal”, “intoxication”, “lens”, “mercury”, “ocular alterations”, “ocular manifestations”, “ocular surface”, “ophthalmological findings”, “optical coherence tomography”, “optic nerve”, “poisoning”, “retina”, “toxicity”, “visual alterations” and “visual evoked potentials”. We completed the selection of pertinent literature until the inception of the manuscript (October 2022) based on title, abstract and full content information.

3 Results

3.1 Ocular surface disease and anterior segment alterations

The lacrimal functional unit, a term first introduced by Stern et al., is a unit composed of the ocular surface (corneal, conjunctival and limbal epithelium plus the overlying tear film), all tear-producing glands and cells, in addition to the immune cells and nervous fibers that work together to maintain the health of the cornea (Stern et al., 2004), which is the major refractive surface of the visual system and the most sensitive tissue in the body, being densely innervated at its external layers by the first division (ophthalmic) of the trigeminal nerve.

Neurotoxicity induced by mercury may target this rich innervation as previously reported by Sabelaish and Hilmi who described loss of corneal sensation in most affected patients after subchronic organomercury poisoning, however, no objective test was performed to confirm this finding (Sabelaish and Hilmi, 1976). Nevertheless, Cañadas et al., from our group, evaluated 22 male workers who were accidentally exposed to mercury vapor for 14 consecutive days and described diminished corneal sensitivity as well as decreased nerve density and branch density of sub-basal corneal nerves, and reduced density of dendritic cell in corneal stroma determined by non-contact Belmonte gas esthesiometry and *in vivo* confocal microscopy, respectively, and thus impairing both nerve function and nerve regenerative activity (Cañadas et al., 2021). Most of these patients referred dry eye symptoms, mostly severe, using the Ocular Surface Disease Index questionnaire (OSDI) and showed increased tear osmolarity compared to control healthy subjects, however, no alteration in tear quality and ocular surface integrity was found. Tear production was not significantly affected in those workers, although 8 patients showed low lysozyme tears levels, particularly with some elevated cytokines in tears, such as interleukin (Il)-6, lL-12p70, regulated on activation normal T-cell expressed and secreted (RANTES), and vascular endothelial growth factor (VEGF) and with high urine mercury levels. So far, this is the only report on ocular surface disease in subacute mercury intoxication in humans and taking this evidence together, a primary neurogenic inflammation mechanism triggering a proinflammatory cascade of cytokines may explain ocular surface

disease in mercury poisoning (Cañadas et al., 2021; Pollard et al., 2019; Yang et al., 2020).

Less frequently, band keratopathy, mercury deposits on the corneal stroma and anterior capsule of the lens (mercurialentis) have also been reported in some cases of chronic mercury intoxication (El-Sherbeeny et al., 2006). In addition, Korbas et al. in studies with zebrafish larvae (*Danio rerio*), suggested that methylmercury may accumulate in the secondary fiber cells of the lens after reaching high intraocular levels by being able to cross the blood-aqueous barrier (Korbas et al., 2008; Korbas et al., 2013). Furthermore, Domínguez-Calva et al. showed that mercury has a cataractogenic potential by inducing non-amyloid aggregation of human lens proteins (γC and γS crystallins proteins) (Domínguez-Calva et al., 2018).

3.2 Retina, optic nerve, and visual alterations

Accumulation of mercury in the retinal pigment epithelium, inner plexiform layer, ganglion cells and vessel walls of the inner retina and of the optic nerve was described by Warfvinge and Bruun in squirrel monkeys up to 3 years after mercury vapor exposure (Warfvinge and Bruun, 2000). Similarly, Phamphlett et al. found that mercury may appear in fetal retinal ganglion cells, optic nerve glial cells, peripapillary retinal pigment epithelium, and endothelial cells of mice after prenatal exposure to mercury vapor (Pamphlett et al., 2019). Korbas et al. demonstrated that methylmercury may target both optic nerve (Korbas et al., 2008) and outer segments of photoreceptors cells (Korbas et al., 2013) in zebrafish larvae. Additionally, it has been suggested that even though there are three forms of mercury, retinal and optic nerve distribution seems not to differ in experimental animals when administered as mercury vapor, organic mercury, or inorganic mercury (Pamphlett et al., 2019).

Visual impairment due to mercury toxicity may occur as a direct eye damage as demonstrated by the IOBA's Retina group and other researchers (Bridges et al., 2007; Korbas et al., 2013; Pastor-Idoate et al., 2021) in addition to visual cortex injury (da Costa et al., 2008; Saldana et al., 2006; Ventura et al., 2004; Ventura et al., 2005; Yorifuji et al., 2013), causing night vision dysfunction, decreased color vision and contrast sensitivity, central visual impairment, visual field (VF) defects such as concentric constriction, and optic atrophy (El-Sherbeeny et al., 2006; Bridges et al., 2007; Pastor-Idoate et al., 2021; da Costa et al., 2008; Saldana et al., 2006; Ventura et al., 2004; Ventura et al., 2005; Yorifuji et al., 2013; Cavalleri et al., 1995; Cavalleri and Gobba, 1998; Urban et al., 2003; Rodrigues et al., 2007; Fillion et al., 2011; Dos Santos Freitas et al., 2018; Feitosa-Santana et al., 2008; Feitosa-Santana et al., 2007; Feitosa-Santana et al., 2018; Barboni et al., 2008).

Some studies have reported retinal pigment epithelium (Bridges et al., 2007) and photoreceptor damage (Korbas et al., 2013). Recent evidence by Pastor-Idoate et al., from our group, showed a primary involvement in electroretinogram (ERG) of both the inner (oscillatory potentials) and outer retina, mainly reduced scotopic rod response, in a long-term group of affected patients (Pastor-Idoate et al., 2021). Nevertheless, 30-Hz flicker, single flash cone response and multifocal ERG and pattern ERG alterations also appeared when deeper and more extensive VF defects developed, suggesting that cone dysfunction and ganglion macular cells damage can occur secondarily, thus causing color vision impairment, mainly in the blue-yellow range using Roth 28 Hue test. In addition, prolonged latencies, and reduced amplitudes of P100 in visual-evoked potentials compared to controls were found when severe VF was altered (Pastor-Idoate et al., 2021), as previously reported (El-Sherbeeny et al., 2006). In summary, although neurologic and visual pathway involvement was clear, there were also data suggesting the existence of a direct functional retinal damage and retinal participation in mercury poisoning.

Ekinci et al. reported in 31 industrial mercury battery workers blue-yellow color vision impairment but reduced retinal nerve fiber layer thickness (RNFLT) and choroidal thickness (CT) (Ekinci et al., 2014) on optical coherence tomography (OCT), data not confirmed by Pastor-Idoate et al. (Pastor-Idoate et al., 2021) who found normal RNFLT, CT, and central retinal thickness. Additionally, Bilak et al. demonstrated reduced volumes of the inner plexiform and ganglion cell layers on OCT in patients exposed to mercury from amalgam dental fillings compared to controls, however, RNFLT and CT decreases were neither significant nor clinically relevant (Bilak et al., 2019). An important aspect of the case series by Bilak et al. and Ekinci et al. is that they were done in patients chronically exposed to mercury and ocular electrophysiologic studies were not performed in contrast to the clinical analysis done by Pastor-Idoate et al. in patients with acute/subacute exposure to mercury. Blood concentrations may have been lower and evidently exposure times were different.

On the other hand, Cavalleri et al. (1995) and Jedrejko and Skoczyńska (2011) also observed color vision alteration in the blue-yellow range in workers exposed to mercury vapor, whereas Ventura et al. (2005) and Feitosa-Santana et al. (2008) found both blue-yellow and red-green alterations in patients with chronic mercury vapor intoxication, suggesting alterations in both the retina and the optic nerve. Lacerda et al. investigated two Amazonian populations, 10 Riverines exposed to organic mercury by eating fish and 34 gold-miners exposed to mercury vapor, and described that both groups had similar color vision impairment compared to control groups using Farnsworth–Munsell test, however, visual perimetry impairment was greater in riverines than in gold-miners using Förster perimeter, which may be due to higher exposure to mercury in riverines (Lacerda et al., 2020).

There is no consensus about the reversibility of mercury intoxication. Previous studies described that color vision loss may be reversible (Cavalleri and Gobba, 1998; Urban et al., 2003), however, recent reports strongly suggest irreversible damage in both chronic methylmercury consumption and in workers chronically exposed to mercury vapor (Feitosa-Santana et al., 2007; Feitosa-Santana et al., 2008; Feitosa-Santana et al., 2018). Similarly, Ventura et al. and Costa et al. also found that contrast sensitivity is irreversibly impaired in long-term occupational mercury intoxication (Ventura et al., 2005; Costa et al., 2008).

3.3 Other ophthalmic manifestations

Mercury has also been suggested to be linked to glaucoma (Vennam et al., 2020). Ceylan et al. found significantly higher blood mercury levels in 32 patients with pseudoexfoliation

syndrome compared to controls (Ceylan et al., 2013). Pseudoexfoliation syndrome is considered a systemic disease characterized by accumulation of extracellular material, named pseudo-exfoliative, in many organs including the eye and orbit, mainly on the anterior lens capsule and/or the pupillary border and which may impair aqueous drainage and thus high intraocular pressure and glaucoma (Plateroti et al., 2015). Pseudoexfoliation origin is not fully established and is more frequent in Northern European countries. Trace elements have been suggested to have roles in its pathogenesis, however no pseudoexfoliation glaucoma association was significantly found in these patients (Ceylan et al., 2013). Similarly, Lee et al. (48) based on data from the Korean National Health and Nutrition Examination Survey (KNHANES) did not find significant associations between blood levels of mercury and open angle glaucoma prevalence (Lee et al., 2016).

Less frequently, eyelid tremor, nystagmus and abnormal saccadic lateral conjugate eye movements have also been reported in some cases of chronic intoxication (El-Sherbeeny et al., 2006; Rustagi and Singh, 2010; Fowler and Zalups, 2022).

4 Conclusion

In summary, mercury intoxication is a major public health concern and patients suffering from systemic mercury poisoning, in addition to central nervous system damage, may exhibit a direct ophthalmic involvement, mainly as an ocular surface disease and targeting primarily the inner and outer retina with secondary impairment of the optic nerve. It also has a potential cataractogenic effect, however more studies are needed to confirm this hypothesis. Ophthalmic changes may lead to potential irreversible damage to the visual system, hence raising people awareness and leading to massive interventions to reduce major sources of human mercury exposure according to the Minamata Convention on Mercury by shifting energy production from coal burning to clean energy, eliminating gold and mercury mining, and phasing down of mercury use in some products or processes.

Author contributions

CdlS contributed to redaction of the review. JCP and MC have equally contributed as senior author to the supervision of the final manuscript. All authors approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Sex-related differences in retinal function in Wistar rats: implications for toxicity and safety studies

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Introduction: Wistar Han rats are a preferred strain of rodents for general toxicology and safety pharmacology studies in drug development. In some of these studies, visual functional tests that assess for retinal toxicity are included as an additional endpoint. Although the influence of gender on human retinal function has been documented for more than 6 decades, preclinically it is still uncertain if there are differences in retinal function between naïve male and female Wistar Han rats.

Methods: In this study, sex-related differences in the retinal function were quantified by analyzing electroretinography (ERG) in 7-9-week-old (n = 52 males and 51 females) and 21–23-week-old Wistar Han rats (n = 48 males and 51 females). Optokinetic tracking response, brainstem auditory evoked potential, ultrasonic vocalization and histology were tested and evaluated in a subset of animals to investigate the potential compensation mechanisms of spontaneous blindness.

Results/Discussion: Absence of scotopic and photopic ERG responses was found in 13% of 7-9-week-old (7/52) and 19% of 21–23-week-old males (9/48), but none of female rats (0/51). The averaged amplitudes of rod- and cone-mediated ERG *b*-wave responses obtained from males were significantly smaller than the amplitudes of the same responses from age-matched females (–43% and –26%, respectively) at 7–9 weeks of age. There was no difference in the retinal and brain morphology, brainstem auditory responses, or ultrasonic vocalizations between the animals with normal and abnormal ERGs at 21–23 weeks of age. In summary, male Wistar Han rats had altered retinal responses, including a complete lack of responses to test flash stimuli (i.e., blindness), when compared with female rats at 7–9 and 21–23 weeks of age. Therefore, sex differences should be considered when using Wistar Han rats in toxicity and safety pharmacology studies with regards to data interpretation of retinal functional assessments.

KEYWORDS

retinal function, toxicity, Wistar Han, sex, electroretinography (ERG)

1 Introduction

Due to their longevity, small body size, slow growth rate, and low incidence of spontaneous tumors, Wistar Han (WH) rats are currently one of the most used strains in biomedical research (Weber et al., 2011; Gauvin et al., 2019). This strain of rat has also been recommended for use in toxicological testing in drug development in the United States (Son et al., 2010; Gauvin et al., 2019) and Europe (Gauvin et al., 2019). Sometimes visual functional tests, e.g., electroretinography (ERG) or visual discrimination behavioral tests, are included as an add-on endpoint for assessing potential retinal toxicity of new molecules (Rosolen et al., 2005; Brock et al., 2013). Ophthalmologic and histopathologic examinations have shown a higher incidence of corneal opacities and mineralization in WH rats compared with Sprague-Dawley rats (Hayakawa et al., 2013). Spontaneous microscopic lesions have also recently been reported in retinas in this strain with 5.0%–45.7% of rats examined displaying retinal degeneration and retinal rosettes/folds (Cloup et al., 2021). In previous pilot studies, as many as 11%–12% of adult male WH rats were identified as having virtually no ERG responses to a series of test light flashes, indicating a loss or decrease in visual function. Although these animals behave normally, as observed during cage-side observations, and had no findings with standard eye examinations, some of them were found to be blind based on our ERG assessments. In pharmacology or neurological studies, rats with significant photoreceptor loss (O'Steen et al., 1995) and rats with reduced visual acuity (Prusky et al., 2000) are all impaired in the Morris water task experiments, compromising the interpretation of experimental data that are dependent on visual function. It is also essential for toxicologists to be familiar with spontaneous ocular morphological and functional alternations that may occur in WH rats used in safety assessment studies. Although the visual responses at retina (Heiduschka and Schraermeyer, 2008), brain (Thomas et al., 2005), visual acuity threshold (Prusky et al., 2002) and susceptibility to light damage (De Vera Mudry et al., 2013) have been compared between pigmented and albino rats, no comparative study has quantified the visual or retinal function of WH rats in large groups of male and female WH rats.

Visual impairment or blindness can alter sensory, memory, social, and survival behavior through various compensatory mechanisms. Since the 1980s, evidence has accumulated showing that blind individuals can have better hearing than those with normal vision, due to intramodal plasticity in the cortex and subcortical auditory structures (Niemeyer and Starlinger, 1981; Liotti et al., 1998; Bavelier and Neville, 2002). Alterations in auditory brainstem responses have also been observed in blind adults (Jafari and Malayeri, 2014) and children (Jafari and Malayeri, 2016). However, these forms of intramodal compensation have not been documented in blind or vision-impaired rodents.

To fill the knowledge gaps, the current study screened male and female adult WH rats using regular ophthalmic examinations and ERG. Additionally, optokinetic response tracking (OKR), brainstem auditory evoked potential (BAEP), and ultrasonic vocalization (USV) were performed or recorded to compare the potential differences between normal-sighted and blind animals. The resulting structural plasticity in the retina, visual, and auditory pathways in the brain was also examined using conventional histology methods.

2 Materials and methods

2.1 Animals

All activities involving animals conformed to the guidelines established by the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research, and the animal use protocol was approved by the Pfizer Institutional Animal Care and Use Committee (IACUC). Adult male and female WH rats (Crl:WI [Han], Charles River Laboratories, Raleigh, NC) were obtained at an age of approximately 6–10 weeks of age. The animals were group-housed (2–3/cage) in Techniplast cages with Enrich-n'Pure bedding (The Andersons Inc., Maumee, OH) with a room temperature of 20°C–26°C and humidity of 30–70 %, under a 12 h:12 h light-dark cycle. They were provided with *ad libitum* reverse osmosis purified water and a regular irradiated Teklad Global Rodent Diet (Envigo, 2916C). ERG and OKR tests were performed on all animals between 8:00 a.m. and 3:00 p.m. Three cohorts of WH rats were ordered (see Supplementary Table S1) and assigned to four groups in this study as summarized in Table 1. Group 1 and 3 consisted of 2 separate sets of male rats, one 7–9 weeks of age ($n = 52$) and the other 21–23 weeks of age ($n = 48$). Group 2 and 4 consisted of the same set of female rats ($n = 51$) evaluated at 7–9 weeks of age and then again at 21–23 weeks of age.

2.2 Ophthalmologic examination

A standard qualitative ophthalmic examination was conducted either prior to ERG testing for group 2 animals (females) at

TABLE 1 Incidence of blindness in Wistar Han rats.

Group	Age (week)	Sex	# Of animals with abnormal ERG	Total # of animals tested	Incidence of blindness (%)	*p*-Value compared with group 1 animals
1	7–9	Male	7	52	13	N/A
2		Female	0	51	0	0.0126
3	21–23	Male	9	48	19	0.5877
4[a]		Female	0	51	0	0.0126

[a]The same animals as group 2 tested at 21–23 weeks of age.

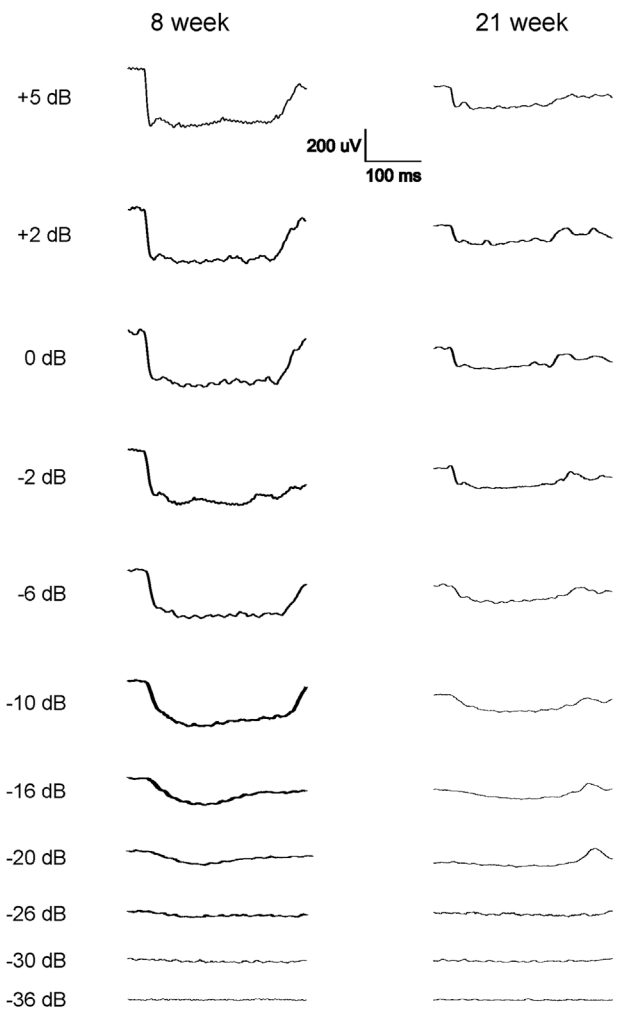

FIGURE 1
Representative abnormal scotopic ERG waveforms from some of the 7–9 weeks and 21–23-week-old male WH rats in response to a series of light flashes (−20 to 5 dB). Each waveform is the average of 3-9 responses to the same intensity flash. Following the flash stimulation of the eyes, no standard *a*-wave could be elicited, instead long (about 300 ms) negative waveforms were seen when the flash stimuli reached −20 dB, without further increase when the flashes were intensified. No standard *b*-wave was present in these animals.

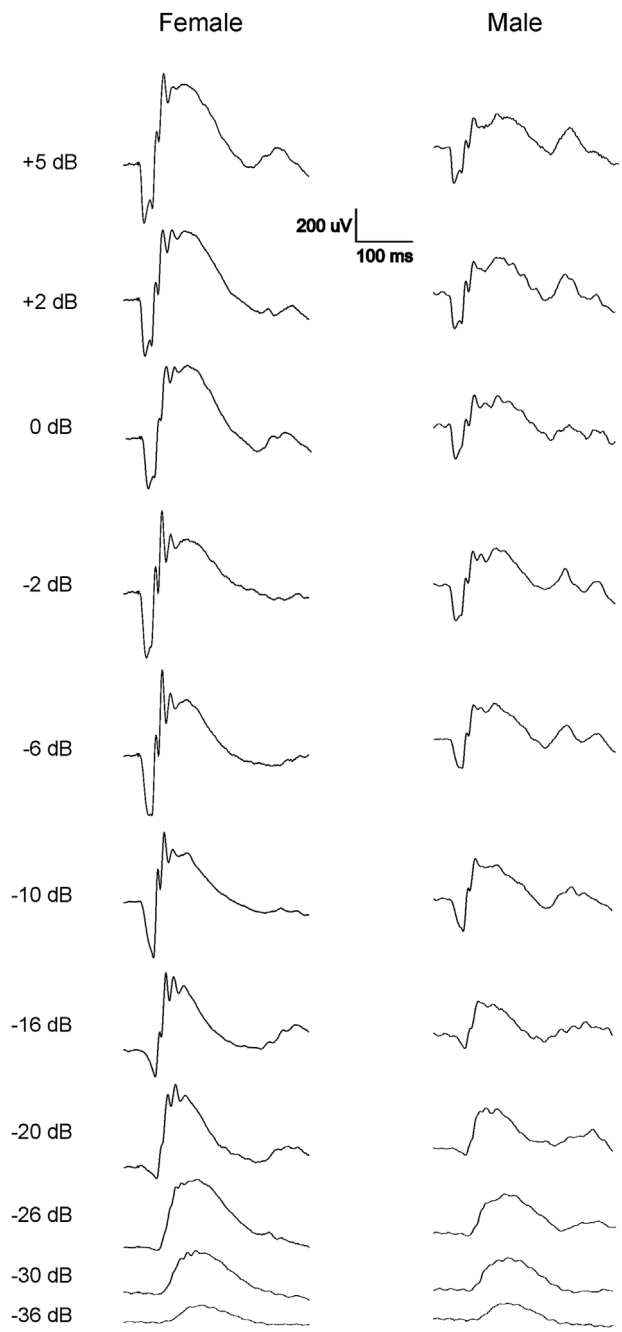

FIGURE 2
Representative normal dark-adapted ERG waveforms from 7-9-week-old female (left) and male (right) Wistar Han rats in response to flashes from −36 to 5 dB. Note that both *a*- and *b*-waves are larger in females compared with males at all tested flash levels.

7–9 weeks of age or after the ERG assessment for groups 1 and 3 animals (males) at 7–9 weeks of age and 21–23 weeks of age, respectively. The visible ocular and adnexal anatomy were evaluated. Mydriacyl (1.0% tropicamide, Akorn Operating Company LLC, Lake Forest, IL) was applied topically to each eye to assist in the examination. In ambient lighting, indirect ophthalmoscopy was utilized to examine the retina, optic disc, and blood vessels, and a handheld slit lamp biomicroscope was employed to examine the anterior chamber.

2.3 Electroretinography

Full-field ERGs were tested at 7–9 and 21–23 weeks of age using a LKC system (LKC Technologies, Gaithersburg, MD), as previously described (Liu et al., 2015). Briefly, the male and female rats were kept in the dark for a period of 2–8 h prior to ERG testing in order to enhance retinal sensitivity (Behn et al., 2003). The animals were anesthetized with a 2.0%–2.5% concentration of isoflurane in oxygen. A dim red light, generated by an Energizer red LED 315 headlamp (Intensity: ~5 μW/cm^2; wavelength: 620–645 nm; Energizer Holdings, Inc., MO), was briefly used to aid in animal manipulation and electrode placement. The body temperature was

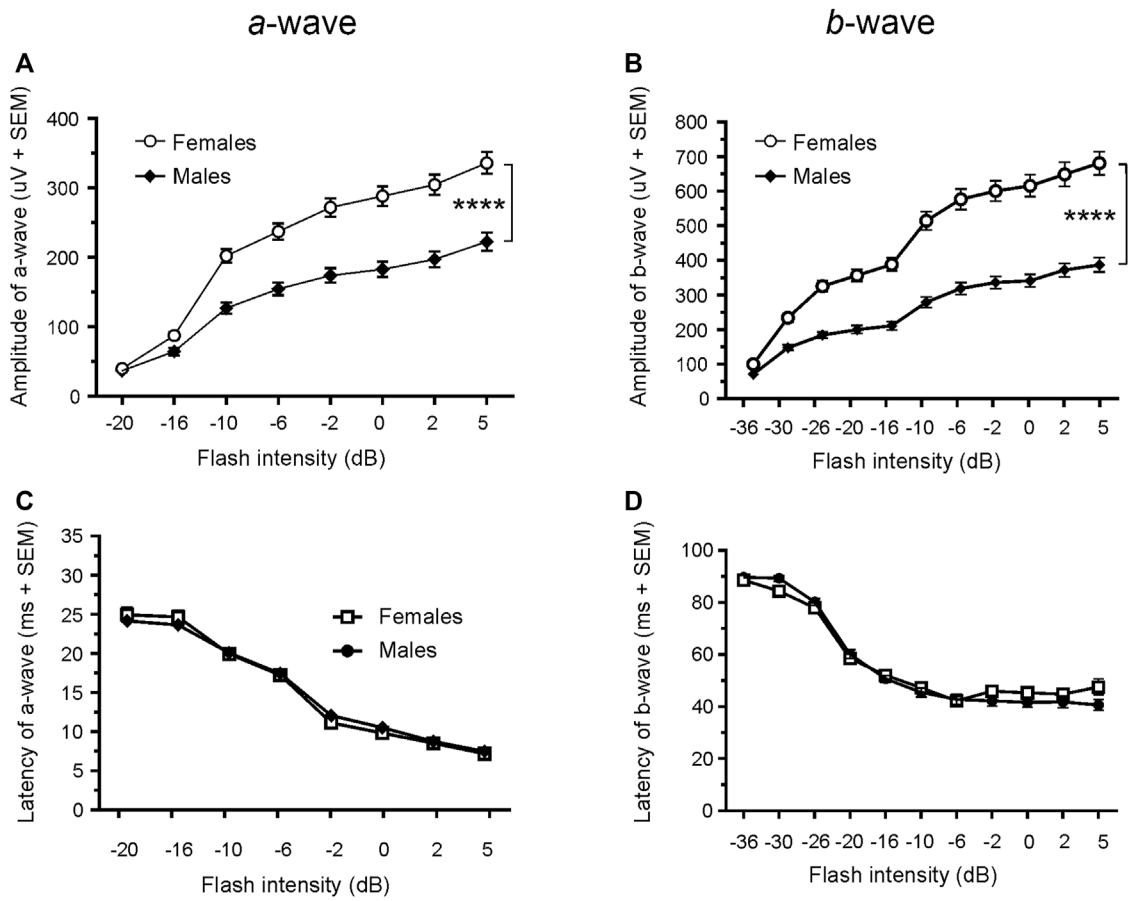

FIGURE 3
Comparison of scotopic *a*- and *b*-wave luminance responses between male and female WH rats with normal ERG signals at 7–9 weeks. Male Wistar Han rats had lower mean amplitudes of rod-mediated ERG *a*-wave **(A)** and *b*-waves **(B)** tested with −36 to +5 dB flashes that were statistically significant when compared with subset of female Wistar Han rats with normal ERG, but there were no statistically significant differences in the latencies of rod-mediated luminance response *a*- or *b*-waves **(C, D)** between the two groups of animals. SEM = standard error of the mean. * Indicates significant differences between male (filled circle) and females (open circle) at the same flash intensities of stimulation (2-way ANOVA, F (1,94) = 36.98, ****$p < 0.0001$ for **(A)** and F (1.94) = 56.02, $p < 0.0001$ for **(B)**.

maintained using a heated pad connected to the ground. One drop of local anesthetic was administered to prevent blinking, and 1% tropicamide was applied to induce pupil dilation. ERG lens electrodes (Medical Workshop, Groningen, Holland) were placed on both eyes using artificial tears (GenTeal Tears, Alcon, Geneva, Switzerland) as a coupling agent. After disinfecting the skin with an alcohol pad, a platinum needle reference electrode (Natus Neurology, West Warwick, RI) was inserted subcutaneously between the eyes on the forehead. After scotopic testing, the animals were exposed to standard facility lighting (~250 lux) for 10 min to allow for light adaptation prior to photopic ERG testing.

A UTAS BigShot Visual Electrodiagnostics System was used to evoke and acquire ERG signals that were high–pass filtered at 0.3 Hz and low–pass filtered at 500 Hz. ERG protocols were adapted from Rosolen et al. (2005) to test scotopic and photopic luminance responses of the retina. Photopic responses were obtained with the background Ganzfeld illumination of 30 cd/m^2 (white light generated by the BigShot system and calibrated by LKC Inc.). ERG waveforms were analyzed using LKC Technologie's software and the guidelines of the International Society for Clinical Electrophysiology of Vision (ISCEV) (Rosolen et al., 2005). The amplitude of the *a*-wave was measured from baseline to trough and its latency was measured from stimulus to *a*-wave trough. The amplitude of the *b*-wave was measured from *a*-wave trough to *b*-wave peak and its latency was measured from the stimulus to *b*-wave peak.

2.4 Optokinetic tracking response

Visual acuity was measured in the animals with normal ($n = 9$, male) and abnormal ($n = 9$, male) ERGs in group 3 (Table 1) using an optokinetic testing apparatus (OptoMotry; Cerebral Mechanics, Inc., Lethbridge, AB, Canada) at 21–23 weeks of age. It tested if the animal had reflexive head movement as the responses to rotating strips displayed on four computer monitors (optokinetic reflex) surrounding the animal (Chowers et al., 2017). A standard stepwise protocol was adapted, and the final score was calculated by the program, and the test videos were captured for post-experiment

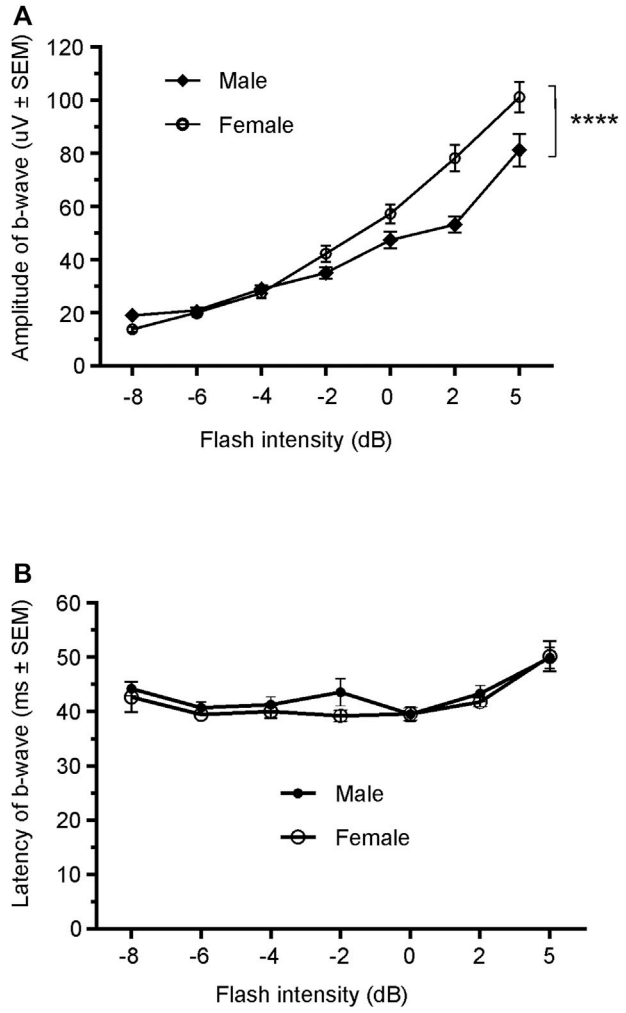

FIGURE 4
Comparison of photopic *b*-wave luminance responses between male and female Wistar Han rats with normal ERG signals at age of 7–9 weeks. Male Wistar Han rats had lower mean amplitudes of cone-mediated ERG *b*-wave **(A)** tested with −8 to +5 dB flashes that were statistically significant [2-way ANOVA, F (1,657) = 19.14, ****$p < 0.0001$] when compared with the female Wistar Han rats. But there were no statistically significant differences in the latencies of cone-mediated luminance response *b*-waves **(B)** between the two groups.

review and confirmation. Three observers' judgments were pooled for determination of animal's OKR responses.

2.5 Brainstem auditory evoked potential

Rats with normal ($n = 9$, male) and abnormal ($n = 9$, male) ERGs from group 3, used for OKR test, were also tested for BAEP at 21–23 weeks of age. The animals were anesthetized with 2.5% isoflurane and placed on a heated pad to maintain a body temperature of approximately 37.5°C. Acoustic stimuli were created using a digital stimulator (WPI DS8000, World Precision Instruments, Sarasota, FL) in the form of click stimuli with a 100 µs duration and a monopolar waveform. The stimuli (75 dB) were delivered bilaterally to the rat's external auditory canals via earplugs. Six hundred and fifty stimuli were administered at a 5 Hz frequency. Auditory potentials were recorded from the right ear only through a subcutaneous Grass® platinum needle electrode (F-E2, Natus Neurology, Galway, Ireland) placed at the vertex (active) and parietal-occipital area ventrolateral to the right ear (Alvarado et al., 2012). The signals were amplified 10,000 times, bandpass filtered between 300 Hz and 3,000 kHz, and sent to an Axon Digitizer (1550B, Molecular Devices Corp, Sunnyvale, CA) for analog-to-digital conversion. The responses were averaged 650 times, and the averaged waveforms were analyzed within a 20 msec post-stimulus window. Clampfit software (Molecular Devices, ver. 10.6) was used for measurements and analysis of amplitude and latency of evoked auditory responses. The peak amplitudes and latencies of waves II, III, IV, and V were determined relative to the onset of the acoustic stimulus (Alvarado et al., 2012).

2.6 Ultrasonic vocalization

Rats with normal ($n = 8$, male) and abnormal ($n = 8$, male) ERGs from group 3, used for OKR and BAEP tests, were also tested for USV at 21–23 weeks of age. To reduce social isolation effects on USVs (Brudzynski and Ociepa, 1992), rats were pair-housed in 8 cages for 24-h continuous recording of USVs. In the test cage, an ultrasound microphone was inserted and fixed in the center of the short wall to capture USV signals emitted by the rats. The emissions were captured by the UltraSoundGate condenser ultrasonic microphone (CM16, Avisoft Bioacoustics, Berlin, Germany), which is sensitive to frequencies between 15 and 180 kHz and has a flat frequency response between 25 and 140 kHz (±6 dB). The microphone was connected to a computer via an UltraSoundGate IH8 (Avisoft Bioacoustics), and acoustic data were recorded by Avisoft Recorder software (version 2.95, Avisoft Bioacoustics), using a sampling rate of 250,000 Hz in 16-bit format and a recording range of 0–125 kHz (Hwang et al., 2022). Fifty and 22 KHz signals were analyzed off-line.

2.7 Histology

One to 3 weeks after behavioral testing (OKR, USV and BAEP tests), the 18 male rats were selected for necropsy and tissue collection. These animals were deeply anesthetized using isoflurane and then euthanized by exsanguination. The brains were rapidly and carefully removed, sliced in half coronally, and then fixed overnight in 4% neutral buffered formalin. The following day, the specimens were trimmed coronally at the level of the striatum, corpus callosum, and motor cortex, as well as at the level of the mid-cerebellum and medulla oblongata (levels 2 and 6, as described in (Bolon et al., 2013)). The two most rostral sections of each brain level were processed and embedded into the same paraffin block, and 5 µm sections were taken. The eyes were enucleated immediately after the brain was collected and fixed in Davidson's fixative. The eyes were then processed into slides for microscopic evaluation. For each eye, a horizontal section was taken just below the optic nerve and at least five step sections were taken at 100 µm intervals, starting from below the optic nerve and

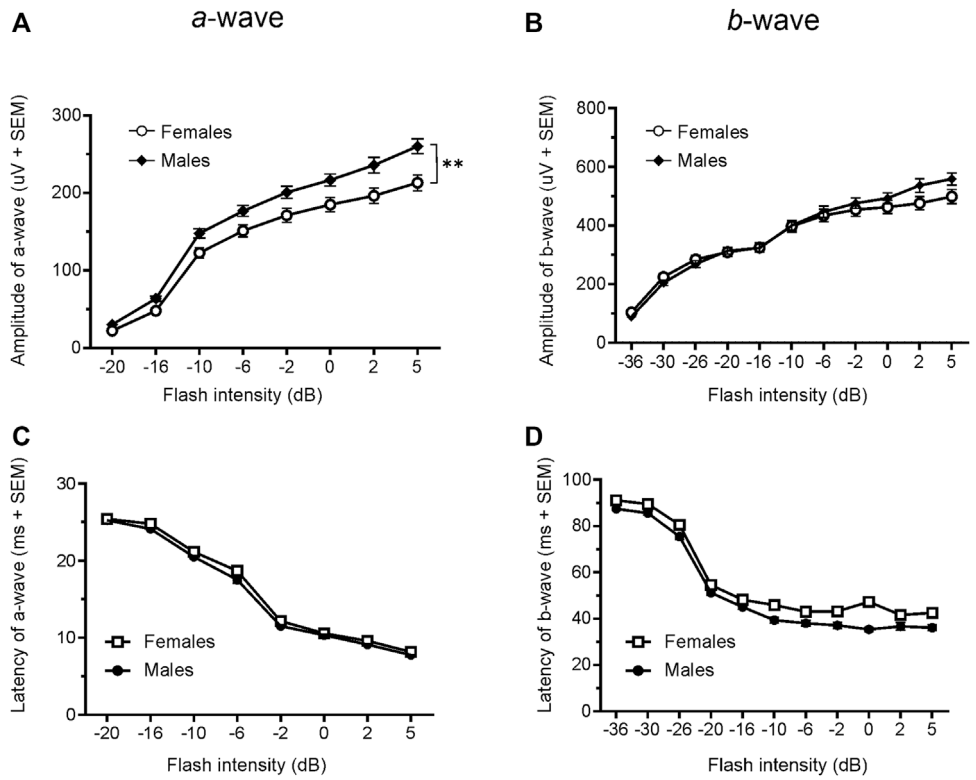

FIGURE 5
Comparison of scotopic *a*- and *b*-wave luminance responses between male and female Wistar Han rats with normal ERG signals at 21–23 weeks. Female Wistar Han rats had lower mean amplitudes of rod-mediated ERG *a*-wave **(A)** but not *b*-waves **(B)** tested with −36 to +5 dB flashes that were statistically significant [2-way ANOVA, F (1,88) = 8.210, $^{**}p = 0.0052$] when compared with a subset of male Wistar Han rats. But there were no statistically significant differences in the latencies of rod-mediated luminance response *a*- or *b*-waves **(C, D)** between the two groups of animals.

proceeding toward the optic disk. All brain and eye sections were stained with hematoxylin and eosin (H&E) for microscopic evaluations.

2.8 Data analysis and statistics

For ERG data, a two-way analysis of variance (ANOVA) with repeated measures was performed to compare and assess the luminance responses to light flashes (Inamdar et al., 2022), using GraphPad Prism (Version 9.0.0, GraphPad Software, San Diego, CA). Student *t*-test was used to compare the differences in ERG, OKR, USV, and BAEP parameters between normal sighted animals and those with abnormal ERGs. Fisher exact test was used for rate or incidence comparison. The statistical significance of the comparisons was determined at a level of $\alpha = 0.05$ (Liu et al., 2015).

3 Results

3.1 Abnormal ERG in male Wistar Han rats

The scotopic and photopic luminance responses to a series of flashes were tested in four groups of WH rats aged 7–9 and 21–23 weeks. For female animals, ERGs were tested at 2 ages within the same animals (group 2 and 4). Interestingly, some animals in both age groups displayed abnormal ERG waveforms, characterized by a large negative inflection followed by a flat line (Figure 1), without clear *a*- or *b*-waveform as seen in normalsighted animals (Figure 2). In addition, these waveforms did not increase in amplitude as the flash stimuli were intensified (Figure 1). Notably, this type of abnormal ERG waveform was only observed in males in groups 1 and 3 (13% and 19%, respectively), but not in age-matched females (0%, $p = 0.0126$, Fisher exact test, Table 1).

3.2 Normal ERG response comparison between male and female Wistar Han rats

Since only male WH rats manifested abnormal ERG waveform, we wondered whether there were also any differences between males and females that had normal ERG responses. Therefore, we compared scotopic *a*-wave, *b*-wave, and photopic *b*-wave parameters between male and female animals at 7–9 weeks (45 males vs. 51 females) and 21–23 weeks (39 males vs. 51 females) of age. At 7–9 weeks, male WH rats (group 1) had lower mean amplitudes for rod-mediated scotopic ERG *a*-wave (Figures 2, 3A) and *b*-wave (Figures 2, 3B), which were statistically significant when compared with female WH rats. However, there were no differences in the latency of scotopic *a*-

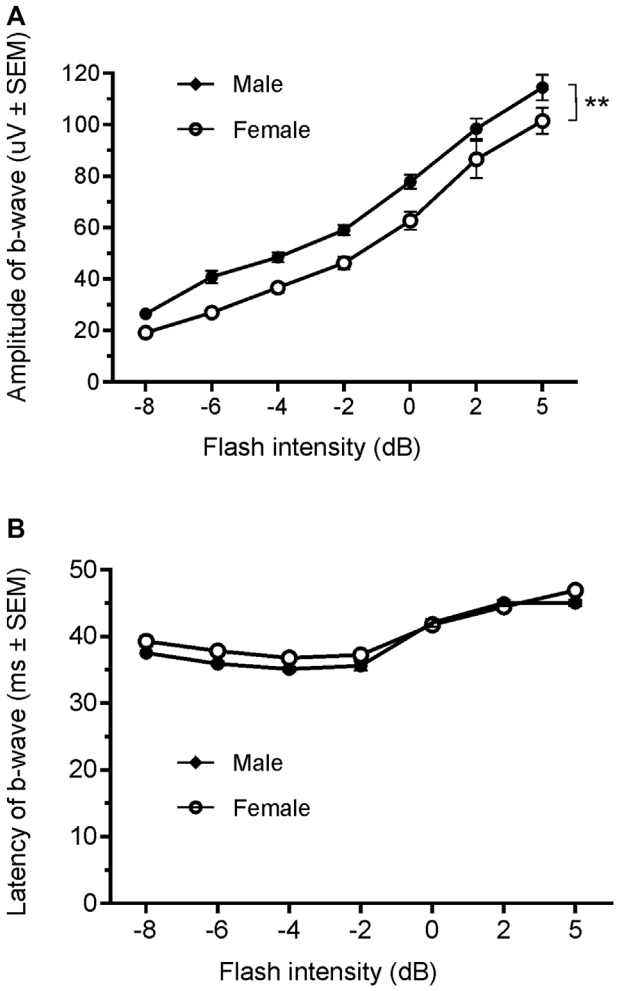

FIGURE 6
Comparison of photopic *b*-wave luminance responses between male and female Wistar Han rats with normal ERG signals at age of 21–23 weeks. Female Wistar Han rats had lower mean amplitudes of coned-mediated ERG *b*-wave **(A)** tested with −8 to +5 dB flashes that were statistically significant [2-way ANONA, F (1,88) = 11.21, $^{**}p$ = 0.0012] when compared with the male Wistar Han rats. But there were no statistically significant differences in the latencies of cone-mediated luminance response *b*-waves **(B)** between the two groups.

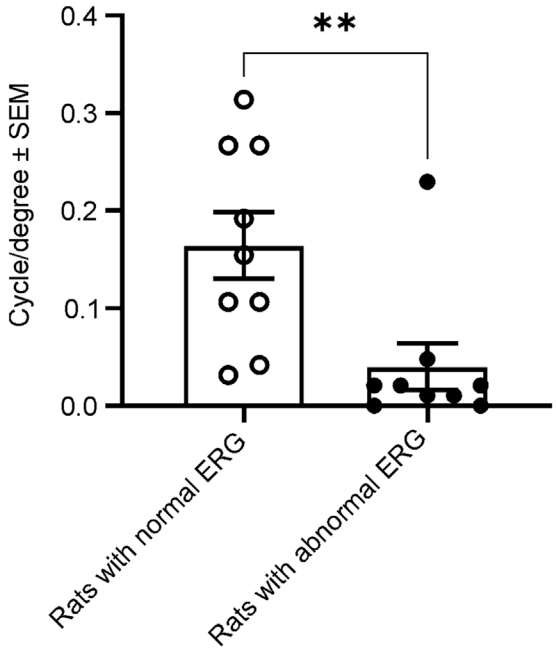

FIGURE 7
Comparison of spatial frequency thresholds of the acuity measured with OKR between animals with and without normal ERG waveforms at 21–23 weeks. The animals with abnormal ERG waveforms had significantly smaller grating thresholds (0.04 cycle/degree), compared with the animals with normal ERG (0.17 cycle/degree, *t*-test, t (Bavelier and Neville, 2002) = 2.978, $^{**}p$ = 0.0089).

or *b*-waves between males and females (Figures 3C, D). The mean amplitudes of scotopic oscillatory potential were significantly lower in males compared to females, with a 43% difference ($p < 0.0001$, *t*-test). The male WH rats also had lower mean amplitudes for cone-mediated photopic *b*-wave (Figure 4A), but no differences in the latency of photopic *b*-waves between males and females (Figure 4B). At 21–23 weeks, in contrast to the comparative results obtained at weeks 7-9, male WH rats (group 3) had slight but significantly larger mean amplitudes (Figure 5A) and similar latency (Figure 5C) of *a*-wave, and similar *b*-wave amplitude and latency of rod-mediated scotopic ERG responses. Likewise, cone-mediated *b*-wave amplitude was larger in males compared with females (Figure 6A). There were no differences in the latency of *b*-weave ERG parameters tested at this time point (Figures 6B).

3.3 Normal ERG responses comparison between 7–9 and 21–23 weeks in female Wistar Han rats

Given the opposite difference of ERG responses between male and female animals at 7–9 and 21–23 weeks, we longitudinally compared the animals ERG responses in cohort 2 female animals (group 4 vs. group 2). Interestingly, the amplitudes of both scotopic ERG *a*- and *b*-wave, though not the latencies, were significantly decreased in 21–23 weeks compared with 7–9 weeks (*a*-wave: 213.0 μV vs. 335.9 μV at 5 dB; b-wave: 498.3 μV vs. 698.6 μV at 5 dB, all $p < 0.0001$, Figures 3A vs 5A and Figures 3B vs 5B).

3.4 Visual acuity in weeks 21–23

To evaluate whether animals with abnormal ERG would exhibit normal visual-dependent behavior, we performed a visual acuity behavior test (OKR) in male rats at 21–23 weeks of age. We measured and compared visual acuity between 9 animals with normal and 9 animals with abnormal ERG waveforms. In the nine rats with normal ERG waveforms, the average visual acuity was 0.165 ± 0.102 cycle/degree (mean ± SD), whereas the mean visual acuity was only 0.040 ± 0.073 cycle/degree in 9 animals with abnormal ERG waveforms. Thus, the animals with abnormal ERG waveforms resulted in statistically significantly smaller mean visual

acuity scores compared with the animals with normal ERG waveforms. (Figure 7).

3.5 BAEP in week 23

To determine if the animals with abnormal ERG have altered hearing function to compensate for poor vision, we measured and compared brainstem auditory-evoked potentials in the same group of animals tested for visual acuity (3.4). There were no statistically significant differences in the amplitudes or latencies of waves II, III, IV, and V between the animals with normal ERG and those with abnormal ERG waveforms (Supplementary Table S2; Supplementary Figure S1).

3.6 USV comparison in weeks 21–23

Ultrasonic vocalization, an important mean of communication between rats, was evaluated to investigate if there was compensation in USVs in the blind animals. We recorded the USV from 8 rats with normal and 8 with abnormal ERG previously used for BAEP test continuously for 24 h. The poor-sighted animals had similar circadian patterns and 24-h total USV counts as the normal-sighted animals in both 50-kHz and 22-kHz USV call counts (all $p > 0.05$, Supplementary Figure S2).

3.7 Clinical and ophthalmic observations

No signs of abnormal behavior or morbidity were observed in any animal throughout the 3-month period. The ophthalmological analyses revealed no abnormalities in the retina and other components of the eyes in group 2 animals (females) at 7–9 weeks of age or groups 1 and 3 animals (males) at 7–9 weeks of age and 21–23 weeks of age, respectively.

3.8 Histology

There were no abnormal microscopic findings in the retina, brainstem, and visual and auditory-related areas in the rats with abnormal ERG responses at 21–23 weeks of age (Supplementary Table S3).

4 Discussion

In this study, we evaluated retinal function in male and female WH rats at two ages to determine the presence of spontaneous retinal functional deficits in the albino strain, and to explore any potential compensations in other sensory systems. Our results showed that a fraction of male WH rats had abnormal ERG signals and poor visual-mediated tracking responses at both 7–9 weeks and 21–23 weeks of age, without any changes in retinal or brain morphology. Even in normal-sighted rats with normal ERG signals, we found that the scotopic and photopic luminance responses were smaller in male WH rats compared with age-matched females at 7–9 weeks, but not at 21–23 weeks. Here, we chose the ages to mimic the duration of regular 3-month sub-chronic toxicity studies (Galijatovic-Idrizbegovic et al., 2016), which usually starts at 5–9 weeks of age (Baldrick, 2008). We did not observe evidence of compensations in brainstem auditory potential, ultrasonic vocalizations, or auditory morphology in the visual pathway of blind rats at 21–23 weeks of age (to mimic the time point at which histopathology is routinely evaluated). In conclusion, these findings confirm the presence of spontaneous retinal ERG deficits in 13% of adult male WH rats at 7–9 weeks of age and ERG and OKR deficits in 19% of adult male WH rats at 21–23 weeks of age, respectively.

The most notable outcome of our study is that a subset of naïve male WH rats showed abnormal ERG responses when their eyes were stimulated with flashes. As depicted in Figure 1, the amplitude of the scotopic ERG barely increased as the stimuli grew brighter, a phenomenon similar to the waveforms reported previously in 8.5-week-old albino rats with retinal dystrophy (Dowling and Sidman, 1962). The missing amplitudes of both *a*- and *b*-waves in these rats could be a result of weaker or no activity of photoreceptors, or minimal input from photoreceptors (Dowling and Sidman, 1962) into the post-photoreceptor circuits in the neuroretina, such as bipolar cells. In addition to the abnormal ERGs, we also evaluated the visual acuity of rats with and without normal ERG waveforms at 21–23 weeks of age. The sighted animals had an average acuity of 0.17 c/d, which is slightly lower than the values (0.36 c/d) reported for male WH rats at 7–9 weeks (Redfern et al., 2011). This difference might be due to observer bias. Despite this apparent decrease, the animals with abnormal ERGs had significantly lower acuity values, providing further evidence of vision impairment in this subset of male WH rats. Our data analysis of other animals with normal ERG waveforms, similar to the results reported for 8–26 week-old Sprague-Dawley rats (Chaychi et al., 2015), confirmed that the average ERG luminance responses in male WH rats were significantly smaller than those of females (Figures 2, 3) at 7–9 weeks old ($p < 0.01$), suggesting functional differences in photoreceptors, particularly the rod photoreceptors. Interestingly, our microscopic evaluations showed no noticeable thinning or reduction of the photoreceptor nuclei layer in 21–23 weeks old male rats with abnormal ERGs compared to those with normal ERGs. This is consistent with a recent review paper, which found retinal degeneration in control WH rats only after 52- and 104-week toxicity studies (Cloup et al., 2021). Likewise, the routine ophthalmic examination did not find any abnormality in the eyes of male and female WH rats at 7–9 weeks of age or male HW rats at 21–23 weeks of age. For the female animals in group 4, no ophthalmic examination was repeated at 21–23 weeks of age, since the ophthalmic examination is less sensitive compared with histopathology or ERG in spontaneous (Taradach et al., 1981), light-induced (Jaadane et al., 2015) or systemically administered drug-induced (Huang et al., 2015) retinal damages. We hypothesized that visual functional impairment occurs before any morphological changes can be seen in these animals. We also don't attribute the current observation to well-documented light-induced retinal damage often seen in albino rats [see review (De Vera Mudry et al., 2013)], since in our vivarium environment, 12 h on/12 h off cyclic illumination was applied during all the study course, which is less damaging to the retina than constant illumination (De Vera Mudry et al., 2013). We and animal vendor also used ~300 lux lighting 1 m above the floor (Supplementary Table S4), which was approved safe and no phototoxic retinopathy concern for rats (Bellhorn, 1980). Furthermore, the animal cages were rotated vertically in the rack

on a weekly basis as suggested (Rao, 1991). Rather, it may be inherited and related to albinism. It is well established that albino rats, such as Sprague-Dawley and WH, have impaired visual acuity (Prusky et al., 2002) and altered visual signal transmission latency from the retina to the superior colliculus (Thomas et al., 2005) compared with pigmented strains. These investigators did not further differentiate between sex, though. In humans, the influence of biological sex on retinal function as measured with the ERG has been known for over 60 years (Karpe et al., 1950). ERGs are typically reported to have larger amplitudes in women compared to men (Birch and Anderson, 1992; Brule et al., 2007). Estrogens have been demonstrated to be neuroprotective against a variety of insults in both *in vitro* and *in vivo* models of neurodegenerative diseases. It is believed that the differences in retinal function and structure between the sexes may be governed by differences in sex hormone profiles. The presence of estrogen receptors mRNA (Wickham et al., 2000) and protein (Kobayashi et al., 1998) in various layers of the rat retina (Kobayashi et al., 1998; Kumar et al., 2008) suggests that this hormone plays an important role in maintaining normal retinal function in females (Yamashita et al., 2010; Yamashita et al., 2011). Additionally, the menstrual cycle and accompanying hormonal fluctuations, particularly estrogen, have been observed to potentially modulate several ocular structures, including the retina in humans (Barris et al., 1980; Bassi and Powers, 1986). Preclinical experiments demonstrated that estrogen protects against postischemic tissue damage in rat retina (Nonaka et al., 2000), and glutamate-induced cytotoxicity in the retinal photoreceptor cells (Nixon and Simpkins, 2012) and ganglion cells (Kumar et al., 2005). In a light-induced photoreceptor degeneration rodent model, estrogen reduced rod and cone photoreceptor cell damage functionally and structurally (ARVO Annual Meeting Abstracts, March 2012). Other sex-dependent differences, such as retinal pigment epithelia or neurotransmitters (glutamate and GABA (Blaszczyk et al., 2004)) in the retina might play a role in our observation, but none of them has been compared between retinas of male and female albino rats.

The next intriguing question is how blind animals handle communication and orientation without the use of their major sensory function. In other words, whether or not the blinded animals had altered sensory functions as compensation. To answer these questions, we recorded USVs continuously for 24 h, and the animals with abnormal ERGs appeared to have similar circadian patterns to those with normal ERGs in both 50-kHz and 22-kHz call counts. The data suggest that in these blind rats, the eye may still retain its ability to detect light cues for coordinating circadian rhythms, similar to blind mole-rats (Hetling et al., 2005). However, it was not known if there was compensation in other sensory channels, such as USV or auditory function. According to our 24-h recording, the spontaneous USV call count per 30 min and total count of 50-kHz (Schwarting, 2018) and 22-kHz (Simola, 2015) over 24 h didn't show any significant difference between these animals and other normal-sighted animals as groups. For BAEPs to click stimuli, the sources of waves I, II, III, IV, and V of the potential are the cochlear nerve, cochlear nuclei, superior complex, dorsal and rostral olive extrusion, and lateral lemniscus, respectively (Shaw, 1988; Chen and Chen, 1991). BAEP increase during the postnatal period and are sensitive to brainstem lesions such as tumors, trauma, hemorrhage, ischemia and demyelination (Legatt, 2002). Our results indicate the auditory function in the brainstem level of the animals with abnormal retinal or visual function appears the same as those in the normal-sighted animals. This study is the first to investigate compensatory mechanisms of WH rats with impaired vision. We did not observe compensatory responses in USVs and BAEPs as well as the histology of auditory and visual pathway in these animals. Further studies need to be performed to explore additional systems or functions potentially altered in these animals. The mechanism underlying the retinal functional differences and potential compensation remains to be elucidated in further studies. Transcriptomic analysis might provide more details (e.g., immune response, inflammation, apoptosis, Ca2+ homeostasis or oxidative stress (Kozhevnikova et al., 2013). Other sensory modalities, for example, the olfactory function, which has been found age-related (Kraemer and Apfelbach, 2004), might be worth exploring for possible sensory compensation in blind rats.

In conclusion, our study shows 13%–19% incidence of retinal functional deficits in naive males WH rats at 7–23 weeks of age. Therefore, sex differences should be considered when using Wistar Han rats in toxicity and safety pharmacology studies with regard to data interpretation of retinal functional assessments. In addition, pigmented rats, such as Long-Evans rats with less spontaneous (Heiduschka and Schraermeyer, 2008) or light-induced (Wasowicz et al., 2002) visual impairments, could be considered for stand-alone retinal toxicity tests (Heiduschka and Schraermeyer, 2008; Perlman, 2009; Liu et al., 2015; Shibuya et al., 2015), although it is not a standard toxicity study strain and has less information available for other non-ocular tissues. Pre-screening the male WH rats in the pre-dose phase of the planned toxicity studies with ERG endpoint is also recommended.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Pfizer Institutional Animal Care and Use Committee (IACUC).

Author contributions

C-NL, KW, and MB contributed to conception and design of the study. CT and S-KH collected and analyzed data. BJ performed the ERG data statistical analysis. RS performed eye examination and data analysis. BM performed and interpreted histologic evaluation. C-NL wrote the first draft of the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

All authors were employed by Pfizer at the time of the study.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ftox.2023.1176665/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Representative waveforms of brainstem auditory evoked potentials (BAEPs) in a rat with normal ERG signals (solid trace) and an animal with abnormal ERG waveforms (dotted trace). All four characteristic waves of the rat BAEP (II, III, IV, and V) were comparable between the two groups (Unpaired t-test, $t(16) = 0.2437–0.9041$, all $p > 0.05$).

SUPPLEMENTARY FIGURE 2
Comparison of USV calls between animals with and without normal ERG waveforms. The two groups of animals had similar circadian patterns of 50-kHz **(A)** and 22-kHz **(B)**. Each data point is the total count of 30 min recording. There are no significant differences in the total count of 50-kHz **(C)** or 22-kHz **(D)** USV calls during the 24-hour recording course. ($P > 0.05$, Unpaired t-test, $t(6) = 0.2503$, $p = 0.8107$ for 50 kHz, and $t(6) = 1.691$, $p = 1.6910$ for 22 kHz). Filled bar = light off; open bar = light on.

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Unmet needs and future perspectives in hydroxychloroquine retinopathy

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Retinopathy is a well-recognized toxic effect of hydroxychloroquine treatment. As hydroxychloroquine retinopathy is potentially a vision-threatening condition, early detection is imperative to minimize vision loss due to drug toxicity. However, early detection of hydroxychloroquine retinopathy is still challenging even with modern retinal imaging techniques. No treatment has been established for this condition, except for drug cessation to minimize further damage. In this perspective article, we aimed to summarize the knowledge gaps and unmet needs in current clinical practice and research in hydroxychloroquine retinopathy. The information presented in this article may help guide the future directions of screening practices and research in hydroxychloroquine retinopathy.

KEYWORDS

hydroxychloroquine, perspectives, retinal toxicity, screening, unmet needs, natural history, definition

1. Introduction

Hydroxychloroquine, a widely used drug for the treatment of numerous rheumatologic and dermatologic disorders (e.g., rheumatoid arthritis and systemic lupus erythematosus), may cause a form of retinal toxicity called hydroxychloroquine retinopathy. The pathogenic mechanism of retinal toxicity is poorly understood. Impaired autophagy and defective phagocytosis of photoreceptor outer segments has been suggested as the pathogenic mechanism of chloroquine/hydroxychloroquine toxicity (1, 2), whereas the role of melanin, whether harmful or protective, remains controversial. Hydroxychloroquine retinopathy is reported to be irreversible, progressive, and vision threatening if detected late. Substantial progress has been made in the diagnosis of hydroxychloroquine retinopathy with modern retinal imaging techniques such as spectral-domain optical coherence tomography (OCT) and fundus autofluorescence (FAF) imaging. National guidelines also play a crucial role in retinopathy detection by identifying high-risk patients and providing recommendations on screening modalities and frequency. The guidelines recommend four screening tests: OCT, FAF, automated visual fields, and multifocal electroretinogram (mfERG). The most recent AAO guidelines designated OCT and automated visual fields as primary screening tests (3).

Despite efforts to standardize screening practices and improved knowledge of disease phenotypes and natural disease course, early detection and management of hydroxychloroquine retinopathy remain challenging. This perspective article aimed to highlight the unmet needs in hydroxychloroquine retinopathy, including the consensus definition of retinal toxicity, hydroxychloroquine blood levels and pharmacogenomics, animal models of disease, roles of ophthalmologists, and use of artificial intelligence (AI) in screening.

2. Toward a consensus definition for retinal toxicity

Published studies and clinical guidelines provide substantially different definitions for retinal toxicity and discrepant data on the sensitivity of screening tests. In particular, previous studies have shown disparities in the role of visual field testing (4, 5), leading to divergent recommendations. Elucidating the early natural history of the disease and its manifestations in mainstream diagnostic tests are central in reaching a consensus on the definition of retinal toxicity.

The most recent American Academy of Ophthalmology (AAO) guidelines (2016) specify a broad diagnostic criterion for toxicity: "at least one objective test abnormality confirming a subjective test abnormality." However, the most recent Royal College of Ophthalmologists criteria require two abnormal test results to identify "definite toxicity," which must include at least one objective structural test result but need not include visual field testing if both OCT and FAF imaging provide objective evidence of toxicity. This disparity is based on the role and cost of visual field testing, with recent data suggesting that automated visual field testing may fail to detect scotomas despite structural changes on OCT (4). In contrast, some reported cases showed characteristic ring scotoma on visual field testing with no or subtle changes on OCT (5). As FAF imaging may not detect very early disease, visual field testing remains an important primary test in the 2016 AAO guideline (3, 6).

Because OCT is highly sensitive in detecting characteristic outer retinal changes in the parafoveal or pericentral areas, it has a central role in defining toxicity. According to recent evidence, retinal toxicity can be recognized using OCT alone, through the identification of outer retinal thinning on several OCT systems, as well as typical photoreceptor or retinal pigment epithelial damage on B-scans. Given the rapid image acquisition of OCT without the need for pupil dilation, its acceptability to patients, and its relatively low cost, future definitions of toxicity will likely be mainly based on objective structural data from OCT images. Consequently, establishing an OCT-based consensus definition for toxicity may be an important short-term goal.

The definition of toxicity may be formed on the basis of a few critical points in the disease course (Figure 1): (i) the threshold at which structural (e.g., OCT) abnormalities are detected; (ii) the threshold at which functional deficits are detectable. A consensus on the definition of retinopathy would enable standardized testing protocols, diagnostic criteria and comparisons between study populations. Further, the threshold for management should be carefully defined for patients with hydroxychloroquine retinopathy. Clinicians may allow the continuation of hydroxychloroquine for the primary treatment indication until the agreed threshold is reached, even if retinal toxicity is present on the basis of retinal imaging findings alone. In clinical practice, the drug may be discontinued at a threshold at which disease progression does not occur, provided that functional deficits are early or mild and do not affect daily activities such as driving and reading.

Abbreviations: AAO, American Academy of Ophthalmology; AI, artificial intelligence; FAF, fundus autofluorescence; OCT, optical coherence tomography; QoL, quality of life.

Further discussion is required to reach a consensus on the terms used to describe hydroxychloroquine retinopathy, particularly in early disease. A clearer description of the early natural history of disease, in particular relating to distribution and progression in the context of mainstream diagnostic tests, may help to further refine the classification system.

A few studies have noted discrepancies in retinopathy severity when using different imaging modalities (7). Currently, the degree of photoreceptor damage around the fovea is used for distinguishing between early and moderate stages; however, the appropriate test for classification and whether the degree of damage precisely corresponds to the extent of retinopathy are unclear. The staging of disease severity also depends on the sensitivity of diagnostic tests. Currently, no single test can be universally applied for the precise classification of retinopathy. For example, subtle outer retinal changes may not be identifiable using FAF imaging (8), and distinguishing between early and moderate stages solely based on OCT B-scans may be challenging. Alternatively, the area or extent of outer retinal damage can be used to classify the severity of retinopathy. A consensus should be reached on disease classification based on commonly used modalities to standardize the nomenclature among studies and further characterize the disease, thereby allowing a more direct comparison of study results.

3. Hydroxychloroquine levels in blood

Serum hydroxychloroquine levels have been evaluated with respect to adverse drug effects. A previous study measured blood hydroxychloroquine levels after a loading phase with variable doses in three groups of patients with rheumatoid arthritis (9). The results revealed a correlation between serum hydroxychloroquine levels and gastrointestinal adverse events (9).

Although measurement of blood hydroxychloroquine levels is relevant in determining clinical efficacy, its role in ascertaining the chronic toxic effects of drugs is unclear. A single high measurement of serum hydroxychloroquine level may not necessarily reflect steady-state pharmacokinetics; therefore, a series of measurements prospectively performed over many years is required to evaluate the utility of blood hydroxychloroquine levels as a risk factor for hydroxychloroquine retinopathy. However, in patients undergoing extremely high-dose hydroxychloroquine adjuvant chemotherapy over shorter durations, often as part of clinical trials (10–15), measurement of blood hydroxychloroquine levels may be valuable in the short term for evaluating whether a particular measurement may predict hydroxychloroquine retinopathy development.

Two recent studies on the relationship between serum hydroxychloroquine level and retinopathy development reported conflicting results (16, 17). In a case–control study involving 23 patients with confirmed hydroxychloroquine retinopathy and 547 controls, blood hydroxychloroquine levels were not significant predictors in univariate analysis (16). However, in another study that identified 23 (of 537) patients with confirmed retinopathy, serum hydroxychloroquine levels (mean or maximum) predicted later retinopathy (17). Further data analysis revealed that patients in the lowest tertile of time-adjusted serum hydroxychloroquine levels (0–739 ng/mL) had a 1.2% risk of retinopathy, those in the middle tertile (740–1,180 ng/mL) had a 3.1% risk, and those in the highest tertile (1,181–3,466 ng/mL) had an 8.5% risk, and the differences were statistically significant for the trend. Logistic regression

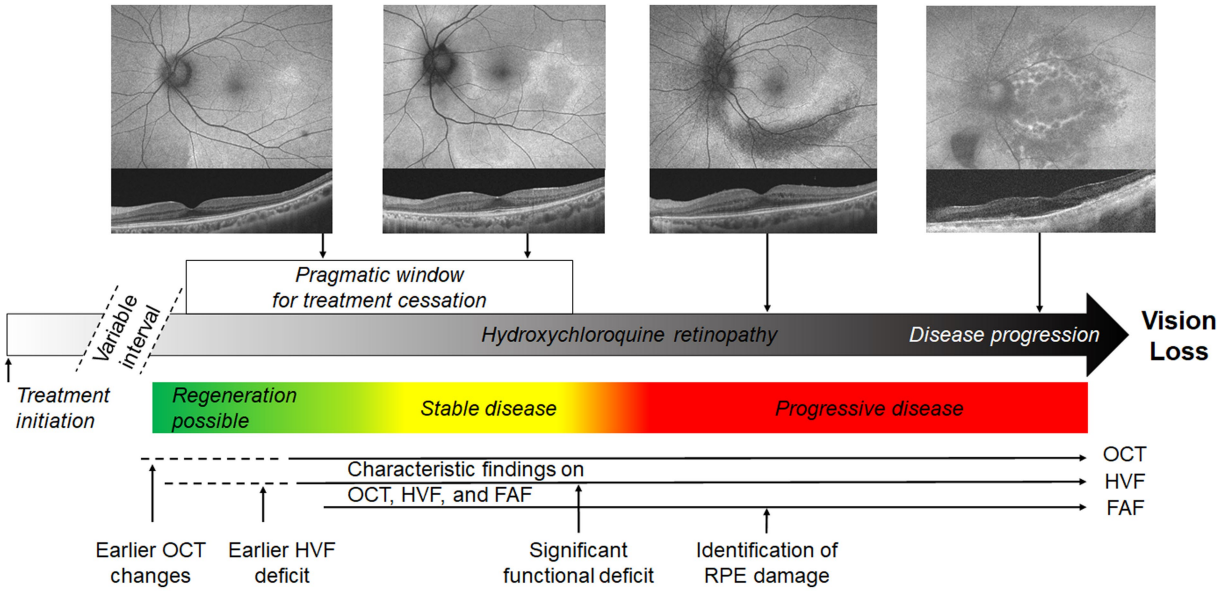

FIGURE 1
Schematic representation of possible definitions of toxicity in hydroxychloroquine retinopathy. The pragmatic interval of treatment cessation is indicated by the white box, defined as the interval between sufficient evidence of retinopathy to the point at which a significant functional deficit occurs. This limit is not specified and requires a consensus. Colored boxes represent the most likely disease behavior after treatment cessation, although regeneration is not invariable in early disease. The approximate intervals at which disease is detectable using mainstream screening tests is indicated. FAF, fundus autofluorescence; OCT, optical coherence tomography; HVF, automated visual fields.

analysis identified that the relationship between serum hydroxychloroquine levels and retinopathy remained significant after adjustment for therapy duration (18). Although the level was reported to be useful for prediction of retinopathy development, its clinical utility for prediction of future progression after drug cessation has not been validated. As the retinopathy is known to progress in advanced stages even after drug cessation, its predictive role in eyes with advanced disease stages may be limited and should be investigated further in future studies.

There have been a few reports on the rapid-onset retinal toxicity of hydroxychloroquine, in which cases occurred within 3 years of use (19, 20). Ozawa et al. reported abnormally high blood levels of hydroxychloroquine in one patient (20). The result suggests that hydroxychloroquine blood level may be useful for understanding and predicting rapid-onset disease.

Considering the established risk factors for retinal toxicity, further studies are required to determine the significance of serum hydroxychloroquine levels in definition or prediction of the risk of retinopathy. Furthermore, the relationship between the daily dose and blood levels of hydroxychloroquine should be validated. If this relationship is proven, serum hydroxychloroquine levels may be used to adjust the daily doses to achieve a balance between efficacy in treating the primary disease and the risk of retinal toxicity. This may help individualize hydroxychloroquine treatment to maximize the therapeutic effects while minimizing the toxicity risk.

4. Pharmacogenomics

Patients with identical exposure to hydroxychloroquine may have differing susceptibility to retinopathy. Some patients may develop retinopathy even with low-dose hydroxychloroquine use (5) or at a much faster rate than anticipated (21). This disparity in susceptibility may be explained by disease modifiers, including genetic or environmental factors. Pharmacogenomics investigates how the genetic factors affects a person's response to drugs and even drug-related side effects. The identification of risk alleles for retinal toxicity may be useful in further reducing the risk of toxicity and the cost of screening by seeking alternative medications for high-risk patients.

An initial report suggested that certain *ABCA4* missense variants associated with Stargardt disease may predispose patients to hydroxychloroquine-or chloroquine-induced retinal toxicity (22), although a further study demonstrated a protective effect (23). In another study, 99 patients with >5 years of hydroxychloroquine exposure underwent genetic testing of 960,919 single nucleotide polymorphisms, and 13 common macular dystrophy genes were sequenced in a separate cohort of 44 cases and 53 controls (24). Furthermore, whole-exome sequencing was performed in 16 cases and 17 controls for all genes associated with retinal dystrophy, chloroquine pathway metabolism, and autophagy. In this large series, genetic tests did not reveal an association with hydroxychloroquine retinopathy.

Large collaborative studies using an unbiased approach, such as a genome-wide association study, may be required to detect genetic traits that confer an increased risk. The success of such studies depends on the allele frequency of unknown genetic variants that influence toxicity, the strength of their effect, and the sample size. Even large collaborative studies may fail to identify a genetic locus if the effect size is small. Studies will likely require age-matched controls treated with hydroxychloroquine but without toxicity as confirmed by rigorous screening procedures, as well as age-matched controls without hydroxychloroquine exposure.

If a genetic locus that confers risk of hydroxychloroquine-induced retinal toxicity is identified, the relative influence of this predictive genetic locus should be evaluated with respect to other known risk factors for retinopathy (e.g., drug use duration and tamoxifen use). A further promise of pharmacogenomic investigation is improved understanding of the pathophysiology of hydroxychloroquine retinopathy. Identification of candidate loci is likely to motivate a variety of functional studies to further delineate the influence of such loci on drug pharmacokinetics or on the local effect of hydroxychloroquine on retinal pigment epithelial or photoreceptor cells.

5. Development of animal or cellular models

The development of specific therapies for hydroxychloroquine retinopathy, to prevent disease formation or protect against further degeneration, has been hampered by the lack of validated disease models. Rodents lack an anatomical macula, which is the classic site of retinopathy. An experiment with albino rats identified a dose-dependent decrease in B-wave amplitude on full-field electroretinography after chloroquine exposure (25). In the 1970s, rhesus monkeys were intramuscularly injected with chloroquine for 4.5 years without causing fundus, retinal angiographic, or electrophysiological abnormalities. The use of OCT and FAF imaging may enable a more sensitive and earlier detection of the disease, considerably shortening the observation period of such studies (26). However, primate studies have not been repeated, perhaps because the perceived rarity of retinopathy does not justify the resources required for these investigations. The existence of validated disease models would enable investigation into the role of potential therapies in stopping or slowing down disease progression. The increasing use of hydroxychloroquine, emerging prevalence data, and ability to detect retinopathy at earlier stages may together lead to renewed interest in establishing disease models to better characterize the pathogenesis of hydroxychloroquine retinopathy and to develop new therapies.

In vitro or cell-based models (ARPE19) may be useful for determining the effect of hydroxychloroquine exposure on cellular omics (gene expression) in the short term (27). These models may help elucidate the pathways to toxicity. Single-cell expression assays (e.g., RNA-Seq) can enhance the understanding of cellular responses to drug exposure. However, cell culture techniques cannot be used to model clinically relevant chronic exposure to hydroxychloroquine, or the interdepedence of the retina and RPE which may be relevant in this disease.

6. Changing roles of ophthalmologists

The role of ophthalmologists in the management of hydroxychloroquine retinopathy have not been addressed extensively in the literature. Understanding the role of ophthalmologists in hydroxychloroquine retinopathy requires considering the unusual nature of the disease: (i) hydroxychloroquine retinopathy generally occurs many years after therapy initiation (sometimes after >20 years), (ii) ophthalmologists do not prescribe hydroxychloroquine, and (iii) ophthalmologists are not involved in using hydroxychloroquine for treating the primary disease. This scenario helps explain the responsibilities of health-care professionals involved from therapy initiation to drug cessation and those of ophthalmologists, including establishing links with relevant physicians and departments, establishing screening services, training colleagues in data interpretation, improving patient education, and auditing service outcomes.

As hydroxychloroquine can be initiated for various clinical indications, it may be prescribed by several different specialists. Ophthalmologists should provide further information to patients about hydroxychloroquine retinopathy at their first involvement with screening services, including the nature and timing of screening tests.

Ophthalmologists can ensure safe dosing according to the patient's body weight to reduce the risk of toxicity, and this may require providing recommendations to the prescribing physician. Recommendations on potentially revising the hydroxychloroquine dose after any substantial weight loss may also be helpful. Ophthalmologists will determine the timing of annual screening visits for the evaluation of dosing, renal function, and concurrent tamoxifen use, and this should be communicated to prescribing physicians along with the baseline ophthalmological findings.

The results of screening tests should be communicated to the patient and prescribing physician. If definite retinopathy exists, a recommendation to stop treatment can be made to the prescribing physician, who can subsequently discuss treatment options with the patient. To facilitate this discussion, a description of disease severity (early, moderate, or severe) is helpful. Patients with early retinopathy but with severe systemic disease may elect to continue hydroxychloroquine because the benefits of systemic treatment are immediate (and perhaps more profound) and the toxic effects on the retina are slow. Patients should be actively involved in the decision to stop hydroxychloroquine therapy as guided by clear information from the ophthalmologist and prescribing physician. Considering the functional effects of retinopathy (e.g., on the ability to drive or work) may be helpful. Clear communication between ophthalmologists and patients may also minimize anxiety in at-risk patients.

The ophthalmologist's role extends to understanding the organizations and individuals responsible for referrals and referral pathways including rheumatologists, dermatologists, and other specialist services. Rheumatologists, dermatologists should be able to access screening services, track the use of screening services in patients at risk under their care, and have access to screening outcomes. Ophthalmologists are responsible for ensuring rigorous screening procedures, including image quality, controlled reporting, and auditing outcomes - as have been established screening for diabetic retinopathy.

On the diagnosis of hydroxychloroquine retinopathy, the ophthalmologist should provide the necessary support depending on each patient's retinopathy stage, social circumstances, visual function, emotional distress, and ocular and systemic comorbidities. Low-vision services may be required for patients with substantial visual impairment, and registration of visual impairment may be necessary for those with advanced visual field loss. Although further visits would not change the clinical course after drug cessation, they may be necessary for patients who are particularly concerned about disease progression and those with moderate-to-advanced disease. Patients at risk of retinopathy should not be discharged if they fail to attend an

appointment, and a fail-safe mechanism is required to ensure that at-risk patients are screened.

7. Role of AI in retinopathy screening

Artificial intelligence (AI) analysis of digital retinal images is a rapid and noninvasive method of identifying and characterizing the pathological features of macular and retinal diseases (28). In particular, deep learning algorithm using convolutional neural networks can be developed to extract generalized features from digital images. By using training datasets, these tools enable the recognition of pathology through supervised and unsupervised methods. However, unsupervised techniques may yield novel subclinical imaging biomarkers of disease because the methods are not biased by assumptions (29). The use of AI is particularly suited to screening in which early disease manifestations may be subtle and easily missed by human observers. Convolutional neural networks have been trained to perform comparably to human graders of diabetic retinopathy images (30). OCT interpretation of images has been demonstrated in macular disorders such as age-related macular degeneration and diabetic macular edema (28).

Although only one study has evaluated the utility of AI in hydroxychloroquine retinopathy detection, it has great potential to increase the sensitivity of early retinopathy detection (31). The earliest known OCT finding in hydroxychloroquine retinopathy was localized outer nuclear layer thinning before the development of qualitative changes in the outer retinal layers, more easily detectable by human observers. The subjectivity of OCT image interpretation may explain the conflicting data on the natural history of hydroxychloroquine retinopathy, with some studies indicating that visual field changes may precede OCT abnormalities (5) and others reporting the opposite finding (4). Recent clinical studies have shown that OCT maps of retinal thickness may help human observers detect localized retinal thinning in patients relative to age-matched controls (32). Automated OCT segmentation tools and software may facilitate this process. However, AI may detect the disease at an earlier stage using as yet unknown retinal/OCT biomarkers or through integrated observations of large populations. Accordingly, AI may play a crucial role in further delineating the natural history of early hydroxychloroquine retinopathy based on objective structural outcomes.

AI may facilitate the identification of objective thresholds before which retinopathy is not progressing, permitting patients to continue benefiting from hydroxychloroquine therapy until this threshold is reached. This represents a clinically meaningful, evidence-based, patient-centered endpoint. The use of AI will also minimize inappropriate treatment cessation, which is one of the main risks of screening. Furthermore, patients with very early retinopathy may continue to benefit from hydroxychloroquine therapy if their retinal function is not yet threatened. Moreover, if AI can be harnessed to detect the earliest stage of disease, reclassification of retinopathy stages (normal, preclinical retinopathy [beyond human detection], preperimetric retinopathy [structural deficit but no functional deficit], and retinopathy with functional deficit) would be necessary. Further, the cost of hydroxychloroquine retinopathy screening can be substantially reduced by personalizing the intervals between screening visits, thereby reducing the overall number of screening episodes for a given population of at-risk patients.

The major barrier to the development of AI tools for detecting hydroxychloroquine retinopathy is the number of OCT images required to train an algorithm. Moreover, the clinical manifestations of hydroxychloroquine retinopathy seem to be partially dependent on ethnicity. A previous study with a multiethnic cohort of patients with diabetic retinopathy showed that AI required 100,000 images for training (33). However, for hydroxychloroquine retinopathy, acquiring a dataset of this size would require multinational collaboration and curation.

8. Conclusion

No consensus has been reached on the definition and classification of early hydroxychloroquine retinopathy. As early detection of retinopathy remains challenging, personalized screening according to the retinopathy risk based on hydroxychloroquine blood levels or pharmacogenomics could help to further refine screening by identifying patients at greater risk. The roles of ophthalmologists are changing, and better communication with prescribing physicians and patients are important for appropriate management and regular monitoring. Finally, advances in AI and AI-assisted screening programs for retinopathy should be integrated into health-care systems, which require future research.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

IY and SA: conception, design, and data collection. IY, PC, and SA: analysis, interpretation, obtain funding, overall responsibility, and approve the submitted version. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Ocular surface complications following biological therapy for cancer

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Novel and highly effective biological agents developed to treat cancer over the past two decades have also been linked to multiple adverse outcomes, including unanticipated consequences for the cornea. This review provides an overview of adverse corneal complications of biological agents currently in use for the treatment of cancer. Epidermal growth factor receptor inhibitors and immune checkpoint inhibitors are the two classes of biological agents most frequently associated with corneal adverse events. Dry eye, Stevens-Johnson syndrome, and corneal transplant rejection have all been reported following the use of immune checkpoint inhibitors. The management of these adverse events requires close collaboration between ophthalmologists, dermatologists, and oncologists. This review focuses in depth on the epidemiology, pathophysiology, and management of ocular surface complications of biological therapies against cancer.

KEYWORDS

biological therapy, cancer, cornea, immunotherapy, ocular surface, targeted therapy

Introduction

The emergence of biologicals as antineoplastic therapies began in the 1990s. Such agents inhibit the growth and survival of cancer cells, but can also induce severe side effects that affect multiple body systems. The various cell types present in the cornea each have distinct receptor expression profiles that makes the cornea susceptible to adverse outcomes during use of biological agents. This review will summarize the corneal complications of biological agents used in oncology and discuss the pathogenesis and clinical management of these adverse events.

Tyrosine kinase inhibitors

Tyrosine kinases regulate cell proliferation and apoptosis by transducing intracellular signaling cascades. Their inhibitors, known as tyrosine kinase inhibitors (TKi), include agents that can suppress uncontrolled cell proliferation in various types of cancer. As the use of TKi to treat cancer has increased in recent years, awareness of ocular side effects from TKi

has also increased. Among all TKi in clinical oncology practice, epidermal growth factor receptor inhibitors (EGFRi) have been most commonly reported to be associated with keratitis (Saint-Jean et al., 2018).

Epidermal growth factor receptors (EGFRs) are highly expressed on the ocular surface and periocular tissues, and adverse effects of EGFR inhibition on the cornea should not be surprising. Breakdown of the corneal epithelial barrier is often an initial harbinger of keratitis. Reduced epithelial cell proliferation in the cornea during EGFRi treatment results in loss of epithelial regeneration, impaired healing from environmental exposures such as dryness and exposure to particulate matter, and ultimately leads to corneal inflammation. Inhibition of the EGFR cascade also disrupts hair follicle growth cycle, resulting in trichomegaly which can add insult to the cornea due to trichiasis. Suppression of EGFR also inhibits the proliferation and repair of the meibomian glands. When meibum secretion is diminished, the tear film evaporates more rapidly, further compromising corneal epithelial repair (Ho et al., 2013; Huillard et al., 2014).

One such EGFRi, cetuximab, has been strongly associated with induction of keratitis (Table 1). Cetuximab is now approved to treat metastatic colorectal cancer and head and neck squamous cell carcinoma. Trichomegaly, conjunctivitis, and blepharitis are the most common ocular side effects reported as associated with cetuximab (Fraunfelder and Fraunfelder, 2012). According to post-marketing surveillance in Japan, the incidence of ocular adverse events linked with cetuximab was approximately 2.6%, and the severity of most adverse events was less than grade 2 (Ishiguro et al., 2012; National Institutes of Health, 2022). However, in select case reports, cetuximab was associated with severe keratitis (Specenier et al., 2007).

Afatinib is another EGFRi that is now used as a first-line treatment for non-small cell lung cancer. Common side effects associated with afatinib include dry eye and ulcerative keratitis (McKelvie et al., 2019). In the LUX-lung 3 trial for metastatic lung adenocarcinoma with *EGFR* mutations (Sequist et al., 2013), the prevalence of keratitis in these patients was 2.2%. Notably, approximately 0.4% of patients had grade 3 keratitis, leading to the discontinuation of the therapy (Yang et al., 2015). Cases of trichomegaly and keratitis have also been reported with other EGFRi, for example, erlotinib and gefitinib, as well as other TKi (Zhou et al., 2016; Rawluk and Waller, 2018).

Artificial tears and lubricating ointments are frequently used to protect and rehydrate an injured corneal epithelium. Additionally, topical corticosteroids can be applied to block inflammation, which may confer rapid relief of pain (Huillard et al., 2014). In large epithelial defects, bandage contact lenses may be prescribed to protect the cornea and alleviate pain. However, if TKi-associated keratitis proves unresponsive to these measures, it may be necessary to discontinue the EGFRi (Johnson et al., 2009). Treatment of such cases with EGF-containing eyedrops is a unique approach still not validated in a human clinical trial. However, Kawakami et al. reported dramatic improvement associated with starting topical human recombinant EGF in a patient with severe filamentous keratitis after beginning cetuximab treatment for colorectal cancer. The keratitis cleared just 3 weeks after starting topical recombinant EGF, despite continuation of the cetuximab (Kawakami et al., 2011). EGFR inhibitors have also been found to induce skin toxicity through upregulating keratinocyte cytokine release (CCL2, CCL5, CCL27, and CXCL14) that leads to chemokine-driven skin inflammation, which may deter patients from taking the medication (Lichtenberger et al., 2013). Nonetheless, skin toxicity can also be an important predictor of drug response, making it difficult for clinicians to decide whether to discontinue treatment due to cutaneous and/or ophthalmological side effects, which requires collaboration between medical specialties.

Immune checkpoint inhibitors

Immune checkpoints occur when costimulatory T cell receptors bind to "checkpoint" proteins on the surface of tumors that results in sending an "off" signal to the T cells, thus reducing host immune responses to the cancer. Immune checkpoint inhibitors (ICI) are agents that block this process, thus rendering tumors susceptible to host immune attack. The development of ICI has greatly benefitted the progression-free survival and in select instances, the rate of cure for patients with what were previously difficult or frankly untreatable malignancies, often including metastatic disease (Goleva et al., 2021). The primary checkpoint proteins targeted in this pathway are cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and programmed cell death protein 1(PD-1), along with the PD-1 binding partner, programmed death ligand 1 (PD-L1). However, cutaneous, neurological, cardiac, and ocular adverse events, the latter including ocular myasthenia, uveitis, and dry eye, have been associated with ICI therapy since their introduction as treatment for multiple types of cancer (Vanhonsebrouck et al., 2020; Huang et al., 2021; Park et al., 2021; Chiang et al., 2022a; Chiang et al., 2022b; Kao et al., 2022; Lee et al., 2022).

Dry eye affects between 1% and 24% of patients on ICI. The mechanism for dry eye in persons on ICI therapy, as proposed by Hiro et al., is thought to be loss of self-tolerance and induction of autoimmunity, resulting in primary lacrimal dysfunction and clinical sicca syndrome (Hori et al., 2020). A similar mechanism has been proposed for the cornea with disruption of immune privilege, and subsequent T cell infiltration at the ocular surface. Among U.S. Food and Drug Administration (FDA)-approved ICI, nivolumab and pembrolizumab had the highest incidence of ocular adverse effects, followed by atezolizumab and ipilimumab (Fang et al., 2019; Hori et al., 2020) (Table 1).

During the phase II trial of nivolumab in subjects with ipilimumab-refractory melanoma, three patients (3%) treated with 3 mg/kg nivolumab suffered either grade 1 or 2 dry eye (Weber et al., 2016). In the KEYNOTE-010 clinical trial, which compared pembrolizumab to docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer, out of 1,034 study subjects, ten (1.5%) treated with pembrolizumab experienced grade 1, 2 dry eye, while only one person treated with docetaxel reported dry eye (Herbst et al., 2016). While most documented dry eye occurrences are grade 1 or 2, there have been reports of more severe dry eye necessitating withdrawal of the ICI. A 58-year-old man with metastatic melanoma developed bilateral superficial punctate keratitis after receiving six courses of nivolumab treatment. Despite punctal plugs to increase the tear film, and

TABLE 1 Biological anti-cancer agents–their indications and reported ocular adverse effects.

Drug	Indications	Corneal adverse event
Cetuximab	Colorectal cancer, squamous cell carcinoma of the head and neck	Conjunctivitis
Afatinib	Non-small cell lung cancer	Ulcerative keratitis
Erlotinib	Non-small cell lung cancer, pancreatic cancer	Trichomegaly, keratitis
Gefitinib	Non-small cell lung cancer	Keratitis
Nivolumab	Melanoma	Dry eye, keratitis
Pembrolizumab	Non-small cell lung cancer	Dry eye

use of topical cyclosporin, one cornea perforated. Three weeks following the withdrawal of nivolumab, and concurrent with institution of topical loteprednol (0.5%), and topical autologous serum, the perforation healed (Nguyen et al., 2016).

Treatment with ICI has also been associated with Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), which can result in severe cicatrizing keratoconjunctivitis leading to blindness. ICI-related SJS/TEN was reported in a case series involving eight individuals with SJS and an ALDEN score greater than four. Five patients exhibited ocular involvement, and three individuals exhibited grade 3 ocular involvement. Of the three patients with severe ocular involvement, two were being treated with pembrolizumab, and one with atezolizumab. In this series, patients with nivolumab-associated SJS/TEN exhibited little to mild ocular involvement (Ma et al., 2021).

ICI has also been hypothesized to be associated with corneal transplant rejection. An 85-year-old asymptomatic woman with a history of bilateral penetrating keratoplasty presented with bilateral diffuse keratic precipitates and subepithelial infiltrates 3 months after starting immunotherapy with pembrolizumab for a metastatic urothelial cell carcinoma. The corneal transplant rejection was treated with topical dexamethasone drops, but relapsed 2 weeks after the drops were discontinued. After consulting with an oncologist, pembrolizumab was discontinued (Vanhonsebrouck et al., 2020).

Conclusion

As the clinical use of biological anti-cancer agents expands, the frequency of associated side effects is also expected to increase. This article briefly overviews corneal adverse effects associated with biological agents, particularly EGFRi and ICI. More research is needed to pinpoint the molecular basis for these adverse events. Ophthalmologists and other medical professionals should be aware of corneal adverse events in patients receiving biological agents for cancer. In order to prevent sight-threatening complications, the management of corneal adverse events requires close coordination between oncologists, ophthalmologists, and dermatologists so that the important benefits of anti-cancer therapies are balanced against the potential loss of vision in the small but significant number of treated patients who develop keratitis.

Author contributions

KS-KM wrote the first draft of the manuscript. P-FT and TY-JH contributed to and edited the draft. JC conceived the topic, edited the manuscript, and finalized the paper for submission. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Ocular surface disease: a known yet overlooked side effect of topical glaucoma therapy

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Ocular surface disease (OSD), a disorder affecting the lacrimal and meibomian glands and the corneal and conjunctival epithelium, is a well-known complication of topical glaucoma therapy. OSD can present as a new or pre-existing condition that virtually any anti-glaucoma formulation can exacerbate. As such, both glaucoma and OSD frequently coexist. Typical OSD symptoms include ocular discomfort, redness, burning, and dryness, whereas signs include periorbital and eyelid skin pigmentation, conjunctival scarring, and superficial punctate keratitis. Pressure-lowering eyedrops can cause toxic, allergic, and inflammatory reactions on the ocular surface. The latter can result from either preservatives or direct toxicity from the active molecule. Although usually mild, OSD can cause significant symptoms that lead to poor quality of life, decreased compliance to therapy, glaucoma progression, and worse visual outcomes. Given the chronic nature of glaucoma, lack of curative therapy, and subsequent lifelong treatment, addressing OSD is necessary. This manuscript aims to provide an up-to-date overview of OSD's signs, symptoms, and pathogenic mechanisms from glaucoma therapy toxicity.

KEYWORDS

alpha-adrenergic agonists, beta blockers, carbonic anhydrase inhibitors, dry eye disease, nitric oxide-donating prostaglandin analogs, ocular surface disease, prostaglandin analogs, rho-kinase inhibitors

1 Introduction

Glaucoma is a group of diseases leading to progressive optic neuropathy, characterized by visual field and optic nerve head changes (Aguayo Bonniard et al., 2016). It is the primary cause of irreversible blindness worldwide, with a prevalence ranging from 2.0% in Europeans to 7.3% among individuals of African descent (Sun et al., 2022). Unfortunately, the lack of a cure renders glaucoma therapy lifelong (Aguayo Bonniard et al., 2016). Moreover, disease progression requiring more than one anti-glaucomatous agent occurs in approximately 40% of glaucoma patients. The latter results in chronic exposure and toxicity to the active molecules and associated preservatives (Anwar et al., 2013).

Ocular surface disease (OSD) represents a spectrum of diseases, including conjunctivitis, lid disease, allergic manifestations, superficial punctate keratitis, and dry eye disease (DED)

TABLE 1 Mechanisms of action of pressure-lowering medications.

Drug class	Mechanisms of action	Examples	References
Increased uveoscleral and trabecular meshwork outflow			
PGAs	- Causes relaxation of the ciliary muscle by binding to the FP and EP-1, -2, -3, and -4 receptors causes relaxation of the ciliary muscle	Latanoprost, bimatoprost, unoprostone, travoprost, and tafluprost	Nilsson et al. (2006); Alm and Nilsson. (2009); Agarwal and Agarwal. (2018); Karli et al. (2018)
	- Degrades the ECM by activating MMPs-1, -2, -3, and -9 in ciliary body smooth muscle cells		
Decrease aqueous humor production			
Beta blockers	- Antagonize the effects of catecholamines on β2-adrenoreceptors in the ciliary epithelium	*Cardioselective*: betaxolol	Nyborg and Nielsen. (1995); Kiland et al. (2004); Servat and Bernardino. (2011)
	- Vasoconstriction of ciliary arteries	*Non-selective*: timolol, carteolol, levubonolol, and metipranolol	
CAIs	- Inhibit carbonic anhydrase isoenzymes present in the ciliary processes	Dorzolamide and brinzolamide	Sugrue et al. (1990); Mincione et al. (2007); Shahidullah et al. (2009)
	- Inhibition of HCO_3^- and CO_2 interconversion		
Alpha agonists	- Activation of α1- and α2-adrenoreceptors inhibits adenylate cyclase, causing a decrease in cAMP.	Apraclonidine and brimonidine	Bausher and Horio, (1995); Servat and Bernardino, (2011)
Increased trabecular meshwork outflow			
Cholinergics	- Acts on iris muscles' muscarinic receptors, causing pupillary and ciliary muscle contraction, decreasing resistance to aqueous humor outflow	Pilocarpine, bethanecol, and carbachol	Kam and Sullivan, (2011); Zhang et al. (2017)
Mixed mechanisms			
ROCK inhibitors	- Inhibits ROCK and NE transporters in the trabecular pathway, which reduces aqueous humor production, increases trabecular outflow, and decreases EVP.	Netarsudil, fasudil, and ripasudil	Serle et al. (2018); Batra et al. (2021)
NO-donating PGA	- LBN is metabolized into butanediol mononitrate, a NO-donating moiety, and latanoprost acid (increases uveoscleral outflow)	Latanoprostene bunod	Lo et al. (2022)
	- NO increases TM outflow via TM and Schlemm's canal relaxation		

PGAs, prostaglandin analogs; FP, F prostanoid; EP, E prostanoid; ECM, extracellular matrix; MMPs, matrix metalloproteinases; CAIs, carbonic anhydrase inhibitors; HCO_3^-, bicarbonate; CO_2, carbon dioxide; cAMP, cyclic adenosine monophosphate; ROCK, rho-kinase; NE, norepinephrine; EVP, episcleral venous pressure; NO, nitric oxide; LBN, latanoprostene bunod; TM, trabecular meshwork.

(Fechtner et al., 2010; Saade et al., 2015; Gomes et al., 2017). Both glaucoma and OSD are prevalent in the elderly and frequently coexist in the same patient (Fechtner et al., 2010). Up to 66% of patients with severe OSD have glaucoma (Fechtner et al., 2010), whereas the prevalence of OSD in patients using topical anti-glaucoma agents is as high as 59% (Zhang et al., 2019). Patients with prior DED, those exposed to preserved pressure-lowering medications (PLMs), and those requiring ≥1 agent (Saade et al., 2015), are at an increased risk of experiencing worse OSD symptoms. Furthermore, dry eye symptoms can result in increased patient depression, anxiety, and poor quality of life (QoL), which, in turn, is associated with poor compliance to glaucoma therapy and an increased risk of glaucoma progression (Stringham et al., 2018; Tirpack et al., 2019; Rodriguez-Garcia et al., 2022). Thus, addressing OSD in patients with glaucoma is necessary.

This review discusses the pathogenic mechanisms and diagnosis of ocular surface toxicity induced by anti-glaucoma agents, with emphasis on the newer drugs: the rho-kinase (ROCK) inhibitors and nitric oxide (NO)-donating prostaglandin analogs (PGAs).

2 Mechanism of action of pressure-lowering medications

The mechanism of action of PLMs can be divided into those decreasing the aqueous humor production and those increasing the trabecular meshwork or uveoscleral outflow. Regarding the latter, PGAs are the first-line PLM for managing ocular hypertension (OHT) and glaucoma. They exert their effects by binding to E prostanoid and F prostanoid receptors, leading to ciliary muscle relaxation and increased aqueous humor outflow through the uveoscleral pathway (Yamagishi-Kimura et al., 2022). PGAs also induce the expression of matrix metalloproteinases (MMPs) that disrupt the extracellular matrix (ECM), leading to increased trabecular meshwork outflow. Hence, PGAs lower intraocular

pressure (IOP) by increasing flow through both pathways (Heo et al., 2020).

Table 1 describes the mechanism of action of each PLM class. However, in the following subsections, we will discuss the mechanism of action of the newer classes of PLMs: the ROCK inhibitors and the NO-donating PGAs.

2.1 Rho-kinase (ROCK) inhibitors

Rho-associated coiled-coil-containing protein kinase (ROCK) is the most studied downstream effector of RhoA, a guanosine triphosphate (GTP)-ase member of the Rho subfamily of the Ras protein family. Rho is activated in the GTP-binding state. The process is aided by bioactive receptors like endothelin-1 and transforming growth factor (TGF)-β, which activates GTPase activating proteins (GAP) and guanine nucleotide exchange factors (GEFs), leading to Rho activation through GTP-binding (Berrino and Supuran, 2019).

ROCKs exhibit two isoforms (ROCK-I and ROCK-II) expressed in many body organs, with varying extents of the subtype involved in each organ and cell tissue. Within the eye, ROCKs are expressed in the trabecular meshwork. In a calcium-independent manner, ROCKs contract the trabecular meshwork by phosphorylating LIM kinases and myosin light chain (MLC) phosphatase. This creates resistance to aqueous humor outflow in the trabecular meshwork (Berrino and Supuran, 2019; Bhargava et al., 2022). ROCK inhibitors decrease actin fiber density in the trabecular meshwork and increase endothelial cell permeability in Schlemm's canal, ultimately counteracting this outflow resistance (Moura-Coelho et al., 2019).

Netarsudil, a specific ROCK inhibitor, has an amplified impact as it is also a norepinephrine transporter (NET) inhibitor that further decreases aqueous humor production and venous pressure in episcleral vessels (Bhargava et al., 2022).

2.2 Nitric oxide (NO)-donating prostaglandin analogs (PGAs)

Under physiologic conditions, NO is expressed by trabecular meshwork cells, Schlemm's canal, and the ciliary body. NO reduces IOP by increasing aqueous humor outflow from the trabecular meshwork by activating the soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) pathway and by decreasing aqueous humor production through ion channel modulation. cGMP regulates the action of various downstream effectors, including protein kinase G (PKG), that relaxes vascular smooth muscle (Mao et al., 2020). Via the NO/sGC/cGMP pathway, PKG activates MLC phosphatase, which, in turn, dephosphorylates MLC, leading to the relaxation of trabecular meshwork and Schlemm's canal cells. The hindmost decreases aqueous humor outflow resistance (Gao et al., 2017). In addition, inducible NO synthase can be activated in trabecular meshwork cells when anterior chamber perfusion pressure becomes elevated (Wu and Ma, 2012).

Latanoprostene bunod is a NO-donating prostaglandin F2α analog recently approved by the FDA (2017) for managing glaucoma (Krauss et al., 2011). When topically administered, the compound is split into the conventional drug and NO, potentiating the IOP-lowering effect (Krauss et al., 2011; Mao et al., 2020). Thus, NO-donating PGAs offer two mechanisms of action. The NO component promotes outflow through the conventional pathway, and the prostaglandin component facilitates flow through the uveoscleral pathway space (Cavet et al., 2015).

3 General mechanisms of ocular surface toxicity caused by pressure-lowering medications

The lacrimal functional unit (LFU) is comprised of the eyelids, meibomian glands (MGs), the main and accessory lacrimal glands, the lacrimal drainage system, the ocular surface (cornea and conjunctiva), and the intertwined innervation (Baudouin et al., 2013). A healthy ocular surface relies on the LFU, which is responsible for the adequate production, distribution, and clearance of the tear film. The latter, in turn, preserves homeostasis of the ocular surface epithelium and protects it from physical damage and exposure (Kawakita, 2018). Dysfunction of one or more components of the LFU 1) hinders the composition of tears, and thus its ability to protect the surface epithelium; 2) disrupts the innate and adaptive immune and inflammatory pathways that protect the ocular surface from external stimuli (i.e., exposure, infection); and 3) stimulate the production of proinflammatory cytokines [i.e., interferon (IFN)-γ, interleukin (IL)-1, −6, −8, tumor necrosis factor (TNF)-α, intercellular adhesion molecule (ICAM)-1, among others] by the ocular surface immune and epithelial cells (Roy et al., 2022).

In this regard, PLMs for the management of OHT and glaucoma have been shown to cause damage to the LFU through a myriad of mechanisms, including a decrease in conjunctival goblet cell (GC) density, squamous metaplasia (Baudouin et al., 2008), conjunctival human leukocyte antigen (HLA)-DR overexpression (Baudouin et al., 2008), and disruption of the degrading-remodeling effect between MMPs and tissue inhibitors of metalloproteinases (TIMPs) in the ECM compounds, including collagen fibers (Karli et al., 2018). Moreover, the need for fixed combinations, commonly required by patients exhibiting disease progression and lifelong treatment, as well as the vehicles and preservatives contained in drug formulations [i.e., benzalkonium chloride (BAK)], will result in a significant number of patients experiencing ocular surface damage (Tiedemann et al., 2019; Rodriguez-Garcia et al., 2022). Additionally, an impression cytology study reported significant overexpression of C-C chemokine receptors (CCR)-type 4 and 5 in the conjunctival epithelium of glaucoma subjects chronically treated with PLMs compared with controls (Baudouin et al., 2008). CCR4 is expressed by the Th2 pathway, which is involved in IgE-mediated allergic diseases, whereas CCR5 is expressed by the Th1 pathway, which has a role in type IV hypersensitivity reactions and the immune response to infections (Baudouin et al., 2005) (Figure 1). These findings suggest that, aside from the inflammatory and toxic mechanisms, allergy may also play a role in the ocular surface damage experienced by glaucoma patients treated with PLMs. Table 2 and Table 3 present the ocular surface

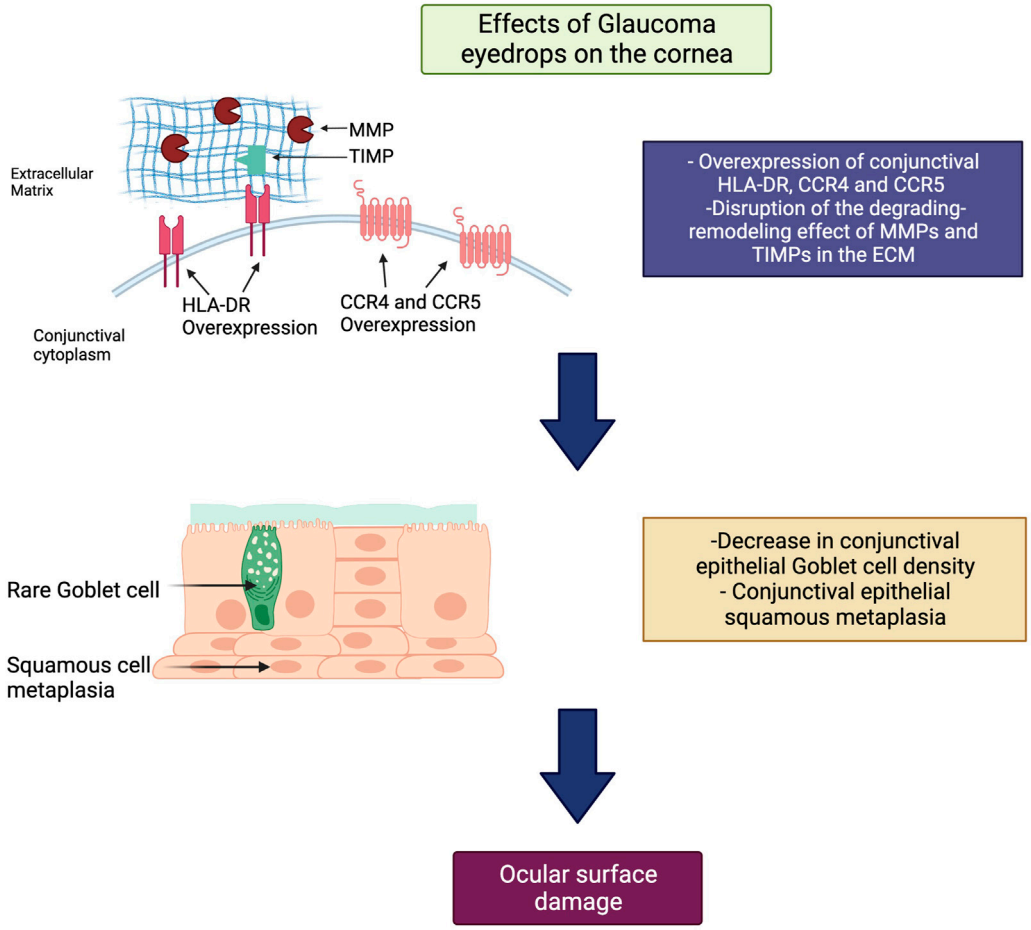

FIGURE 1
General mechanism of ocular surface toxicity due to pressure-lowering medications (PLMs). Within the ocular surface, PLMs cause overexpression of C-C chemokine receptors (CCR)-type 4 and 5, leading to an IgE-mediated allergic reaction on the ocular surface. Also, they cause to disrupt the degrading-remodeling balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in the extracellular matrix (ECM). The latter results in decreased goblet cell density, squamous metaplasia, and ocular surface damage. Footnote: HLA-DR, human leukocyte antigen DR isotype; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitors of metalloproteinases; ECM, extracellular matrix; CCR, C-C chemokine receptor.

disease manifestations of PLMs preserved with BAK and other additives, respectively (Figure 2).

4 Specific ocular surface disease changes caused by pressure-lowering medications

4.1 β-Adrenergic antagonists (β-blockers)

4.1.1 Contact dermatitis

Periocular contact dermatitis is a frequent adverse effect of topical anti-glaucomatous agents, mainly β-blockers (Horcajada-Reales et al., 2015) (Figure 3A). It may present as erythema with or without eczema and crusting of the eyelids. Koch et al. reported sensitization to a single β-blocker despite previous exposure to other β-blockers in three patients (Koch, 1995). This was also reported by Perez-Rodriguez et al. in a patient sensitized to 0.005% latanoprost but not to 0.03% bimatoprost (Perez-Rodriguez et al., 2008) and by Geyer et al. in 15 patients with proven allergy to 0.5% apraclonidine but without cross-reactivity to 0.25% clonidine hydrochloride (Geyer et al., 2000). Contrariwise, other authors report positive patch testing for multiple β-blockers, suggesting cross-sensitization (Horcajada-Reales et al., 2015). While some authors suggest cross-reactivity between multiple β-blockers might result from a common lateral aliphatic chain acting as an antigenic determinant others hypothesize that positive reactions could be related to multiple sensitizations instead of cross-reactivity. Allergic contact dermatitis in patients naïve to other PLMs from the same or other group has been reported with dorzolamide, brimonidine tartrate, and cholinergic agonists (i.e., pilocarpine) (Grey and Warshaw, 2016).

Interestingly, other studies report the development of a clinically apparent allergic reaction but with negative patch testing. Giordano-Labadie et al. reported the case of a patient who developed negative-patch chronic eczema for timolol, carteolol, and befunolol. The

TABLE 2 Human studies reporting ocular surface disease manifestations of BAK-containing pressure-lowering medications.

Drug class (frequency)		Periocular changes	Lacrimal drainage system	Meibomian glands	Conjunctiva	Cornea	References
PGAs	Frequent	Eyelash bristles	Epiphora	MG atrophy	Hyperemia	SPK	Inoue et al. (2012b); Lopilly Park et al. (2012); Sakata et al. (2013); Peace et al. (2015); El Hajj Moussa et al. (2018); Staso et al. (2018); Chang et al. (2021); Singh et al. (2022); Di Maria et al. (2023)
		Skin pigmentation		MG dropout	SQ metaplasia	↓CCT	
				↓TF stability	↓GCD	↑DCC	
				↓MAD		Irregular LTE	
				↓MAA		↑Nerve tortuosity	
						↓ECD	
	Rare	DUES	Fibrosis	None	DICC	PSK	
		Periocular CD	NLDO				
		Periocular ACD					
Beta blockers	Frequent	Periocular CD	↓Schirmer	MG atrophy	Hyperemia	SPK	Jappe et al. (2006); Hegde et al. (2007); Servat and Bernardino, (2011); Frezzotti et al. (2014); Ortiz-Basso et al. (2018); Staso et al. (2018); Kim et al. (2021b); Chang et al. (2021); Singh et al. (2022)
		Periocular ACD		MG dropout	SQ metaplasia	↑DCC	
				↓TF stability	↓GCD	Irregular LTE	
						↓ECD	
	Rare	DI-ectropion	NLDO	None	DICC	PSK	
CAIs	Frequent	Periocular ACD	Epiphora	MG atrophy	Hyperemia	SPK	Delaney et al. (2002); Hong et al. (2006); Hegde et al. (2007); Servat and Bernardino, (2011); Ortiz-Basso et al. (2019); Chang et al. (2021); Singh et al. (2022)
				MG dropout	Allergy		
				↓TF stability	SQ metaplasia		
	Rare	DI-ectropion	NLDO	None	DICC	PSK	
						Corneal failure	
Alpha agonists	Frequent	Periocular ACD	Epiphora	MG atrophy	Hyperemia	SPK	Servat and Bernardino, (2011); Aydin Kurna et al. (2014); Ortiz-Basso et al. (2018); Duru and Ozsaygili, (2020); Chang et al. (2021); Singh et al. (2022)
				MG dropout	Allergy		
				↓TF stability			
	Rare	Periocular CD	NLDO	None	Edema	None	
		DI-ectropion					
Miotics	Frequent	Periocular CD	None	MG atrophy	Hyperemia	SPK	Servat and Bernardino, (2011); Grey and Warshaw, (2016); Zhang et al. (2017); Ortiz-Basso et al. (2018); Chang et al. (2021); Singh et al. (2022)
				MG dropout	SQ metaplasia	Haze	
				↓TF stability			
	Rare	Periocular ACD	NLDO	None	DICC	PSK	
ROCK inhibitors	Frequent	Eyelid erythema	Epiphora	None	Hyperemia	SPK	Wisely et al. (2020); Kim et al. (2021a); Batra et al. (2021); Meirick et al. (2022)
					Hemorrhage	Cornea verticillata	
	Rare	Eyelid wound dehiscence	Transient punctal stenosis	None	None	Reticular bullous epithelial edema	
NO-donating PGAs	Frequent	Skin pigmentation	None	None	Hyperemia	SPK	Kawase et al. (2016); Lo et al. (2022)
	Rare	None	None	None	None	None	

BAK, benzalkonium chloride; PGAs, prostaglandin analogs; MG, Meibomian gland; TF, tear film; MAD, mean acinar density; MAA, mean acinar area; SQ, squamous; GCD, goblet cell density; SPK, superficial punctate keratitis; CCT, central corneal thickness; DC, dendritic cell density; LTE, limbal transition epithelium; ECD, endothelial cell density; DUES, deepening upper eyelid sulcus; CD, contact dermatitis; ACD, allergic contact dermatitis; NLDO, nasolacrimal duct obstruction; DICC, drug-induced cicatrizing conjunctivitis; PSK, pseudo-dendritic keratitis; DI, drug-induced; CAIs, carbonic anhydrase inhibitors; ROCK, rho kinase; NO, nitric oxide.

TABLE 3 Human studies reporting ocular surface disease manifestations of non-BAK preserved pressure lowering medications.*†

Drug class	Findings	Polyquad	Sofzia	Purite	References
PGAs	**Periocular changes**	Skin pigmentation	Skin pigmentation	-	Kammer et al. (2010); Mizoue et al. (2014); Lopes et al. (2015); Peace et al. (2015); El Hajj Moussa et al. (2018); Ortiz-Basso et al. (2018); Muz et al. (2021)
			DUES		
	LD system	↓Schirmer	Not reported	-	
		↓tear film stability			
	Meibomian glands	MG dropout	MG dropout	-	
		↓tear film stability	↓tear film stability		
	Conjunctiva	Hyperemia	Hyperemia	-	
		Edema			
	Cornea	Punctate keratitis	Punctate keratitis	-	
		↓Corneal hysteresis			
Beta blockers	**Periocular changes**	Not reported‡	-	-	Schnober et al. (2015)
	LD system	Not reported‡	-	-	
	Meibomian glands	Not reported‡	-	-	
	Conjunctiva	Hyperemia‡	-	-	
	Cornea	Punctate keratitis‡	-	-	
Alpha agonists	**Periocular changes**	-	-	Eyelid edema	Whitson et al. (2006); Duru and Ozsaygili. (2020)
				Periocular CD	
	LD system	-	-	↓Schirmer	
	Meibomian glands	-	-	Not reported	
	Conjunctiva	Hyperemia	-	Hyperemia	
		Allergic conjunctivitis		Allergic conjunctivitis	
				↓Goblet cell density	
	Cornea	-	-	Punctate keratitis	
				PSK	

*Blank cells indicate that there are no available formulations containing of the drug class and the preservative.
†There are no available formulations of Polyquad-, Sofzia, and Purite-preserved carbonic anhydrase inhibitors, miotics, rho kinase inhibitors, and nitric-oxide donating PGAs.
‡Polyquad-preserved beta blockers only exist in fixed combinations with prostaglandin analogs (travoprost).
BAK, benzalkonium chloride; PGAs, prostaglandin analogs; DUES, deepening upper eyelid sulcus; LD, lacrimal *drainage; MG, Meibomian gland; CD, contact dermatitis; PSK, pseudo-dendritic keratitis.*

authors suggested cross-reactivity occurred after drugs were metabolized to a common aldehyde rather than a reaction to an individual hapten (Giordano-Labadie et al., 1997). Written informed consent was obtained from all patients to publish the clinical images used throughout the manuscript.

4.1.2 Meibomian gland dysfunction (MGD)

Sullivan and coworkers hypothesized that the drug action of anti-glaucomatous agents might contribute to DED development by a direct effect on MGs (Zhang et al., 2017; Han et al., 2018; Han et al., 2020). They performed a series of experiments in which they cultured immortalized human MG epithelial cells (iHMGEC) with different concentrations of several α-adrenergic agonists (brimonidine, clonidine, phenylephrine) (Han et al., 2018), dorzolamide (Han et al., 2020), pilocarpine, and timolol (Zhang et al., 2017). Using clinical doses of 0.5% timolol and 4% pilocarpine (See Section 4.5.2), Zhang et al. demonstrated they both caused significant

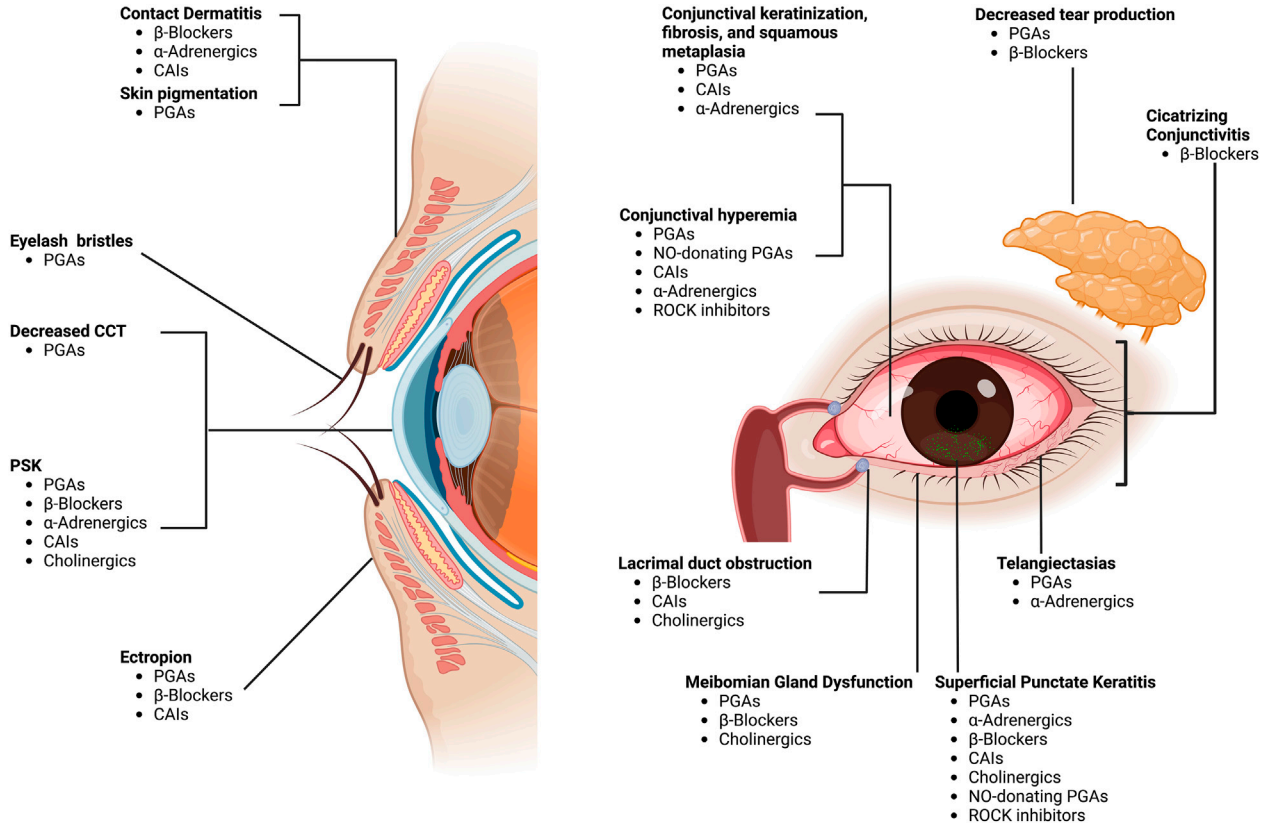

FIGURE 2
Ocular surface adverse effects of pressure-lowering medications. Footnote: CAIs, carbonic anhydrase inhibitors; PGAs, prostaglandin analogs; CCT, central corneal thickness; PSK, pseudodendritic sterile keratitis; NO, nitric oxide; ROCK, rho-kinase. "Created with BioRender.com".

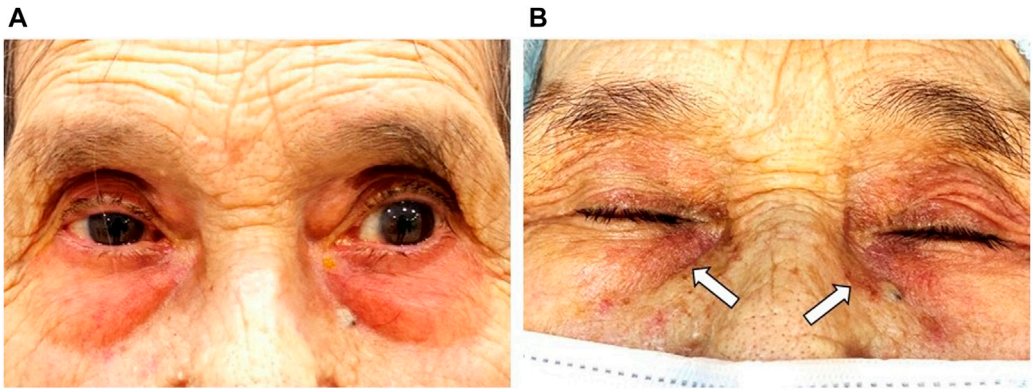

FIGURE 3
A 91-year-old female patient with a history of chronic angle-closure glaucoma treated with benzalkonium chloride (BAK)-containing 0.03% bimatoprost qHs and a fixed combination of 0.5% timolol maleate/2% dorzolamide BID eyedrops for at least 10 years. **(A)** Significant erythema of the periorbital skin and lid margin are consistent with allergic contact dermatitis. **(B)** After 4 weeks of treatment with ocular lubricants and preservative-free (PF) IOP-lowering eyedrops, there was a significant reduction of the periorbital skin and lid margin erythema. However, residual skin pigmentation (white arrows) is observed. Footnote: Written informed consent was obtained from both patients to publish the clinical images.

cell atrophy and death of iHMGEC (Zhang et al., 2017). Regarding timolol, MG dropout might be associated with the blockade of β_3-adrenoreceptors, which has been found in MGs of murine models (Knop et al., 2011). These receptors mediate fat oxidation and increase lipolysis; thus, beta-adrenergic blockade might cause detrimental effects on iHMGEC (Zhang et al., 2017).

TABLE 4 Recent relevant *ex-vivo* and *in-vitro* human studies reporting ocular surface disease manifestations of PLMs.

References	Cell type	Agents used	Study description	Preservatives	Findings
Park et al. (2011)	*Ex-vivo* orbital fat	PGAs	Evaluate the adipocyte density in orbital fat after exposure to preserved PGAs	BAK, PQ	The mean adipocyte density was significantly increased in eyes exposed to preserved PGAs, suggesting adipocyte atrophy
Choi et al. (2012)	Orbital adipocytes	PGAs	Evaluate the effects of PF PGAs in orbital fat	None	LAT, TRV, BIM, and TAF inhibited intracellular lipid accumulation and preadipocyte differentiation
Seibold et al. (2013)	*In vitro* subcutaneous adipocytes	BBs and PGAs	Compare the short-term effects of PF-TIM, various PGAs, and BAK alone on adipocyte cytotoxicity and preadipocyte proliferation	BAK	PF-TIM and BAK alone yielded anti-proliferative effects on pre-adipocytes and cytotoxic effects on mature adipocytes compared with the minimal toxicity caused by PGAs
Lopilly Park et al. (2012)	*Ex-vivo* tears	PGAs and BBs	Proteomic analysis of tears from patients using TIM, or various preserved PGAs, including LAT, TRV, or BIM for >1 year	BAK	Increased levels of MMP-1, MMP-3, MMP-9, IL-1β, IL-6, and decreased levels of TIMP-1 and TIMP-2 in PGA treated eyes compared with TIM
Mohammed et al. (2020)	*Ex-vivo* tears	PGAs	Evaluate the profile of inflammatory cytokines among various preserved and PF PGAs	BAK, PQ	BAK-preserved PGAs induced significant mRNA and protein expression of IL-1β, IL-6, and IL-8 compared with PQ and PF-PGAs
Zhang et al. (2017)	*In vitro* MGs	BBs and miotics	Evaluate the effects of PF TIM and PIL in MG epithelial cells	None	TIM and PIL resulted in dose-dependent atrophy and dropout of MG epithelial cells
Han et al. (2018)	*In vitro* MGs	AAs	Evaluate the effects of various AAs on the structure and function of MG epithelial cells	None	Brimonidine elicits a dose-dependent differentiation of MG epithelial cells, increasing neutral lipidsand lysosome levels
Rath et al. (2019)	*In vitro* MGs	PGAs	Evaluate the effects of various preserved and PF PGAs on MG epithelial cells	BAK, PQ	Cell viability was significantly reduced in BAK-containing PGAs and BAK alone compared with PF PGAs and TRV with PQ
Ammar et al. (2010)	*In vitro* cornea and conjunctiva	PGAs	Percentage of living epithelial cells to different preserved PGAs	BAK, PQ, SZ	PQ and SZ resulted in higher percentages of living cells compared with BAK
Whitson and Petroll (2012)	*In vitro* cornea	PGAs	Evaluate the toxicity of preserved and PF PGAs in the corneal epithelium	BAK, PQ	BAK-containing formulations resulted in significantly greater toxicity and less cell viability
Paimela et al. (2012)	*In vitro* cornea	PGAs	Determine the cytotoxic and inflammatory effects of preserved LAT and TRV	BAK, PQ	PQ-containing TRV activated NF-κB and significantly increased IL-6 and IL-8 compared with BAK
Yuan et al. (2016)	*In vitro* cornea	Miotics	Evaluate the cytotoxic effects of pilocarpine in stromal cells	None	Pilocarpine can induce apoptosis of corneal stromal cells in a dose-dependent manner
Liang et al. (2022)	*In vitro* conjunctiva and cornea	PGAs	Effects of preserved and PF PGAs in a wound-healing epithelial cell model	BAK, PQ, SZ	BAK significantly delayed healing through decreased Ki-67-positive cell numbers and actin disorganization compared to PQ, SZ, and PF-PGAs
Hedengran et al. (2022)	*In vitro* conjunctiva	PGAs	Viability of goblet cells and secretion of cytokines and mucins after exposure to TRV	BAK, PQ	PQ-containing TRV resulted in no goblet cell loss. Both PQ and BAK showed no differences in mucin and IL-6 and IL-8 secretion
Hedengran et al. (2021)	*In vitro* conjunctiva	CAIs, AAs, and miotics	Evaluate the effects of BAK-containing PLMs in conjunctival GCs	BAK	BAK-preserved LAT, followed by DORZ, resulted in significantly less GC density. BRIM did not affect GC survival

PLMs, pressure-lowering medications; PGAs, prostaglandin analogs; BAK, benzalkonium chloride; PQ, polyquad; PF, preservative-free; LAT, latanoprost; TRV, travoprost; BIM, bimatoprost; TAF, tafluprost; BBs, beta blockers; TIM, timolol; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; IL, interleukin; MGs; Meibomian glands; AAs, alpha agonists; SZ; Sofzia; NF-κB, nuclear factor kappa beta; GC, goblet cells.

Arici et al. reported significantly lower TFBUT scores, a surrogate marker of increased evaporation due to MGD, in patients treated with BAK-containing 0.5% betaxolol or 0.5% timolol eyedrops compared with controls (Arici et al., 2000). Table 4 presents the recent relevant *ex-vivo* and *in-vitro* human studies reporting OSD manifestations of preserved PLMs.

4.1.3 conjunctival goblet cell (GC) dropout

β-blockers cause abnormal keratinization, squamous metaplasia, inflammation leading to GC loss, and subconjunctival fibrosis (Singh et al., 2022). An impression cytology study reported that 50% and 55% of samples treated with BAK-containing 0.5% betaxolol or 0.5% timolol, respectively, were classified as grade 2, defined as the presence of large and multinucleated epithelial cells and a marked reduction of GCs, or grade 3, defined as even larger epithelial cells and complete absence of GCs (Arici et al., 2000). Terai et al. performed a histological analysis of human conjunctiva, evaluating the effect of BAK-containing 0.5% timolol and 0.005% latanoprost on MMPs and TIMPs expression and ECM organization (Terai et al., 2009). Compared with latanoprost, timolol-treated eyes exhibited overexpression of CD68 antibodies, an indicator of inflammatory infiltration. The latter suggests that chronic exposure to timolol eyedrops might result in conjunctival scarring and the potential for filtering surgery (trabeculectomy) failure (Terai et al., 2009).

A study performed by Aydin Kurna and coworkers evaluated the effect of different anti-glaucoma formulations, including preserved and preservative-free (PF)-timolol, and preserved formulations of latanoprost, bimatoprost, travoprost, and brimonidine (Aydin Kurna et al., 2014). At the 12-month follow-up, a significant increase in superior-central and inferior-nasal squamous metaplasia was observed in the brimonidine and both preserved and PF-timolol maleate groups. In PGA-treated eyes, an increase in the inferior-nasal squamous metaplasia was only reported in the BAK-containing travoprost group. Regarding GC loss, significant superior-central and inferior-nasal loss were observed in the PF-timolol and BAK-travoprost groups. In contrast, a consequential inferior-nasal loss was documented in the preserved-latanoprost and -brimonidine groups (Aydin Kurna et al., 2014).

4.1.4 drug-induced cicatrizing conjunctivitis (DICC)

DICC, also known as "pseudo-pemphigoid," is the development of conjunctival scarring after exposure to an inciting agent (Singh et al., 2022). It may be non-progressive or progressive, depending on whether the scarring process stabilizes (or not) after the withdrawal of the inciting agent (Singh et al., 2022). Although DICC can be associated with any anti-glaucoma medication, β-blockers are, by far, the most frequently reported. In 41 patients with DICC, β-blocker exposure was reported in 36 cases (88%). Timolol maleate was the culprit in 73% of cases (Thorne et al., 2004). The pathogenic mechanism of DICC consists of an inflammatory and immunological process leading to limbal stem cell deficiency, subconjunctival fibrosis, and fornix foreshortening, mainly of the inferior bulbar and palpebral conjunctiva (Vazirani et al., 2020). However, ocular signs may involve the entire surface, including punctum scarring, periocular hypopigmentation, obstructive MGD, eyelash overgrowing (distichiasis), malposition (trichiasis), and lid margin keratinization (Singh et al., 2022).

Histopathological findings of DICC are very similar to those encountered in other cicatrizing conditions such as ocular mucous membrane pemphigoid (OMMP), notably showing increased proliferation of the basal cells of the conjunctival epithelium, marked infiltration of inflammatory cells such as macrophages, neutrophils, and T-lymphocytes in the acute phase, and fibroblast stimulation resulting in fibrosis in the chronic phase (Elder and Lightman, 1994; Singh et al., 2022). However, a notable distinction between DICC and OMMP can be made with direct immunofluorescence (DIF). While DIF following a conjunctival biopsy of a patient with OCP shows linear deposition of IgA, IgG, IgM, and complement C3 on the conjunctival epithelial basement membrane zone, DIF in pseudo-pemphigoid cases such as DICC is usually negative for these observations and require clinical diagnoses (Singh et al., 2022).

Gibran reported the case of an 85-year-old woman with an 8-year BAK-containing latanoprost and apraclonidine use to manage pseudo-exfoliative glaucoma in her left eye (Gibran, 2004). Symptoms included painful red eye and blurred vision, whereas signs included keratoconjunctivitis sicca, fornix foreshortening, and corneal scarring with active neovascularization in the left eye. The right eye was normal (Gibran, 2004). A similar case of a patient exposed to multiple BAK-containing drugs, including latanoprost, dorzolamide, brinzolamide, pilocarpine, and brimonidine, was also reported by Kahana et al. (Kahana et al., 2007). In both cases, BAK was deemed responsible for the development of DICC (Gibran, 2004; Kahana et al., 2007).

4.1.5 Lacrimal drainage obstruction (LDO)

Topical anti-glaucoma agents can cause isolated canalicular and lacrimal occlusion, as well as a more extensive cicatrizing process known as drug-induced cicatrizing conjunctivitis (DICC, See Section 4.1.4) (Kashkouli et al., 2008). Narioka et al. found a decrease in the lumen width of the nasolacrimal drainage (NLD) system after exposure to 0.5% timolol, located mainly in the middle and lower regions (Narioka and Ohashi, 2007). These findings imply that timolol caused vasodilation of the blood vessels in the NLD system's cavernous body, suggesting that the autonomic nervous system may partially control tear drainage through the NLD system (Narioka and Ohashi, 2007). In a large prospective and controlled case series of 627 eyes from 384 patients, Kashkouli et al. reported significant lacrimal drainage obstruction (LDO) in patients using combined formulations of timolol/dorzolamide and timolol/dorzolamide/pilocarpine. Timolol alone did not cause substantial obstruction, which suggests that fixed combinations of PLMs had an increased risk of LDO (Kashkouli et al., 2008).

4.2 Prostaglandin analogs (PGAs)

4.2.1 Skin pigmentation

Eyelid pigmentation (0%–26%) and eyelash bristles (0%–77%) represent a frequent periorbital manifestation associated with PGAs, with an increased frequency if used for >3 months (S et al., 2018) (Figure 3B). Inoue et al. reported there were no significant differences in the frequency (4%–6%) of eyelid pigmentation after >3 months of latanoprost, tafluprost, bimatoprost, travoprost, or isopropyl unoprostone use (Inoue et al., 2012a). Eyelash bristles, however, occurred significantly less with unoprostone (8%) compared with the other four drugs (26%–54%).

4.2.2 Meibomian gland dysfunction (MGD)

Mocan et al. reported a significantly higher prevalence of MGD in glaucoma patients managed with PGA monotherapy than those

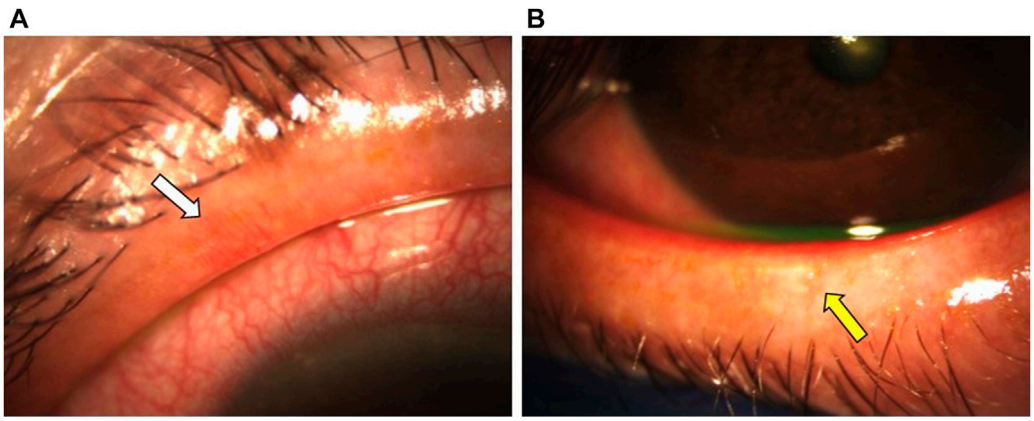

FIGURE 4
A 61-year-old female patient with a 7-year history of primary open-angle glaucoma (POAG) was treated with BAK-containing 0.005% latanoprost qHs eyedrops. **(A,B)** Significant lid margin erythema, telangiectasias (white arrow), meibomian gland clogging (yellow arrow). Footnote: Written informed consent was obtained from the patient to publish the clinical images.

treated with other PLMs (92% vs 58%). The obstructive form of MGD was documented in 96% of patients from the PGA group (Mocan et al., 2016). Moreover, other ocular surface parameters, including the Ocular Surface Disease Index (OSDI), tear-film breakup time (TFBUT), lissamine green staining, and Schirmer scores were significantly worse in patients treated with PGA monotherapy compared to healthy controls (Mocan et al., 2016). The pathogenic mechanisms of MGD in patients treated with PGA remains poorly understood. Some authors suggest that subclinical inflammation of the conjunctiva results in MG dropout and dysfunction (Agnifili et al., 2018). Agnifili et al. reported a significant reduction in the mean acinar area (MAA) and density (MAD), which are respective surrogates of reduced meibum production and glandular dropout, and higher interstice inhomogeneity, which reflects MG and tarsal inflammation, in patients treated with PGAs (Agnifili et al., 2018). These findings were significantly higher in preservative PGA-treated patients than those managed with PF-PGAs. In another study, Arita et al. reported significantly higher lid margin abnormalities (i.e., vascular tortuosity, irregular lid margin, replacement of the mucocutaneous junction, and plugged MG orifices) (Figures 4A,B), which are associated with MGD and conjunctival inflammation, and higher Meibo-scores, implying increased MG dropout, in patients treated with PGA compared with β-blocker treated eyes and healthy controls (Arita et al., 2012) (Figure 5). The authors suggest that the lid margin abnormalities in glaucoma-treated eyes support the hypothesis that subclinical inflammation predates MG alterations (Arita et al., 2012). Recurrent inflammation resulting from prolonged exposure to PGA might lead to meibum stagnation with subsequent keratinization of MG orifices (i.e., obstructive MGD) (S et al., 2018).

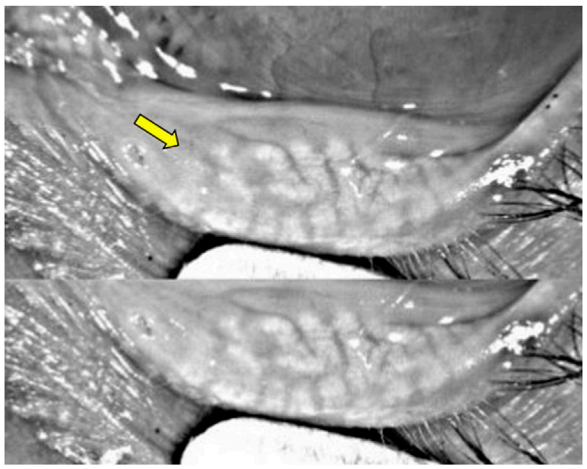

FIGURE 5
Keratograph analysis from a patient with a 12-year history of POAG treated with BAK-containing 0.005% latanoprost qHs eyedrops showing significant meibomian gland dropout (yellow arrow).

4.2.3 Conjunctival hyperemia

A recent meta-analysis performed by Tang et al. reported that the frequency of conjunctival hyperemia was significantly higher in bimatoprost (40%) compared with travoprost (39%) and latanoprost (28%) (Tang et al., 2019). Although unclear, PGAs are suspected of inducing the production of NO synthase, which may lead to conjunctival hyperemia due to their vasodilatory properties (Astin et al., 1994) (Figure 6A).

4.2.4 Conjunctival goblet cell (GC) dropout

Human studies report GC loss after long-term treatment with BAK-containing PGA eyedrops and after short-term exposure to BAK alone (Pisella et al., 2004). On the other hand, Mastropasqua et al. described an increase in GC density after 6 months of therapy with PF-tafluprost. This finding can be explained by the ability of PG to stimulate the secretion and proliferation of mucin in numerous mucosal surfaces, including the conjunctiva (Mastropasqua et al., 2013). Interestingly, the authors report a transient increase in GC density after 1 month of preserved latanoprost. The GC density,

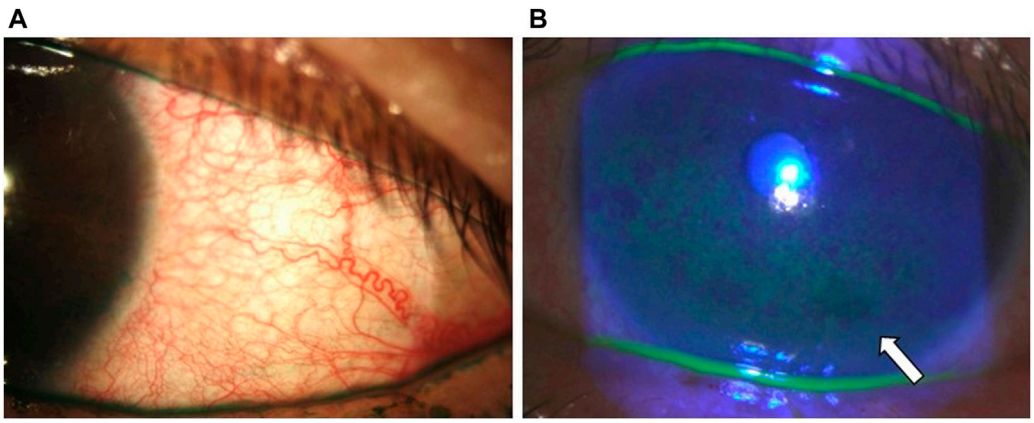

FIGURE 6
Clinical photographs of the patient from Figure 4 showing **(A)** conjunctival hyperemia with lissamine green staining and **(B)** central and inferior corneal staining (white arrow).

however, returned to baseline after 6 months, suggesting that over time, the positive effect of the PG derivative is nullified by the preservative (Mastropasqua et al., 2013).

4.2.5 Tear film

Using the Schirmer test I, Agnifili et al. compared tear production between healthy controls and groups of patients using several anti-glaucoma regimes, including combinations of preserved PGAs with timolol maleate and PF-bimatoprost and timolol (Agnifili et al., 2018). The tear production of healthy controls was significantly higher than in all therapy groups, including the fixed combination of PF-bimatoprost and timolol (18.4 ± 5.5 mm *vs.* 9.8 ± 3.5 mm). Interestingly, the unfixed combination of latanoprost and timolol yielded significantly worse Schirmer scores than the fixed combinations of timolol and different PGAs (latanoprost, travoprost, and bimatoprost) (Agnifili et al., 2018).

4.2.6 Corneal thickness

A significant reduction in the central corneal thickness (CCT) was documented in human eyes after 8 weeks of treatment with either 0.03% bimatoprost, 0.004% travoprost, or 0.005% latanoprost compared with controls. No difference between PGAs was found (Hatanaka et al., 2009). Human studies have shown central corneal thinning after long-term exposure to PGAs, possibly due to increased activity of MMPs relative to TIMPs in the corneal epithelium and stroma (Lopilly Park et al., 2012). Upregulation of MMPs, mainly MMP-2 and -9, has been reported in stromal tissue after corneal ablative procedures and corneal erosions (Jadczyk-Sorek et al., 2022). In another study, Lopilly Park et al. did not find a significant reduction in the CCT of human eyes after 1 year of treatment with PGAs (Lopilly Park et al., 2012). In the same study, however, the authors reported that rabbit corneas exhibited corneal thinning caused by a decrease in collagen type 1 after PGA treatment. Also, a significant increase in MMP-1 and MMP-9 and a reduction in TIMP-1 were found in rabbit corneas (Lopilly Park et al., 2012). Differences in the collagen distribution between the human and rabbit corneas might also explain humans' lack of significant corneal thinning. Table 5 presents the recent relevant *in-vivo, ex-vivo,* and *in-vitro* animal studies reporting OSD manifestations of preserved PLMs.

4.2.7 Pseudodendritic keratitis (PSK)

PSK is uncommon in patients using PLMs. It is characterized by pseudodendritic central and lower corneal lesions surrounded by SPK (Chang et al., 2021). A recent retrospective case series reported 31 events (19 patients) of PSK, 52% of them associated with PGAs and 97% to BAK-containing PLMs (Chang et al., 2021). Management includes using therapeutic soft contact lens, lubricant eyedrops, andto discontinuation, decrease, or change of the PLM used (Chang et al., 2021). Due to their similarities, PSK could get confused with herpetic simplex keratitis. However, the latter is characterized by dendrites with central fluorescein staining and terminal bulbs that can be found anywhere in the cornea (Chang et al., 2021).

4.3 Carbonic anhydrase inhibitors (CAIs)

4.3.1 Contact dermatitis

Delaney et al. reported periocular contact dermatitis in 14 patients after 20.4 weeks of initiating BAK-containing dorzolamide. Of those, 13 patients were using preserved β-blockers for 34.2 months (Delaney et al., 2002). Dermatitis wholly resolved in 8 cases (57%) after discontinuing dorzolamide, and in the remaining 6 (43%), resolution occurred after the topical β-blocker was also stopped. Negative patch testing to β-blockers, BAK, and dorzolamide was reported in all cases. The authors hypothesized that a false-negative reaction, or simply irritation rather than sensitization, might have occurred (Delaney et al., 2002).

4.3.2 Drug-induced ectropion

Hegde et al. reported 13 patients who developed drug-induced ectropion after exposure to dorzolamide (7 cases, 53%), brimonidine (3 cases, 23%), and other agents, including β-blockers, latanoprost, and preserved lubricant eyedrops (Hegde et al., 2007). This effect was reversible after drug discontinuation and a short course of

TABLE 5 Recent relevant *in-vivo, ex-vivo,* and *in-vitro* animal studies reporting ocular surface disease manifestations of PLMs.

References	Cell type	Agents used	Study description	Preservatives	Findings
Krauss et al. (2011)	*In vivo* OHT model in monkeys, dogs, and rabbits	NO-donating PGAs	To compare the hypotensive effects between LAT and NO-donating LAT	BAK	NO-donating LAT was more effective in lowering the intraocular pressure with no ocular side effects reported
Taketani et al. (2014)	*In vitro* mouse adipocytes	PGAs	Evaluate the effects of all PGAs on adipogenesis	None	All PGAs, except unoprostone, promoted lipolysis and suppressed adipogenesis, potentially explaining DUES
Jiang et al. (2022)	*Ex-vivo* mouse MGs	PGAs	Evaluate the effects of LAT on chemokine and cytokine secretion	None	LAT induced inflammation in Meibomian gland epithelial cells and suppressed differentiation by overexpressing of IL-1β, IL-6, TNF-α, MMP-9, among other cytokines
Young et al. (1990)	*Ex-vivo* rabbit conjunctiva	Miotics and BBs	To evaluate the effects of PIL and TIM on the conjunctiva	BAK	PIL produced higher myofibroblastic cell proliferation and thickened conjunctival epithelium and stroma
Pisella et al. (2000)	*In vivo* and *ex-vivo* rabbit cornea and conjunctiva	BBs	Evaluate the ocular surface tolerance to preserved and PF TIM	BAK	The PF-formulation exhibited significantly decreased TFBUT compared with BAK-containing TIM. Furthermore, PF-formulations also had less histological stromal edema
Liang et al. (2011)	*In vivo* rabbit cornea and conjunctiva	BBs and PGAs	Assess the toxicological profile of TRV/TIM and LAT/TIM fixed combinations	BAK, PQ	BAK-containing LAT/TIM produced more hyperemia, chemosis, tearing, and cytotoxicity (assessed by IVCM) compared to PQ-preserved TRV/TIM
Ayaki et al. (2012)	*In vitro* rabbit and bovine cornea	CAIs, PGAs, BBs, and AAs	To evaluate *in-vitro* cytotoxicity on corneal epithelial cells of BAK-containing PLMs	BAK	Decreased cell viability scores across most CAIs, B-blockers, and PGAs, or their fixed combinations
Shin et al. (2015)	*Ex-vivo* rat conjunctiva, cornea, and aqueous humor	AAs	Evaluate effect of brimonidine in the level of various inflammatory markers	PUR	Corneoconjunctival levels of inflammatory markers (TNFa, IL-1a, IL-1b, IL-6) were significantly lower in the brimonidine group, but increased in the aqueous humor
Lee et al. (2015)	*In vivo* rabbit cornea and conjunctiva	PGAs	Evaluate the ocular surface effects of various preserved PGAs	BAK, PQ	Decreased conjunctival goblet cell density, increased corneal pyknotic changes, and increased tear IL-6 were found in BAK-containing formulations
Kim et al. (2015)	*In vivo* mouse cornea	PGAs	Evaluate the effects of preserved and PF PGAs	BAK, PQ	PQ and PF formulations showed less SPK, epithelial desquamation, anisocytosis, and cell shrinkage compared with BAK
Lin et al. (2018)	*In vitro* rabbit, monkey, dog, pig, and human corneas *In vivo* rabbit conjunctiva	ROCK inhibitors	Preclinical characterization of netarsudil comparing its effects with other rho-associated protein kinase inhibitors	None	Netarsudil exhibited significant intraocular pressure reductions with only mild hyperemia
Leary et al. (2021)	*In vivo* dog conjunctiva	ROCK inhibitors	Evaluate the safety and efficacy of netarsudil	BAK	Increased conjunctival hyperemia in treated eyes compared with balance salt solution

PLMs, pressure-lowering medications; NO, nitric oxide; PGAs, prostaglandin analogs; LAT, latanoprost; BAK, benzalkonium chloride; DUES, deepening upper eyelid sulcus; MGs, Meibomian glands; IL, interleukin; TNF, tumor necrosis factor; MMP, matrix metalloproteinase; BBs, beta blockers; PIL, pilocarpine; TIM, timolol; PF, preservative-free; TFBUT, tear film breakup time; TRV, travoprost; PQ, polyquad; IVCM, in-vivo confocal microscopy; CAIs, carbonic anhydrase inhibitors; AAs, alpha agonists; ROCK, rho kinase.

topical steroids in 9 cases. However, the remaining patients who did not receive steroids were successfully managed with surgical correction (Hegde et al., 2007).

4.3.3 Meibomian gland dysfunction (MGD)

Han et al. reported that, compared with the therapeutic concentration (50 μg/mL) of dorzolamide, a 10-fold higher concentration of 500 μg/mL significantly reduces iHMGEC proliferation while increasing iHMGEC differentiation (Han et al., 2020). The authors suggest that dorzolamide might enhance the expression of hypoxia-inducible factor (HIF)-1α, which facilitates iHMGEC differentiation by triggering a cellular response to hypoxic stress. However, the therapeutic concentration of dorzolamide did not elicit such an effect (Han et al., 2020).

4.3.4 Conjunctival hyperemia

he incidence of hyperemia in dorzolamide users ranges from 7% to 21% (Adamsons et al., 1998; Ott et al., 2005); however,

brinzolamide, another CAI, has a lower prevalence (<3%) of hyperemia due to its physiological pH, which enhances tolerability and compliance (Lusthaus and Goldberg, 2017).

4.4 α-Adrenergic agonists

4.4.1 Meibomian gland dysfunction (MGD)

Brimonidine triggers the upregulation of sterol regulatory element-binding protein (SREBP)-1, a lipogenesis regulator that synthesizes lipid, cholesterol, and fatty acid production enzymes. Moreover, brimonidine enhances the conversion of SREBP-1 to its mature form, thus promoting lipid accumulation and differentiation iHMGECs (Han et al., 2018). The authors conclude that dry eye symptoms associated with brimonidine, including blurry vision, ocular discomfort, and DED, might be related to corneal toxicity, GC loss, and conjunctival inflammation rather than MG dropout (Han et al., 2020).

4.4.2 Conjunctival allergy

Allergic reactions are another frequent side effect of alpha agonists. Although unclear, the pathogenic mechanism could be related to a volume decrease and subsequent widening of the intracellular spaces between conjunctival cells, leading to an entry portal for external allergens (Yeh et al., 2021). Butler et al. reported an incidence of allergic reaction in 48% of apraclonidine users (Araie et al., 2015). This side effect was more common in women and led to drug discontinuation after an average latency of 4.7 months (Butler et al., 1995). In the case of brimonidine, the incidence of allergy ranges from 4.7% to 25%, occurring at a mean time interval of 6–9 months (Yeh et al., 2021). The prevalence of hyperemia is 13% and 8% for apraclonidine and brimonidine users, respectively (Robin et al., 1995; Mundorf et al., 2003).

4.5 Cholinergic agents

4.5.1 Contact dermatitis

Allergic contact dermatitis has been previously reported in topical pilocarpine users. O'Donnell and Foulds presented a patient with negative patch testing to topical PLM ingredients at days 4 and 7 (O'Donnell and Foulds, 1993). However, using the prick-testing method with unpreserved pilocarpine elicited a 10-mm wheal after 30 min, a finding suggestive of contact urticaria. In a similar fashion, Cusano et al. described of a patient with 1-year history of eyelid dermatitis and negative patch testing, but who developed an inflammatory reaction after repeated open application Test with pilocarpine eye drops (Cusano et al., 1993).

4.5.2 Meibomian gland dysfunction (MGD)

Zhang et al. reported significant cell atrophy and death of cultured immortalized human MG epithelial cells (iHMGEC) with 0.04% pilocarpine, a tenfold lower than the clinical dose (Zhang et al., 2017). The standard 0.4% pilocarpine-induced impaired cellular adherence, perinuclear vesicle accumulation, which heralds cell death, and decreased survival of iHMGEC. Although elusive, pilocarpine-induced MG dropout might be related to its effects on muscarinic acetylcholine receptor 3, present in iHMGEC (Wu et al., 2020).

4.6 Rho-kinase (ROCK) inhibitors

4.6.1 Postoperative eyelid wound dehiscence

It is defined as a break or split in the eyelid after a previously closed surgical incision site (Sandy-Hodgetts et al., 2015). Kim et al. reported the case of an 81-year-old male with glaucoma managed with PLMs, including 0.02% netarsudil. The patient underwent upper eyelid Mohs surgery and lid repair due to basal cell carcinoma. Interestingly, the patient suffered three episodes of wound dehiscence, requiring repair in two (Kim et al., 2021). After the last episode, the patient discontinued the netarsudil eyedrops, and 2 weeks later, he developed granulation tissue, and the wound healed appropriately. In diabetic foot ulcer rat models, overexpression of ROCK1 has been shown to increase wound healing (Wang et al., 2022). Inhibition of MLC-phosphatases by the ROCK pathway leads to long-lasting contraction of myofibroblasts, which is required for wound healing (Saha et al., 2022). Thus, ROCK inhibition with netarsudil could lead to poor wound healing.

4.6.2 Conjunctival hyperemia

The Rho Kinase Elevated IOP Treatment (ROCKET) Trials reported an incidence of conjunctival hyperemia of 50%–53% and 59% in eyes receiving 0.02% netarsudil once and twice daily, respectively. These results were significantly higher than the 8%–11% incidence of conjunctival hyperemia in eyes receiving 0.5% timolol twice daily (Serle et al., 2018). In a rabbit model, Watabe and coworkers demonstrated that ROCK inhibitors caused vasodilation of the ciliary arteries due to a calcium-independent mechanism. This contrasts with the PLMs tafluprost, a PGA, and levobunolol, a β-blocker, which cause relaxation of the ciliary arteries by decreasing calcium concentration in the intracellular space (Watabe et al., 2011). As stated above, ROCKs contract the trabecular meshwork by phosphorylation of MLCs. Thus, conjunctival hyperemia associated with ROCK inhibitors could be related to vasodilation of ciliary arteries due to smooth muscle relaxation secondary to MLC phosphorylation (Watabe et al., 2011).

4.6.3 Subconjunctival hemorrhage

Singh et al. reported a higher incidence of subconjunctival hemorrhage in patients managed with once-a-day 0.02% netarsudil eyedrops compared with twice-a-day 0.5% timolol (17% vs 2%). Among patients managed with netarsudil, the hemorrhage was mild in 92% of cases, self-limiting in 96%, and requiring drug discontinuation in 1% (Singh et al., 2020).

4.6.4 Drug-induced cicatrizing conjunctivitis (DICC)

Meirick et al. reported 16 patients who developed reversible punctum stenosis after an average time of 14 months of 0.02% netarsudil use. Of those, 13 (81%) patients had symptomatic epiphora, leading to drug discontinuation in 7 cases. Histopathological analysis from the conjunctiva and punctum of one patient showed nonspecific lymphocytic inflammation without

eosinophils (Meirick et al., 2022). This contrasts with the findings encountered in eyes with the non-reversible scarring inflammation in DICC, which is typically associated with β-blockers (Singh et al., 2022). Punctal scarring has not been reported with ripasudil, another ROCK inhibitor. Compared with ripasudil, netarsudil has a NET inhibitor function. However, the effect of NET inhibition and punctum scarring remains unknown (Meirick et al., 2022).

4.6.5 Corneal edema

Wisely et al. reported five patients who developed reticular bullous corneal epithelial edema after a mean time of 5.4 (range: 2–8) weeks of netarsudil use (Wisely et al., 2020). Four patients had a prior history of corneal edema due to different causes. The remaining patient had a previous history of anterior uveitis, which predisposes to corneal edema. In all cases, the epithelial edema resolved after a mean time of 7.4 (range: 2–12) weeks of discontinuing netarsudil (Wisely et al., 2020).

ROCKs and tight junctions, including zonula occludins (ZO)-1, oversee osmotic regulation in epithelial surfaces (Chmiel and Gardel, 2022). ROCK inhibitors could lead to epithelial edema by increasing the permeability of tight junctions, thus allowing fluid to percolate from the corneal stroma into the epithelium (Chmiel and Gardel, 2022; Lyons et al., 2022). Corneas with a prior history of developing corneal edema might be more susceptible to ROCK inhibition (Wisely et al., 2020). However, the latter remains unknown.

4.7 Nitric oxide (NO)-donating prostaglandin analogs (PGAs)

4.7.1 Conjunctival hyperemia

The prevalence of hyperemia associated with latanoprostene bunod ranges from 2% to 18% (Lo et al., 2022). Besides the NO synthase induced vasodilation (See Section 4.1.3), another potential mechanism of conjunctival hyperemia could be related to the pro-inflammatory properties of the prostaglandin F2α molecule itself (Kawase et al., 2016).

4.7.2 Superficial punctate keratitis (SPK)

In an open-label clinical study of healthy subjects, Araie et al. reported a prevalence of 54.2% of SPK among healthy users of once-a-day 0.024% latanoprostene bunod. However, in all cases, the SPK was mild and clinically insignificant (Araie et al., 2015). In a recent meta-analysis of randomized controlled trials (RCTs), the prevalence of SPK ranged from 1.1% to 4.3% (Lo et al., 2022).

5 Preservatives

Preservative agents, including BAK, polyquaternium-1 (Polyquad), Sofzia®, and Purite®, influence corneal penetration of the active substance through their surface wetting properties and provide bacteriostatic activity (Servat and Bernardino, 2011). Within the eye, the lipophilic nature of most preservatives renders immediate binding to ocular tissues after application. However, many animal and human studies have shown that preservatives are culprits of inducing or worsening ophthalmic formulations' toxic, allergic, and inflammatory reactions, including PLMs (Kim et al., 2015; Muz et al., 2021).

BAK is the most frequently used preservative in ophthalmic preparations (Muz et al., 2021). It is commonly used as a cationic surfactant, a phase transfer agent in the chemical industry, and a biocidal agent due to its activity against fungi and Gram-positive and Gram-negative bacteria (Muz et al., 2021). However, BAK-preserved formulations have been shown to trigger dose-dependent inflammation, tear instability, increased osmolarity, corneal and conjunctival epithelial cytotoxicity, squamous metaplasia, and GC loss (Kim et al., 2015). To reduce the toxicity of BAK-preserved formulations, less toxic formulations were designed, including Sofzia®, Polyquad, and Purite®. However, PF antiglaucoma formulations are available and should be considered first-line, mainly in patients with preexisting OSD.

Damage to the limbal stem cells (LSCs) and corneal epitheliopathy has been associated with multiple PLMs. However, studies suggest that preservatives, rather than the active ingredient, have been identified as culprits. LSCs, which have a high proliferation, differentiation, and migration capacity, reside in the corneoscleral limbus (Guclu et al., 2021). LSCD results from an impaired function and reduced number of LSCs which, in turn, can lead to corneal conjunctivalization, persistent epithelial defects (PEDs), scarring, and vision loss. The concept of "iatrogenic LSCD" in eyes managed with PLMs was first coined by Schwartz and Holland (Schwartz and Holland, 2001). Güçlü et al. evaluated the limbal epithelium thickness (LET) in patients treated with either one, two, three, or four-drug regimens of BAK-containing anti-glaucoma medications (Guclu et al., 2021). The authors found no difference in the LET between treated groups; however, it was significantly lower compared to non-treated healthy eyes (64.1 ± 9.1 μm *vs.* 76.0 ± 11.5 μm). Moreover, there was a positive correlation between increased LET and increased Schirmer (r = 0.4), TFBUT (r = 0.37), and central corneal epithelial thickness (CCET; r = 0.37) (Guclu et al., 2021). A decreased significant LET is also reported in DED patients (Francoz et al., 2011). PLMs might decrease LET due to increased epithelial turnover or chronic inflammation (Francoz et al., 2011). On the other hand, the association between decreased LET and decreased CCET may result from decreased proliferation, differentiation, and migration of reduced LSCs (Guclu et al., 2021).

A morphologic IVCM study by Mastropasqua et al. analyzed the density of dendritic cells (DCs) and the regularity of the limbal transition epithelium (LTE) in eyes treated with single, double, and triple or more anti-glaucoma eyedrops regimes (Mastropasqua et al., 2015). Eyes managed with preserved β-blockers and preserved PGAs exhibited higher DCs density and worsened LTE irregularity compared with PF-drugs. A higher DC density results from BAK-induced local immune system activation, whereas the LTE irregularity, observed as punctate reflecting elements with IVCM analysis, represents an additional sign of inflammation (Mastropasqua et al., 2015). Moreover, eyes treated with preserved drugs significantly increased HLA-DR and IL-6 compared with PF drugs. This supports the theory that inflammation might be the initial cascade step leading to limbal abnormalities (Mastropasqua et al., 2015).

Superficial punctate keratitis (SPK) encircles a group of corneal epithelial lesions with varying morphology and can be observed as

corneal staining at the slit-lamp. The prevalence of SPK in anti-glaucoma eyedrop users is reported to be as high as 70% (Lajmi et al., 2021) (Figure 6B). Using *in vivo* confocal microscopy (IVCM), Mastropasqua et al. reported that the central corneal DC density significantly increased in patients with preserved compared to those receiving PF PGAs and β-blockers (Mastropasqua et al., 2016). Additionally, the corneal DC density significantly correlated with corneal staining and OSDI scores ($p < 0.001$). These results resemble those found in the limbal epithelium, suggesting an increased inflammatory response in the corneal epithelium with a subsequent increase in signs and symptoms (Mastropasqua et al., 2016). Ye et al. reported a significant association between increased fluorescein staining and epithelial thickness in the central and paracentral cornea, indicating that abnormal staining might predict corneal epithelial thinning (Ye et al., 2022). SPK is also reported in 5%–10% of patients using netarsudil and in 1%–4% of latanoprostene bunod users (Batra et al., 2021; Lo et al., 2022).

6 Ocular surface disease and quality of life in glaucoma patients

Quality of life (QoL) refers to patients' perception of their daily wellbeing (Kumar et al., 2020). Unfortunately, QoL can be severely affected in patients with glaucoma and OSD, which often coexist (Camp et al., 2015; Tirpack et al., 2019). Studies report a significant association between the increased number of PLMs and decreased emotional wellbeing scores, with African American patients experiencing worse QoL scores (Camp et al., 2015). Abegão Pinto et al. prospectively evaluated the change in visual-related QoL, assessed by the Glaucoma Symptom Scale (GSS), in patients with glaucoma after switching from preserved IOP-lowering therapy to PF-timolol/dorzolamide fixed combination (TDFC). After 8 weeks of treatment with PF-TDFC, there was a significant improvement in the GSS-related symptom, function, and total scores (Abegao Pinto et al., 2014). Accordingly, Kumar et al. found significantly worse QoL scores between patients using BAK-containing travoprost and the PF-travoprost and control groups (Kumar et al., 2020). Interestingly, there was no difference between the reported QoL in the PF-travoprost and control groups, suggesting the harmful role of preservatives in OSD and QoL in glaucoma patients (Kumar et al., 2020).

7 Future directions

OSD symptoms are detrimental to glaucoma patients' perceived QoL, compliance to therapy, reliability of diagnostic tests, poor surgical outcomes, and disease progression and visual outcomes (Zhang et al., 2019). Thus, addressing OSD in glaucoma is crucial. A survey-based study reported that 100% of Canadian glaucoma specialists agreed that a good approach to OSD in patients might improve QoL, whereas 97% agreed that it could result in enhanced glaucoma outcomes (Muzychuk et al., 2020). Furthermore, only 22% agreed that OSD is currently being managed appropriately, and 92% agreed that a stepwise approach should be undertaken to address OSD in glaucoma. Accordingly, the authors proposed an algorithm consisting of 1) optimizing topical glaucoma medications by using combined and PF formulations; 2) promoting ocular surface health (i.e., PF-lubricants and gels, omega-3 fatty acid supplementation); 3) enhancing OSD therapy with steroids immunomodulatory drugs (i.e., cyclosporine A, autologous serum); and 4) considering surgical interventions (Muzychuk et al., 2020). In this regard, minimally invasive glaucoma surgery and slow delivery systems such as the bimatoprost implant have emerged as a possible solution for reducing IOP with fewer ocular surface adverse effects compared with traditional surgical and non-surgical IOP-lowering methods (Schoenberg et al., 2015; Schweitzer et al., 2020; Shirley, 2020). A recent prospective cohort study reported a significant reduction in the number of PLMs, a substantial improvement in OSDI and TFBUT scores, and conjunctival hyperemia in patients who underwent combined cataract surgery with trabecular micro-bypass stent(s) implantation (Schweitzer et al., 2020). More randomized prospective trials with extensive follow-up are required to assess the direct impact of MIGS on the ocular surface.

8 Conclusion

OSD is a frequently overlooked condition resulting from glaucoma therapy, with age, use of BAK-containing and multiple anti-glaucoma medications, concomitant systemic comorbidities, and previous DED as the most frequent associated risk factors. Eye care specialists must remain aware of the adverse effects of PLMs and, thus, actively inquire about ocular surface symptoms. Upon diagnosis of OSD, a severity-based, stepladder approach consisting of optimizing glaucoma treatment by switching to fixed and PF combinations, using PF ocular lubricants, prescribing short courses of topical steroids with or without immunomodulatory therapy, and considering early surgical intervention are required to enhance medication adherence and improve glaucoma outcomes.

Author contributions

Design of the work: RR-L, SK, and LW-G. Conceptualization: NA, HM, and VP. Literature investigation and selection: RR-L, NA, HM, SK, MQ-G, LW-G, and CC. Main writing: RR-L. Manuscript reviewing: RR-L, NA, HM, SK, MQ-G, LW-G, and CC. Figures and table editing: RR-L, NA, MQ-G, LW-G, and CC. Critical reviewing: LW-G, CC, and VP. Project coordination: VP. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Chemical toxic exposures and chronic ocular pain

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Chronic ocular pain is a common, debilitating chronic pain condition with significant morbidity and negative impact in patients' quality of life. Several, diverse types of insults to the ocular surface can lead to acute, and under certain conditions to chronic ocular pain, and these include toxic irritants. Exposure of ocular surface to toxic irritants, in addition to direct tissue injury, carries the capacity to generated intense immune and neuronal responses with hyper-excitability, sensitization and chronic pain. Because, chronic ocular pain subsequent to toxic exposures is relatively unrecognized clinical entity, this brief review highlights pertinent concepts of its epidemiology, pathogenesis/pathophysiology, clinical progression, with recommendations for its clinical management that clinicians may find helpful. Suppression of pain signaling, generating neuronal sensitization, and prevention of chronicity of neuropathic pain is particularly emphasized in this respect.

KEYWORDS

chemical injury, toxicity, ocular surface, cornea, ocular pain

Introduction

Chronic ocular pain (COP) is a common and debilitating form of chronic pain, perceived as originating from the ocular surface, and frequently extending to adjacent facial structures; including the eyelids, supra/infra-orbital face, and/or the temporal region (Galor et al., 2015; 2022).

COP manifests as spontaneous pain, frequently accompanied by intensely abnormal sensations (tactile allodynia, allodynia to cold, photo-allodynia/photophobia), as well as orofacial pain, TMJ pain, and headaches. It is associated with significant psycho-social dysfunction and can contribute to an extremely poor quality of life (Galor et al., 2018; 2022; Mehra et al., 2020).

The etiology, pathogenesis, and pathophysiology of chronic ocular pain is variable and includes traumatic, post-surgical, post-LASIK (Theophanous et al., 2015), chemical (Wagoner, 1997; Eslani et al., 2014; Kwok and Chew, 2019; Dua et al., 2020; Hoffman et al., 2020; Soleimani and Naderan, 2020), thermal, or infectious noxious insults (Kaufman, 2008). These insults cause sensitization of the peripheral and central sensory afferent pathways, which generate persistent pain and emotional dysfunction (Guerrero-Moreno et al., 2020; Dieckmann et al., 2022; Galor et al., 2022).

Chronic ocular pain can be persistent and debilitating. It has been suggested that easing the burden of COP requires further exploration of its neurophysiology, diagnostic modalities, preventative and treatment strategies (Mehra et al., 2020; Galor et al., 2022). This requires further elucidating the pathophysiology of COP, identifying relevant pharmacology for its management, as well as identifying targeted therapies onto the ocular surface that may reduce the nociceptive burden and drive to neuronal sensitization.

COP remains extremely difficult to treat partly because of its diverse etiology and pathophysiology, because of insufficient knowledge of its underlying mechanisms, and partly because of the lack of specific treatments. Currently available treatment options include various, mostly systemic, analgesic medications, the use of which is limited by significant untoward side-effects (Goyal and Hamrah, 2016; Galor et al., 2022).

Better understanding of the possible causes of COP, its cause-specific pathogenesis, and treatment options may have a positive impact towards its prevention and therapy. Chronic ocular pain generally remains an underappreciated, underdiagnosed and undertreated disease entity, and many ophthalmologists as well as non-ophthalmologist physicians (such as pain specialists, neurologists, primary care physicians, etc.) are at a loss when they treat patients suffering from eye pain, including chronic pain subsequent to toxic chemical insults to the eye. To their assistance, this review aims to highlight pertinent concepts to ocular pain as a result of toxic chemical irritants to the eye surface. These toxic injuries are common and may have a huge impact on the quality of life of patients, not only from the perspective of loss of vision, but from the perspective of chronic, debilitating pain as well. This brief review will highlight basic concepts pertinent to this condition that should be brought to the attention of non-ophthalmology practitioners.

Epidemiology

Ocular chemical injuries are more likely to occur in the workplace than at home. Toxic chemical injuries account for the second-most common ocular workplace injuries (10%–25%), behind foreign body ocular injuries (35%–50%). Most injuries are reported in the industrial service injury. Men are at least 2 times more likely to suffer a chemical ocular injury than women. Chemical injuries occurring at home are more common in the pediatric population, particularly involving household cleaning agents, medications, and agricultural chemicals (Bizrah et al., 2019). In all settings, splash injuries account for most chemical ocular accidents (Midelfart et al., 2004; Quesada et al., 2020).

The incidence of developing chronic ocular pain from a toxic chemical ocular injury is largely unknown and likely underreported. There are, however, several case reports of patients suffering from chronic ocular pain after chemical injuries from a variety of agents (Memarzadeh et al., 2004; El-Hofi and Helaly, 2019). In a descriptive study conducted on 149 war veteran patients exposed to sulfur-mustard gas, nearly half (43%) described persistent pain many years after the insulting injury (Ghasemi et al., 2009). Clinical outcomes pertaining to vision loss are dependent on the type and amount of chemical agent involved, the duration of chemical exposure, the depth of chemical penetration through the eye, the involvement of extra/intraocular structures, the time to initiation of treatment, the therapeutic course, and the natural healing response (Dua et al., 2020). It is likely that the incidence and extent of chronic ocular pain formation is dependent on the extent of the ocular injury, the therapeutic course taken, as well as the predisposition of a patient for the development of chronic ocular pain—such as a patient with other chronic overlapping pain conditions. It seems that prompt recognition, and appropriate treatment, including management of the pain, acutely as well as in the context of prevention of its transition to chronicity, may be of great importance in ensuring the best possible outcomes.

Pathophysiology

The surface of the eye, particularly the cornea, is the most densely innervated tissue in the body with a nerve density up to 600 times that of the skin, and up to 40 times that of the tooth pulp (Rózsa and Beuerman, 1982; Midelfart et al., 2004; Quesada et al., 2020). Peripheral afferent sensory fibers are located only a few microns below the corneal surface, allowing for significantly direct exposure to topically acting chemical irritants. When exposed to toxic chemicals onto the surface of the eye, these sensory fibers become directly activated generating intense nociceptive signaling and acute pain. Activated peripheral nociceptive nerve fibers are further sensitized by the ensuing inflammation of surrounding tissues (Wenk and Honda, 2003). Neuronal activation, and sensitization as a result of the ensuing inflammatory response, and ongoing pain may have a significant impact in subsequent events with the potential of transition to chronicity.

Because the majority of sensory afferent neurons in the cornea are nociceptors (Lele and Weddell, 1959; Beuerman and Tanelian, 1979), the primary manifestation of toxic chemical irritants to the eye surface is induction of pain sensation as a result of nociceptive signals entering the brain via the trigeminal nerves. Yet, other mechanisms ensue, in addition to the perception and expression of pain.

The central axons of the trigeminal afferents innervating the cornea project onto two distinct regions of the trigeminal subnucleus caudalis (Vc), specifically to the rostral caudalis/interpolaris transition region (Vi/Vc) and the more caudally located Vc junction in the upper cervical spinal cord (Vc/C1) (Marfurt, 1981; Panneton and Burton, 1981; Marfurt and Del Toro, 1987). These areas respond to nociceptive afferent signals originating from the eye surface (Meng et al., 1997). It seems that the Vi/Vc projection neurons and the Vc/C1 projection neurons may respond differentially to different noxious stimuli, yet chemical irritants activate specific neuronal populations in both sites (Bereiter et al., 2000). These neuronal populations also have the capacity to recruit endogenous modulatory mechanisms during corneal pain (Hirata et al., 2000). This may result in attenuation of the initial painful phase in some patients, while in other patients (under different circumstances, determined by the interaction of individual genetic background and type of exogenous noxious insult) there is a transition to chronicity, whenever mechanisms such as neuro-inflammation and prolonged central sensitization develop.

Various animal models of pain after toxic exposure to the eye have been proposed as means to study those mechanisms. These models employ instillations of toxic chemical to the cornea of animals, such as alkali, or other irritants, and subsequent evaluation of pathological, pathophysiological and neurobehavioral parameters. These (mostly rat and cat) animal models utilize either objective behavioral methods of measuring presence of ocular pain (ie: increase in blinking) or measure changes

in neuron firing via surgical insertion of electrodes at various sites along pain pathways (Meng et al., 1997; Wenk and Honda, 2003). These studies facilitate understanding of the pathophysiology and pharmacology of this condition.

Yet, clinically there is an unmet need for specific (and less invasive) diagnostic tools to detect the insulting neural etiologies in patients suffering from COP (Galor et al., 2022), and this applies to pain after toxic chemical exposures, as well. Neuropathic COP after toxicity to the cornea can be diagnosed using a variety of currently available questionnaires, although most are subjective and invalidated. Further diagnostic information can be measured via slit-lamp examination, corneal staining and evaluation of tear production. Direct measures of nociceptor responses in patients include the use of esthesiometry, proparacaine challenge testing, or *in vivo* confocal microscopy, although these tests are largely limited to evaluating the ocular surface (Goyal and Hamrah, 2016). While functional brain mapping has been shown to elucidate visible changes in patients suffering from chronic pain, it is unlikely that these tests provide significant information that would alter clinical management at this time.

Chemical injuries to the surface of the eye cause direct corneal and eye surface injury, including ulcers, as well as inflammation and subsequent events leading to pain, that under some conditions may become chronic (Wagoner, 1997). Yet, chemical injury to the eye has been also shown to extend beyond the cornea, with damage to the retina and optic nerves (Paschalis et al., 2017).

Chemical toxicity by silver nitrate (Wenk and Honda, 2003) has revealed that chemical toxicity rapidly generates acute inflammation with corneal edema and infiltration of neutrophils. Antidromic conduction of acute nociceptive signaling by chemical toxicity may also result in release of neuroexcitatory neuropeptides (such as vasoactive intestinal polypeptide (VIP)) from peripheral terminals of corneal primary afferents adjacent to those exposed to toxic chemicals, thus extending the inflammation (neurogenic inflammation). In contrast, substance P and calcitonin gene-related peptide do not cause any sensitization of corneal afferent neurons (Belmonte et al., 1991; 1994). This notion may be clinically pertinent considering the lack of therapeutic effects of anti-CGRP agents (used for migraines) in the treatment of corneal pain. Yet VIP may contribute to peripheral afferent sensitization and proper strategies aiming at its blockade may be of value clinically and worthy of future investigations.

This inflammatory response subsequent to chemical toxicity is accompanied by increased sensitivity to stimuli (corneal hyperalgesia) by which application of stimuli produces exaggerated responses. This hyperalgesic response is analogous to the acute inflammatory response with hypersensitivity observed in cutaneous tissues after similar noxious toxicity, including edema, neutrophil aggregation and hyperalgesia. In contrast to cutaneous tissue, however, the cornea is not vascularized, therefore corneal inflammation subsequent to chemical toxicity follows a more delayed and prolonged time course and immune cell (mainly neutrophil) infiltration (Wenk and Honda, 2003). The same model indicates that the initial sensitivity may resolve, as a result of recruitment of endogenous descending inhibitory mechanisms from the CNS, while edema and inflammation may persist. Yet, under conditions of more prolonged and extensive nociceptive input, the inhibitory mechanisms may be overwhelmed and more prolonged peripheral sensitization as well as central sensitization may ensue, leading to chronicity of pain.

This has been supported by another animal model of corneal toxicity, induced by repeated topical installations of 0.2% benzalkonium chloride (BAC) onto the left eye of mice (Launay et al., 2016). This model is well-characterized, and simulates conditions like those observed in humans, with reduced tear production ("dry eye") and with actual pain in the ocular surface. BAC results in neuro-inflammation, results in neurotoxicity in the trigeminal nerve and in projection neurons, accompanied by enhanced hypertonic saline-evoked eye wiping behavior (consistent with hyperalgesia) (Launay et al., 2016). This model results in observed inflammation, neurotoxicity and increases neuronal (FOS, ATF3) and pro-inflammatory (IL-6) markers in trigeminal ganglion neurons, accompanied by enhanced hypertonic saline-evoked eye wiping behavior (suggestive of hyperalgesia). Several markers, suggestive of neuroexcitatory changes in the primary (trigeminal) and second orders afferents pathways (Vi/Vc, Vc/C1 regions) and in the glia have been identified, all of which play a primary role in the central sensitization that drives and maintains chronic ocular pain (Launay et al., 2016). These phenomena may be considered as clinically relevant, explaining the chronicity and intractability of pain is susceptible patients with established neuronal sensitization and centralization of their chronic pain, including their resistance to peripherally targeted therapies.

Other models that include chemical toxicity by 0.75 N NaOH alkali solution to the cornea have revealed the development of corneal nerve damage with an acute profound loss of corneal nerve density, which is followed by a delayed but aberrant reinnervation. Corneal nerve damage was accompanied by ocular hyperalgesia in that model. Furthermore, several abnormalities have been observed in the reinnervating nerves such as neuroma formation, increased tortuosity, beading, and abnormal branching, indicating the presence of mechanisms that contribute to neuropathic pain, in addition to nociception and inflammation (Cho et al., 2019). Therefore, the notion of significant neuropathic pain mechanisms, subsequent to toxicity, should be highlighted in the context of pathophysiology and clinical treatments after chemical toxicity to the ocular surface.

So, it seems very likely that the initial noxious insult from toxic exposure to the cornea may initiate activation of the corneal nociceptors, their sensitization, and generation of afferent nociceptive signals, and synaptic excitatory input to central nociceptive pathways that mediate the sensation of pain and the establishment of central sensitization. While varying types of chemicals may induce differentiating histopathological injuries and penetrate a variety of ocular layers, it is likely that a common pain pathway is shared among these injuries. Sensory nerve fibers (primarily unmyelinated C-fibers, but also A-δ fibers) originating from the ophthalmic division of the trigeminal nerve are activated directly via chemical activation of free nerve endings, polymodal receptors, via ectopic transmission, or damage to nerve axons inducing pathological pain signal transmission. These signals may originate at any of the terminal ocular branches of the trigeminal nerve, transmit to the trigeminal brainstem complex, then project centrally to the posterior thalamus and a variety of cortical targets including the insular cortex, amygdala, somatosensory and prefrontal cortex (Goyal and Hamrah, 2016). Peripheral sensitization, ensuing from local inflammatory and immunoreactive mechanisms secondary to

toxicity, as well as central neuronal sensitization, and neuroinflammation, may further sustain chronicity of pain, as well as parallel events, such as photophobia, and emotional dysfunction (Digre and Brennan, 2012; Galor et al., 2015; 2022). These are overall difficult to treat once established. The mechanism of conversion from acute nociceptive pain to chronic neuropathic ocular pain is not fully elucidated and may be more complex than the formation of chronic neuropathic pain in other peripheral nerves. Peripheral sensitization ensues largely after lasting pathological activation of affected nerves deregulates regular transmission activity. Injured terminal neurons can form microneuromata resulting in ectopic pain transmission (Goyal and Hamrah, 2016). Aberrant neural modulation may also originate at the level of cell bodies in the trigeminal ganglia, similarly to the pathological modulation at the level of the dorsal root ganglia in other peripheral neuropathies. Central sensitization to pain is also a key component of the overall development of chronic pain. Central sensitization begins at the level of the second-order neuron synaptic transmission, via upregulation of largely glutamate receptors. Further central sensitization ensues with activation of neuroinflammatory cascades at the level of the brain, with the activation of astrocytes and microglia. This chronic sensitization eventually leads to maladaptive neuroplasticity which is evident with the development of both experienced patient symptoms and objective findings on neuroimaging (Seifert and Maihofner, 2011; Vehof et al., 2013; Crane et al., 2017). While temporal maladaptive developments have not fully yet been elucidated, there is compelling evidence to suggest that the central sensitization leading to chronic pain development is dependent on plasticity of the mesolimbic-prefrontal circuit, then neocortical reorganization. The functional implications of aversive motivational learning and memory formation associated with neuroplasticity in dopaminergic projections (primarily from the ventral tegmental area), and glutamanergic projections (primarily from the amygdala and hippocampus) are likely the mesolimbic changes adapting to constant pain signalization from the periphery (Mansour et al., 2014). The development of chronic pain has also been associated with central changes co-existing in patients with depression, a development likely directly linked due to the high neuro-inflammatory role of pain-related central sensitization (Krishnadas and Cavanagh, 2012). There are fewer amygdala connections with serotonergic projections in patients with chronic pain as well as with depression (Zheng et al., 2022). Both concomitant states have been associated with global cortical gray matter volume alterations (Ma et al., 2022). Furthermore, the two separate conditions may augment each other, worsening the states of both depression and chronic pain (Surah et al., 2014).

In order to guide treatment efforts, grading the extent of the ocular injury is done via either the Roper-Hall classification and/or the Dua et al. classification systems. The Roper-Hall classification system is based on the extent of corneal injury and limbal conjunctival ischemia. It does not consider direct conjunctival injury. The Dua et al. classification system factors in conjunctival injury (percentage of injury) and limbal injury (graded by surface-area "clock hours"). The Dua et al. classification system does not, however, factor in corneal injury. A significant drawback to both classification systems is that neither system takes into consideration the depth of chemical injury, which is of particular significance when considering the pathological etiology for patients' presentations of COP (Bizrah et al., 2019). Furthermore, while both classification systems have been trialed independently and comparatively to prognosticate recovery and lead treatment efforts, there has not been an assessment of utilizing these classification systems for the evaluation and management of COP.

Common toxic chemical ocular injuries

Numerous toxic chemicals in existence can cause ocular damage upon direct exposure to the ocular surface. However, similar pathological reactions are seen within certain groups of chemicals. There are largely 3 different groups of toxic chemicals that induce specific reactions: acids, bases, and "other" chemicals. It is unknown currently whether a certain group of chemicals induces COP with a higher incidence than another group of toxic chemicals.

Alkali agent injuries

Base, or alkali, chemicals account for more significant ocular injuries (80%) than acidic chemicals. Alkali injuries are particularly significant if the pH of the chemical irritant is > 11.5 (Dua et al., 2020). Alkali chemicals are more prone to causing significant ocular injuries due to their ability to penetrate deeper into the eye than acidic or other chemicals. Alkali chemicals can react with cellular membranes causing saponification and lysis of membranes (via hydroxyl ion release and conversion of cell membrane lipids to salts). This will subsequently cause destruction of the ocular epithelial layer and allowance for penetration directly into the anterior chamber of the eye. Further allowance of penetration into the anterior chamber is mediated by the basic chemical's ability to hydrolyze the ocular surface interfibrillar glycosaminoglycans and disrupt the intact extracellular matrix. If enough of the corneal surface is damaged, alkali toxins have been known to induce a temporary paradoxical anesthesia of the eye due to significant nerve destruction. Lime-plaster is the most common cause of alkali chemical ocular injury. The toxin within lime is $Ca(OH)_2$, a chemical compound commonly used in plastering or cementing. The calcium component produces a soap upon saponification within the epithelium, which hinders further penetration into the eye. Ammonia (NH_3) is a compound used mostly in manufacturing, in fertilizer, and in the chemical industry. It has the fastest rate of penetration through the ocular surface. Another compound with a fast penetration rate is lye (NaOH), which is a common chemical component in drain cleaning products. Other alkali chemicals commonly encountered include potash (KOH), used in the chemical industry, or $Mg(OH)_2$, which is found in some fireworks (Morgan, 1987; Lusk, 1999; Dua et al., 2020).

Acidic agent injuries

Although very capable of causing catastrophic ocular pathology and subsequent chronic pain, acidic chemicals are generally less damaging when contacting the human eye than alkali chemicals. While most alkali chemicals are capable of deep penetration past the anterior chamber of the eye, acidic chemicals are generally stabilized before penetrating the anterior chamber. Acidic compounds

primarily cause denaturation and coagulation of the corneal epithelium. Certain acids, such as Hydrofluoric acid (HF), can penetrate deeper into the anterior chamber of the eye. HF is used for rust removal, brick cleaning, pottery glazing, as a lab reagent, and is sometimes used in the semiconductor industry. Yet, the most common ocular injury from acidic chemicals is due to sulfuric acid (H_2SO_4). Sulfuric acid is primarily found in car batteries, fertilizer, or used in the metal industry. Other acidic chemicals that commonly cause ocular injuries include HCl (used in the steel industry or chemical manufacturing) or nitric acid (used in chemical manufacturing) (Morgan, 1987; Lusk, 1999; Dua et al., 2020).

Other chemical agent injuries

Besides acids and bases, other chemicals can cause devastating ocular injuries and subsequent COP. Some common offenders include hydrocarbon fuels (such as gasoline or kerosene) or hydrocarbon solvents (used as cleaning/dissolving substances or paint thinners). These chemicals generally cause superficial pain upon irritation of the cornea, but they do not generally further penetrate the eye. At low concentrations typically encountered, irritant lacrimators (such as pepper-spray) similarly cause corneal nerve stimulation without further structural injury. Cyanoacrylate adhesives (found in a variety of glue preparations) can cause corneal damage from abrasions due to rapid adherence of the eyelids to the cornea. At household strength, H_2O_2 is usually no more than just an irritant to the corneal surface. Similarly, alcohol solutions generally cause limited irritation. Formaldehyde, which is used as a preservative, causes mild irritation initially, but it can potentially penetrate the anterior chamber of the eye if it is not promptly washed out (Morgan, 1987; Lusk, 1999; Dua et al., 2020).

Evaluation and treatment of patient with neuropathic corneal pain

There are no known definitive ophthalmologic interventions that prevent the development of chronic neuropathic ocular pain after injury from chemical exposure. It is these authors' belief that the best way to prevent chronic neuropathic ocular pain after injury from chemical exposure is to promptly eliminate the toxic irritant from the ocular surface, to address ocular surface inflammation, and to offer acute pain control. It is important to prevent further ocular pathology, to promote ocular healing, and to manage patient co-morbidities.

After ensuring the patient is hemodynamically stable and without injury to their airway that may require stabilizing interventions, promptly addressing the ocular health of a patient suffering from a chemical splash injury is of paramount importance. Copious irrigation of the eye is, by far, the most important acute intervention to remove the noxious, toxic agent and to prevent complications after toxic chemical exposure to the eye. Irrigation is recommended for at least 30 min. Irrigation with water is an acceptable method, although more balanced solutions (such as lactated Ringer's) may prevent worsening ocular edema (Dua et al., 2020). Amphoteric chelating agents (ex: EDTA) may be used to neutralize both acid and alkali solutions without causing harmful exothermic reactions. If the injury is secondary to an alkali solution, where deeper penetration is likely, it may be beneficial to utilize hyperosmolar amphoteric solutions (v. iso-osmolar), to achieve deeper irrigating effects and pH control (Soleimani and Naderan. 2020). If the insulting agent is hydrofluoric acid based, irrigating with Hexafluorine ® (an acid (H+) and fluoride (F-) ion absorbent) may yield superior irrigating results. Oil or EDTA may be a preferred irrigating solution for quicklime (calcium oxide) ocular burns (Lorenzana-Blanco et al., 2023). Irrigating efforts should not, however, be delayed in anticipation of obtaining a particular irrigating solution.

While ocular irrigation is imperative to initial treatment efforts of patients with ocular chemical injuries, there must be an emphasis towards ensuring total chemical decontamination of both patients and caretakers. The Occupational Health and Safety Administration (OSHA) outlines an evidence-based decontamination plan that can protect patients and caretakers from further injury after exposure to chemical agents (Department of Labor Logo United States department of Labor, 2023). Prevention of contamination is imperative and involves the utilization of safety equipment (disposable garments, goggles, etc.) and safe work practices (ex: remote sample handling, safe container-opening techniques, etc.). These practices extend to caretakers that respond to patient injuries. Decontamination methods then include physical removal of contaminations (water rinsing, vaporization, scrubbing, steaming), chemical inactivation, disinfection/sterilization, irradiation, and chemical removal (dissolving, surfactant utilization, solidification techniques). Finally, it is important to test for the effectiveness of decontamination, utilizing visual observation, wipe sampling, solution analysis, and permeation testing. Ongoing research continues in improving community awareness towards proper chemical decontamination efforts, the development of novel decontaminants, and addressing novel chemical toxicities. Of note, there is also a priority for future research towards developing methods of decontamination of hair, which may serve as a reservoir for further ocular injury after initial ocular irrigation efforts (Collins et al., 2020; Collins et al., 2021).

After irrigation, an ophthalmologist or emergency medicine provider should instill anesthetic eye-drops and remove any obvious particulate matter on the ocular surface. Adequate acute pain control is imperative for a thorough ocular assessment and continued therapeutic efforts. While not studied directly in the setting of COP, there is evidence to suggest that chronic post-traumatic pain may be avoided by preventing significant central sensitization to pain with adequate pain control in the acute phases of tissue injury (McGreevy et al., 2011). Acute pain control can be achieved with local anesthetic eye-drops and supplemented with oral or IV systemic pain medications. While topical NSAIDS are discouraged in chemical ocular eye injuries, it is unknown whether they are detrimental to ocular healing when administered systemically. Opioid based medications may be significantly beneficial in the treatment of acute ocular pain, which has a primarily nociceptive pathophysiology. However, opioids are unlikely to be beneficial in the treatment of COP, once the pain origins become neuropathic in nature. After achieving adequate analgesia, the ophthalmologist should then perform a thorough ocular assessment, examining the eyelids, cornea, pupil, ocular surface epithelium, stroma, intra-ocular anterior surface

segments, iris, lens, intra-ocular pressure, and fundus. The extent of the injury is classified by either the Dua classification or Roper-Hall classification system (Dua et al., 2020). The eye should be swabbed for cultures to rule out infections. Any necrotic tissue is then debrided. Broad spectrum topical antibiotics (ex: fluoroquinolone) is often instilled to prevent infections in the presence of epithelial defects (Dua et al., 2020).

At this juncture the ophthalmologist's therapies will be targeted to reduce further ocular inflammation, prevent further corneal melting, and management of potential increases in intra-ocular pressure (IOP). Steroid eye drops (dexamethasone or prednisolone) can be utilized to reduce inflammation and minimize proteolytic enzyme release within the cornea. It is of interesting note that topical NSAIDs are discouraged to manage inflammation, as they may contribute to further corneal melting. Sodium citrate eye drops, and oral tetracycline have also been shown to suppress the release of damaging proteolytic enzymes. IOP is managed with eye drops or laser therapy (iridotomy, trabeculoplasty, or cyclophotocoagulation). In certain instances, high IOP requires surgical management (MIGS, trabeculectomy, or shunt insertion) (Bizrah et al., 2019; Dua et al., 2020).

After initial ocular stabilizing interventions and inflammation control, the ophthalmologist's therapies are concentrated on the promotion of corneal re-epithelialization. Minimal ocular corneal irritation may be treated with solely lubricating eye-drops, however more extensive corneal damage would benefit from additional interventions. Current strategies for the promotion of corneal re-epithelialization include topical autologous peripheral blood-serum therapy, topical umbilical cord serum therapy, topical platelet rich plasma (PRP) therapy, or amniotic membrane transplantation. If there is a deficiency in limbal stem cells from extensive chemical injury, a limbal stem-cell transplantation can be considered to promote re-epithelialization (conjunctival-limbal autograft, *ex-vivo* cultivated limbal epithelial transplantation, or a simple limbal epithelial transplantation). Finally, a variety of corneal transplants (keratoplasty or keratoprosthesis) may be considered (Bizrah et al., 2019; Dua et al., 2020).

As it pertains to the evaluation and management of a patient presenting to a pain physician for concerns of chronic ocular pain after chemical injury, a thorough review of the patient's relevant medical history and ophthalmologic interventions is of paramount importance. Before considering COP, an ophthalmologist should rule out other underlying ocular pathologies that may be contributing to the patient's pain. Other neuropathic conditions, such as post-herpetic neuralgia or trigeminal neuralgia, should also be considered (Moshirfar et al., 2022). The patient should have a recent slit-lamp examination with the use of dyes (such as fluoresceine) to evaluate the general health of the corneal surface. A simple Schirmer's test should be recorded to test for the state of the lacrimal system and assess and quantify actual dryness. A proparacaine challenge is an in-office test that can be utilized to differentiate between peripheral and central neuropathic pain. If the pain is peripheral in nature—or originating at the level of corneal nerves—it should be effectively reduced by topical anesthesia with a topical local anesthetic (proparacaine), while central neuropathic pain would largely remain unchanged. Some centers are capable of esthesiometry, an exam which detects the mechanical nociceptor response and quantifies pain fiber function. *In-vivo* confocal microscopy can also be ordered to image the cornea at a cellular level and study regenerative changes of corneal nerves (ex: nerve sprouting, nerve fiber density, or neuroma formation) (Goyal and Hamrah, 2016; Dieckmann et al., 2017; Moshirfar et al., 2022).

The management of COP is currently largely underdeveloped. This also applies to COP after toxic chemical injuries to the eye. Therapies are largely concentrated at treating neuropathic-type pain, not-specific to corneal nerves. Specific evidence-based therapies are lacking in current literature. Nevertheless, a multi-step approach at managing the patient's pain is likely most beneficial. Therapeutic strategies aim to promote ocular surface healing, decrease inflammation, induce appropriate nerve regeneration, manage pain, and manage patient co-morbidities. An interdisciplinary approach is beneficial, as an ophthalmologist may manage ocular healing, a psychiatrist may manage concurrent psychological pathologies that frequent accompanies neuropathic eye pain and may contribute to overall illness and worsened pain control, a pain specialist may manage analgesic efforts, and a general practitioner may manage any other co-morbidities that can contribute to poor healing (Crane et al., 2017). Local therapies such as lid hygiene and warm compresses may be beneficial, as well as therapies geared at increased tear production, like preservative free artificial tears, and/or increased tear retention (i.e., moisture goggles). Anti-inflammatory medications such as topical corticosteroids, oral cyclosporine, tacrolimus, anakinra, topical and/or oral azithromycin or oral doxycycline may also prove helpful. Systemic pharmacotherapy remains one of the most effective methods of pain control for patients with COP. Serotonin-norepinephrine reuptake inhibitors (SNRIs) such as duloxetine (20–60 mg per day), venlafaxine (37.5–150 mg per day), or the newer milnacipran (12.5–50 mg per day) may be helpful and tend to have a better side effect profile when compared to the tricyclic antidepressant (TCA) class medications. However, TCAs can be very effective in treating COP, and nortriptyline (25 mg–100 mg nightly) is an excellent choice as it has less side-effects than amitriptyline (25 mg–100 mg nightly). Another first-line medication would be the anticonvulsant, carbamazepine (400–1,200 mg per day divided into 2-3 doses). A gabapentinoid medication (gabapentin 600 mg–3,600 mg per day divided into 3 doses, or pregabalin 75 mg–200 mg per day divided into 2-3 doses) may be a beneficial complementary medication. Low-dose naltrexone (LDN) is an evolving therapy that has been shown to be effective in treating neuropathic pain by attenuating neuroinflammation and central sensitizing mechanisms leading to chronic pain. If the patient's pain is refractory to first line pharmacotherapy, LDN may be instituted at doses 1.5 mg–4.5 mg per day. Other medications to consider for refractory COP may include mexiletine (225 mg QD–675 mg per day) or low-potency opioid medications, such as tramadol (50–100 mg once or twice a day) in extreme cases, although opioids should be better avoided (for several reasons including their propensity to induce opioid induced hyperalgesia).

Alternative therapies may be beneficial, particularly if used in conjunction to systemic pharmacotherapy. These therapies may include acupuncture, transcranial magnetic stimulation, or percutaneous electrical nerve stimulation, such as TENS applied peri-orbitally, or scrambler therapy. Nerve regenerative therapies are also described, such as autologous serum eye drops at a concentration of

20%–100%. While other types of regenerative therapies such as platelet rich plasma, nerve growth factor, and umbilical cord serum eye drops may be beneficial, they are not easily accessible at this time in the United States (Goyal and Hamrah, 2016; Dieckmann et al., 2017; Moshirfar et al., 2022). Other interventional therapies that have showed to be potentially effective in the treatment of chronic ocular pain (although not specific to chemical-induced COP) include trigeminal nerve stimulation, intrathecal pump therapy, or nerve blocks. Nerve blocks include peri-ocular nerve blocks of nerves adjacent to the eye, sphenopalatine ganglion blocks or superior cervical ganglion blocks in patients with parasympathecially- or sympathetically-mediated pain, respectively (Galor et al., 2018). Botulinum toxin injections may be useful for patients suffering from COP, particularly with concomitant migraines. More research is needed to determine the efficacy of these interventional therapies for the treatment of COP. Furthermore, there may be associated syndromes with COP that warrant multidisciplinary treatment. Patients suffering from COP may concurrently be suffering from other chronic pain conditions, including depression, fibromyalgia, chronic joint pain, and migraines (with or without ocular auras). It is unknown whether some of these conditions can be caused by COP, but there are underlying shared factors among patients suffering from these chronic pain conditions that may be exacerbated by the development of COP. Furthermore, permanent vision loss is linked with an increased risk for serious injuries, depression, anxiety, delirium, and overall poor quality of life (Haring et al., 2016; Crane et al., 2017; Baksh et al., 2021).

Conclusion

COP is a debilitating chronic pain condition that is often underdiagnosed and undertreated. It is a common occurrence after significant ocular damage from toxic chemical exposure. Patients with COP following chemical injuries may present to a chronic pain provider, and it is imperative to understand the basic epidemiology, pathophysiology, diagnostic modalities, and treatment options for these patients. Further research is needed to better elucidate the formation of chronic ocular pain after toxic chemical exposure. Additionally, further therapeutic options should be researched to better manage patients with chronic ocular pain.

Author contributions

MG: Literature search and preparation of the manuscript. KS: Literature search and preparation of the manuscript. DH: Literature search, preparation of the manuscript; senior and corresponding author. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The environment and dry eye—manifestations, mechanisms, and more

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Dry eye disease (DED) is a multifactorial condition that often presents with chronic symptoms of pain (that can be characterized as "dryness," "burning," and "irritation," to name a few) and/or fluctuating or poor-quality vision. Given its multifactorial nature, several pathophysiologic mechanisms have been identified that can underlie symptoms, including tear film, ocular surface, and/or corneal somatosensory nerve abnormalities. Research has focused on understanding how environmental exposures can increase the risk for DED flares and negatively impact the tear film, the ocular surface, and/or nerve health. Given that DED is a common condition that negatively impacts physical and mental functioning, managing DED requires multiple strategies. These can include both medical approaches and modulating adverse environmental conditions, the latter of which may be a cost-effective way to avoid DED flares. Thus, an understanding of how environmental exposures relate to disease is important. This Review summarizes research on the relationships between environmental exposures and DED, in the hope that this information will engage healthcare professionals and patients to consider environmental manipulations in their management of DED.

KEYWORDS

dry eye disease, ocular surface pain, environment, toxicology, temperature, humidity, air pollutant, allergy

1 Introduction

Dry eye disease (DED) is a common ocular condition and a major cause of chronic ocular surface pain and/or fluctuating and poor-quality vision. It is a multifactorial condition, characterized by tear film instability, high or unstable osmolarity, ocular surface inflammation, and/or somatosensory abnormalities. DED does not have a "gold standard" definition, but instead is often referred to as an umbrella term under which various disease phenotypes fit (Galor et al., 2020; Villani et al., 2020). Given this complexity, it is not surprising that heterogeneity exists with respect to the pathophysiological pathways underlying the disease (Craig et al., 2017; Ganesalingam et al., 2019). Of these, this Review will focus on how environmental exposures may impact DED onset, severity, and persistence.

This Review is needed as less is known about the relationships between DED and adverse environmental exposures compared to other disease contributors. For example, T-cell-mediated inflammation has been studied in individuals with DED and comorbid Sjögren's

syndrome (SS), neurovascular instability has been examined in individuals with DED and rosacea, and neuropathic mechanisms have been probed in individuals with DED and comorbid migraine or fibromyalgia. Other studies have focused on behavioral factors (e.g., contact lens use and smoke exposure) and medications (e.g., antihistamines, antidepressants, and antihypertensives) as they relate to DED.

In comparison to these established relationships, less is known about the etiology of DED in response to adverse environmental conditions. Given that DED impacts physical and mental functioning, understanding the factors that contribute to the disorder is essential and can help providers improve care algorithms and deliver precision medicine. Furthermore, certain environmental manipulations may be more cost-effective than medical therapy in controlling severe and/or refractory symptoms. This Review will summarize the current knowledge on the toxicological mechanisms of environmental exposures as they relate to DED manifestations.

2 Body

2.1 Symptoms and signs of DED

When examining studies on DED, it is important to understand the constellation of symptoms and signs that fall under the disorder. The diagnosis of DED is made by clinical examination, based on the presence of symptoms (e.g., that can be assessed with various validated questionnaires), slit lamp findings, and in-clinic point-of-care tests. Given that different risk factors may relate to different aspects of DED, it is important to examine disease definitions when reviewing epidemiological studies on DED.

For symptoms, ocular surface pain is a common complaint patients present with, with common descriptors that include "dryness," "burning," "aching," and "tenderness," to name a few. Pain symptoms can arise from nociceptive sources (activation of nociceptors due to abnormalities in peripheral tissues), neuropathic sources (abnormalities in somatosensory pathways to and from the cornea), or a combination of both (Stucky et al., 2001; Basbaum et al., 2009). Ocular surface pain, whether secondary to DED or other causes, is a major source of morbidity, and DED-associated chronic ocular surface pain is a leading cause of ophthalmic healthcare costs (Yu et al., 2011) and has deleterious effects on the quality of life and productivity (Goyal and Hamrah, 2016; Patel et al., 2019). Considering all symptoms of DED (pain and visual symptoms), cost-of-illness analyses have estimated the burden of DED at nearly $3.84 billion, including indirect costs (loss of work) (Yu et al., 2011).

Ocular surface pain can be quantified using standardized questionnaires, each aimed at eliciting different characteristics of pain. For example, the 5-Item Dry Eye Questionnaire (DEQ-5) assesses the frequency and intensity of dryness and discomfort, along with the frequency of tearing (Chalmers et al., 2010). The Ocular Surface Disease Index (OSDI) assesses the frequency of sensitivity to light, grittiness, and painful or sore eyes, along with visual symptoms, triggers, and quality of life implications (Schiffman et al., 2000). Pain-specific questionnaires have also been developed, most of which use a Likert-type Numerical Rating Scale (NRS), including the Ocular Pain Assessment Survey (OPAS; intensity, descriptors, and quality of life) (Qazi et al., 2016) and the Neuropathic Pain Symptom Inventory modified for the Eye (NPSI-E), the latter of which focuses on neuropathic descriptors of eye pain (Farhangi et al., 2019).

"Signs" of the disease are examined with in-clinic tests that assess ocular structure and function, with certain thresholds used as cut-offs for the clinical diagnosis of DED. These include tests that look for alterations in tear stability (e.g., tear breakup time (TBUT)) and production (e.g., Schirmer's test, with or without anesthesia) and structural integrity (e.g., corneal and conjunctival staining using vital dyes such as fluorescein, lissamine green, and Rose Bengal), and assess corneal function and structure (Mehra et al., 2020; Patel and Sarantopoulos, 2023). Corneal function can be evaluated via corneal sensitivity (qualitatively assessed in the clinic with a cotton tip or floss or quantified in the research arena with an esthesiometer). Structural attributes are examined microscopically via *in vivo* confocal microscopy (IVCM); commonly reported findings include the presence of immune cells within the cornea (e.g., dendritic cells, most commonly noted in individuals with aqueous tear deficiency (ATD) in the setting of auto-immune disease) and corneal nerve abnormalities (e.g., decreased nerve density and increased nerve tortuosity, also common in individuals with systemic auto-immune diseases such as SS) (Hwang et al., 2021).

2.2 Environmental health risks

Risk relationships with environmental factors have been studied for several ocular and systemic conditions (Paschides et al., 1998; Syndulko et al., 1996; D'Amato et al., 2015; Michelozzi et al., 2009). Generally, studies classify exposures as "indoor" or "outdoor" (also known as ambient) when reporting associations. Studies on outdoor exposure are more common, even though we spend most of our time indoors, at least partially due to the availability of national ambient meteorological databases. Some commonly studied factors include air pollutants (e.g., ozone, O_3; nitrogen dioxide, and NO_2), aeroallergens (e.g., pollen, dander, mold, and dust), meteorological conditions [e.g., temperature and relative humidity (RH)], interaction effects (e.g., the effect of temperature and RH simultaneously, also known as heat stress), and behavioral factors (e.g., exposure to smoke, chemicals, medications, and contact lens use). It is important to note that studying the environment is challenging, regardless of the type of exposure—the study of ambient variables requires the integration of patient health data and environmental data with different spatiotemporal scales, which can result in exposure uncertainty (Kumar, 2016). On the other hand, accurate measurements of indoor variables can require special devices like climate control chambers (Calonge et al., 2018) to control indoor exposures or a special set-up to measure exposures.

2.3 Outdoor environment

2.3.1 Temperature

Perhaps the least studied of all ambient variables, toxic exposure to temperature is thought to mainly affect ocular health by its

influence on the tear film (Nagymihályi et al., 2004). Controlled chamber studies have described the direct impact of temperature on the tear film. Specifically, two controlled environment chambers in Europe (Abusharha and Pearce, 2013; Abusharha et al., 2016) exposed individuals to increasing ambient temperatures at constant RH and found that tear film parameters varied by temperature level. One study found that lipid layer thickness increased with increasing temperature (20–40 nm at 5°C and 10°C vs. 40–90 nm at 15°C, 20°C, and 25°C; $p < 0.05$) (Abusharha and Pearce, 2013). Similar findings were noted in the second study (median lipid layer thickness 20–40 nm at 5°C and 10°C vs. 40–90 nm at 15, 20, and 25°C; $p < 0.005$), but interestingly, this second study also found that the evaporation rate increased with temperature (0.06 µL/min at 5°C vs. 0.17 µL/min at 25°C; $p < 0.005$) (Abusharha et al., 2016). These findings are difficult to interpret, as other studies found that a thicker lipid layer led to a lower evaporation rate, thus having a protective effect on the ocular surface (Craig and Tomlinson, 1997; Giraldez et al., 2009). As such, further research is needed to understand how temperature impacts the risk of ocular surface disorders like DED, beyond its effects on lipid thickness.

On an epidemiological level, associations between temperature and DED have varied. A Taiwanese study of 25,818 subjects with known DED (not further defined) found that temperature was positively associated with a DED diagnosis (β = 1.01, 95% CI = 1.00 to 1.02; $p < 0.001$). In this model, RH had an inverse relationship with DED (β= 0.93, 95% CI = 0.91 to 0.95; $p < 0.001$), and NO_2 had a positive relationship (β = 1.08, 95% CI = 1.04 to 1.11; $p < 0.001$) (Zhong et al., 2018). In comparison, a Taiwanese study of 351 patients with known DED (OSDI ≥ 13, TBUT ≤ 5, staining) reported that temperature was inversely related to symptoms (via OSDI; β = −0.84, 95% CI = −1.34 to −0.33; $p < 0.005$) and tear production (Schirmer's: β= −0.73, 95% CI = −1.19 to −0.26; $p < 0.005$) (Ho et al., 2022). Further highlighting the variable findings on temperature, an American study that examined 3.41 million visits at Veteran Affairs (VA) eye clinics between July 2006 and July 2011 found that DED (via ICD9 code; diagnosed in 17.4% of the study population) was most frequently diagnosed in the winter and spring months, compared to the fall and summer (18.7% ± 0.98% and 18.5% ± 4.16%, respectively), with the highest frequency occurring in April (20.9% ± 0.14%) (Kumar et al., 2015). These data suggest that factors beyond temperature alone may impact DED presentation.

In summary, while there is evidence suggesting that the tear film and lipid layer are affected by temperature extremes, the findings are inconsistent as to which extreme of the temperature scale is most harmful. These findings may suggest that the relationship between temperature and DED is non-linear and is instead possibly a "U"-shaped curve, with a "Goldilocks" zone (temperatures below or above this zone having a detrimental impact on tear film health). In fact, the American Society of Heating, Refrigerating, and Air-Conditioning Engineers has recognized the concept and recommended that the indoor temperature be set between 20°C and 25°C (Abdul-Wahab et al., 2015). Further studies are necessary to understand and translate this recommendation to individuals with DED.

Exposure to temperature change is another important factor that may relate to the risk of DED. Studies examining temperature change often utilize the diurnal temperature range (DTR) as a measure of change, which measures the difference between the maximum and minimum daily temperature. A higher DTR has been reported as a risk factor for disease flares across several conditions, from asthma (Xu et al., 2013; Kim et al., 2014; Qiu et al., 2015; Li et al., 2016) to heart failure (Lim et al., 2012). It is hypothesized that exposure to abrupt temperature change may impact the function of immune cells involved in inflammatory and allergic presentations, specifically through altered release of cytokines and cytotoxic proteins (Lobefalo et al., 1999; Graudenz et al., 2006). The aforementioned American study, which studied visits to 3.41 million VA eye clinics across the United States between July 2006 and July 2011, reported on this association with respect to DED—the study found that change in temperature had more influence on DED presentation than absolute temperature throughout each season. The greatest decrease in symptom intensity (via OSDI, DEQ5, and NPSI-E) occurred in winter and summer, when the weather change from the previous season was less abrupt, compared to spring and autumn. The study hypothesized that abrupt meteorological changes may have a detrimental effect on the lacrimal unit (Kumar et al., 2015); however, further studies are necessary to test this hypothesis.

2.3.2 Relative humidity (RH)

Low RH (e.g., desiccating stress) is a well-described risk factor for DED (Smith, 2007). Adequate production and stability of tears is essential to a healthy tear film, and any destabilization in these variables can lead to ocular surface diseases like DED. While the pathway is not entirely understood, studies have implicated a negative association between RH and tear osmolarity (e.g., induction of stress via a hyperosmolar mechanism) (González-García et al., 2007) and alterations in protein oxidation within the tear film and Meibomian lipids (Abusharha and Pearce, 2013) as potential causes of this relationship. In addition to this, RH has also been shown to directly affect tear film evaporation; specifically, aridity affects vapor concentration, which, together with tear film thickness, determines the evaporative flux at the ocular surface (Peng et al., 2014). In this manner, RH may also exert its effects on tear film health by influencing the tear evaporation rate, a finding described in several studies.

Describing these effects, one of the previously described European climate chamber studies also reported on the relationship between RH and tear dynamics. This study examined two conditions: RH set at 40% (normal) and at 5% (desiccating stress). Tear film abnormalities noted in the desiccating (low RH) environment included an increase in tear evaporation, a decrease in tear production, a decrease in lipid layer thickness, and an increase in ocular pain (specific data not provided; $p < 0.05$ for each) (Abusharha and Pearce, 2013). Supporting these human findings, a mouse study reported that exposure to low RH (RH = 18.5% ± 5.1%) for 28 days after an initial equal exposure to normal RH (RH = 50%–80%) led to decreased tear production via cotton thread wetting (baseline: ~2.2 ± 0.2 mm; day 3: 1.4 ± 0.3 mm; $p < 0.005$; day 28: 1.3 ± 0.4 mm; $p < 0.05$) and increased fluorescein staining (baseline: ~1.5 ± 1.5; day 3: 5.8 ± 2.2; $p < 0.0001$; day 28: 4.6 ± 2.3; $p < 0.05$) (Barabino et al., 2005). However, not all studies reported an inverse relationship between DED and RH—one English study of

10 individuals with mild–moderate DED (symptoms, TBUT<10 s, Schirmer<10 mm) and 10 controls exposed groups to varying RH (5%, 40%, and 70%, for 25 min on 3 separate days). As the RH increased from 5% to 70%, tear evaporation rates linearly decreased in both groups (~100 g/m^2/hr at 5%, ~70 g/m^2/hr at 40%, and ~0 g/m^2/hr at 70% for DED vs. ~90 g/m^2/hr at 5%, ~40 g/m^2/hr at 40%, and ~0 g/m^2/hr for controls; $p < 0.05$ between points in each group, respectively), supporting the results of the previous studies. However, tear stability (TBUT) followed a U-shaped curve in both groups with varying RH (4.90 ± 1.66 s at 5%, 6.31 ± 2.21 s at 40%, and 5.90 ± 1.91 s at 70% in the DED group vs. mean 17.80 ± 3.91 s at 5%, 20.70 ± 5.88 s at 40%, and 20.00 ± 5.35 s at 70% in controls), suggesting an optimal value at 40% RH (Madden et al., 2013).

Many epidemiological studies have noted relationships between DED and RH. A Taiwanese study of 25,818 subjects diagnosed with DED found that lower RH (β = 0.93, 95% CI = 0.91 to 0.95; $p < 0.001$) was associated with DED diagnosis, along with temperature and NO_2 (Zhong et al., 2018). A Korean study of 16,824 participants from January 2010 to December 2012 found an inverse relationship between RH and DED symptoms (OR = 0.87; 95% CI = 0.77 to 0.98; $p = 0.03$) and diagnosis (OR = 0.86; 95% CI = 0.76 to 0.97; $p = 0.01$) (Hwang et al., 2016). Supporting this association, a Chinese case-crossover study of 5,062 individuals diagnosed with DED found that lower RH was associated with an increased risk for an outpatient DED diagnosis visit (specific data not provided; $p < 0.05$) (Mo et al., 2019). However, just as observed with the chamber studies, not all epidemiological studies have reported an inverse relationship—one American study of 97 individuals who underwent indoor RH monitoring instead found that RH was positively associated with symptoms (OSDI: r = 0.30, 95% CI = 0.07 to 0.49; $p = 0.01$) and Meibomian gland (MG) dropout (r = 0.27, 95% CI = 0.05 to 0.47; $p = 0.02$), and negatively associated with tear production (Schirmer: r = −0.25, 95% CI = −0.45 to 0.02; $p = 0.03$) (Huang et al., 2020). Of note, the group hypothesized that the noted association between RH and DED was not driven by RH alone, but by the interaction between RH and particle size via the hygroscopic effect (the ability of particulate matter (PM) to absorb water and increase in size under high RH). These findings suggest that, like with temperature, a U-shaped curve may describe the relationship with RH. In fact, the Environmental Protection Agency recommends an ideal RH level of 30%–50% (Wendt et al., 2004), providing credence to a potential "Goldilocks" zone.

2.3.3 Air pollution

Air pollutants can be divided into airborne PM and gas molecules, both of which are generated by indoor and outdoor sources, such as fossil combustion (e.g., automobile emissions and aerosolization of cooking and cleaning products) (Mandell et al., 2020). Air pollutants of special interest to ocular health, as outlined by the World Health Organization (WHO), are O_3, NO_2, sulfur dioxide (SO_2), carbon monoxide (CO), and PM (Versura et al., 1999; Jung et al., 2018).

Air pollutants are hypothesized to impact ocular and periocular components variably, depending on their composition. While all types act as sources of inflammation, ultrafine PM particles can cross the corneal epithelium to induce stress in deeper layers of the eye, while larger particles can settle upon and physically damage (e.g., abrasion) the ocular surface and periocular lid margin (Mandell et al., 2020). Gaseous pollutants, including reactive gases [e.g., NO_2, SO_2, O_3, and volatile organic compounds (VOCs)], react with the tear film and induce a local stress reaction (Mandell et al., 2020). Several mechanisms have been postulated, including the formation of direct irritant reagents at the ocular surface (e.g., solubilization of sulfur-containing compounds to create sulfurous or sulfuric acids) and activation of conjunctival antigen-presenting cells, leading to a pro-inflammatory response (Jung et al., 2018).

One climate control study focused on air pollutants and symptoms and signs of DED in humans and found a decrease in tear stability (via TBUT) after exposure. Specifically, a Danish study exposed 10 individuals to clean (41 μg/m^3 dust) and polluted air (394 μg/m^3 dust) in a randomized order for 3 h and reported a decrease in TBUT compared to baseline (specific data not provided; $p < 0.05$) (Pan et al., 2000). Epidemiological studies have consistently reported positive relationships between air pollution and DED. The Chinese case-crossover study of 5,062 individuals with DED identified that same-day exposure to $PM_{2.5}$ (OR = 1.02, 95% CI = 1.01 to 1.03; $p < 0.01$) and PM_{10} (OR = 1.01, 95% CI = 1.003 to 1.02; $p < 0.01$) was a risk factor for a DED diagnosis visit, along with decreasing RH (Mo et al., 2019). Similarly, the Taiwanese study of 25,818 subjects with DED found that increasing NO_2 (β = 1.08, 95% CI = 1.04 to 1.11; $p < 0.001$) was associated with a DED diagnosis, along with ambient temperature and RH (Zhong et al., 2018). In a similar fashion, a Korean study of 16,824 participants found positive relationships between O_3 levels with DED symptoms (OR = 1.16; 95% CI = 1.02 to 1.30; $p = 0.04$) and DED diagnosis (OR = 1.21; 95% CI = 1.05 to 1.40; $p = 0.008$) (Hwang et al., 2016). Finally, a prospective Korean study of 43 patients with DED undergoing treatment (symptoms, TBUT, staining) noted that O_3 (β = 0.33, 95% CI = 0.16 to 0.49; $p < 0.001$) and $PM_{2.5}$ (β = 0.38, 95% CI = 0.06 to 0.70; $p < 0.02$) levels were positively associated with symptoms (via OSDI), while PM_{10} (β = −0.03, 95% CI = −0.045 to −0.01; $p = 0.001$) was negatively associated with tear stability (TBUT) (Kim et al., 2020).

In summary, studies have overwhelmingly reported a positive association between exposure to different outdoor air pollutants and various aspects of DED.

2.3.4 Airborne allergens

While allergy and DED are separate entities, DED is often comorbid with "ocular allergy" (Leonardi et al., 2021), and DED flares can occur as a result of exposure to allergens, both seasonally and perennially (Friedlaender, 2011; Ortega et al., 2022). One systematic review reported that ~50% of individuals with allergic conjunctivitis (AC) have comorbid DED, and ~20% of individuals with DED have comorbid AC (Akasaki et al., 2022). Other studies have found molecular links between DED and AC—an American study on 75 patients with symptoms or signs of DED reported that 17% of subjects (13/75) had high tear IgE (>1 ng/mL) and that this group was more likely to be exposed to allergens in their home (e.g., pets: OR = 11.5; $p = 0.002$; smoke: OR = 38.6; $p = 0.008$), supporting the idea of an allergic component to DED in some individuals (Dermer et al., 2019). Shared signs have also been noted between DED and allergy, for example, corneal epithelial disruptions assessed with Rose Bengal and fluorescein (Dogru et al., 2008). Overall, these findings suggest that allergens may impact various

aspects of ocular surface health, including tear stability, mediators of inflammation, and mucin abnormalities, leading to sign overlap with DED.

No chamber studies have examined the association between allergens and DED, but epidemiological studies have reported positive links between ocular allergy and DED. In a Swedish study of 89 children aged 7–18 with pollen allergy (positive skin prick test or presence of IgE), ocular pain scores (pain Likert 0–3) increased linearly with pollen grain exposure over 42 days, until exposure to 150 grains/cm^3, where the trend flattened (specific data not provided; $p < 0.05$) (Kiotseridis et al., 2013). In addition to pollen, studies have also examined mold spores, which have been classified as aeroallergens and as bioaerosols across different studies. In a study of 3,485 adults in China, individuals who lived in homes with more signs of mold (severity score quantified by the presence of mold/damp stains, moldy odor, dampness on bed/clothing, window pane condensation in winter, and water damage) had an increased risk of ocular pain compared to those who lived in homes with fewer signs (OR = 3.20, 95% CI = 1.67 to 6.15; $p < 0.01$) (Lu et al., 2016).

Overall, studies suggest a positive relationship between allergens and DED, most notably pain and tear stability. Of interest, environmental studies focusing on allergies have coincided with findings reported for DED—allergic diagnoses and symptoms have been positively linked to temperature, negatively to RH, and positively to air pollution (Reinikainen et al., 1992; Mendell et al., 2002; Wolkoff et al., 2003; Rozanova et al., 2009; Idarraga et al., 2020). Further studies are needed to examine the overlapping pathophysiology between allergy and ocular surface disease and their relationships to the environment.

2.3.5 Atmospheric pressure

Although not as well-studied, atmospheric pressure may also impact ocular health. Atmospheric pressure is especially important in high-altitude areas (e.g., mountainous regions and aircrafts mid-flight), where its value decreases (the amount of gas molecules in the air decreases, making the air less dense than that closer to the ground), as it is hypothesized that lower atmospheric pressure leads to increased tear film evaporation (Tesón et al., 2013). Supporting this idea, the previously discussed American VA-based study found that atmospheric pressure was a risk factor for a DED diagnosis—the risk of a DED diagnosis was 13% higher in patients residing in regions where atmospheric pressure was 1 standard deviation higher than the population mean (incidence rate ratio (IRR) = 1.13, 95% CI = 1.129 to 1.133; $p < 0.001$) (Galor et al., 2014). Further studies are needed to examine this association and to develop appropriate mitigation strategies.

2.3.6 Bioaerosols

Bioaerosols are small biological particles (0.001–100 µm in diameter) that are present in both ambient outdoor and indoor air and are characterized as another form of air pollutant in some studies. These molecules originate from endotoxins, glucans, mycotoxins, allergens, bacteria, and fungal spores and are made airborne by the handling of industrial/agricultural products (soil, plants, animals, etc.). Similar to other airborne particles (PM and allergens), concentrations of bioaerosols vary by meteorological conditions, seasonality, and by human and animal activity (Rock et al., 2021).

It is hypothesized that lipolytic enzymes and polar lipids secreted by eyelid-colonized bacteria may influence meibum composition and health, and thus relate this exposure to the risk of surface disorders like DED (Dougherty and McCulley, 1986). Unfortunately, there is a large paucity of studies that focused on this relationship, with only a few epidemiological studies having been conducted. Only one study has specifically examined bioaerosols in the context of DED—another Australian study obtained swabs from the inferior conjunctival fornix and lid margin of 66 individuals with DED (symptoms, TBUT <10s, staining >3 on Oxford) and 18 controls and found more colony-forming units (CFUs) in the DED vs. the control group (106 ± 82 CFUs vs. 12 ± 18 CFUs; $p < 0.0001$). Moreover, within the DED group, individuals with ($n = 15$) versus without ($n = 51$) MG dysfunction (eyelid thickening, irregularity, telangiectasia, gland loss, capping, or abnormal meibum) had higher CFUs on average (95 ± 66 CFUs vs. 12 ± 18 CFUs; $p < 0.05$) (Albietz and Lenton, 2006).

Given the lack of data, further studies examining both the relationship between DED and airborne bioaerosols as well as the molecular mechanism of injury are necessary. This is especially important given that there are no existing recommended indoor, outdoor, or occupational bioaerosol concentration standards in the United States.

2.3.7 Important considerations for outdoor variables

While we have summarized studies examining the relationships between environmental factors and DED, there are considerations to keep in mind when analyzing these results. First, environmental factors affect one another, making it difficult to analyze the effect of an individual exposure with respect to ocular diseases like DED (D'Amato et al., 2015; Vocks et al., 2001; Pfab et al., 2010; Hong et al., 2016; Levetin and Van de Water, 2008; Ju et al., 1998; Mimura et al., 2014; Park et al., 2020). For example, higher temperatures can promote aeroallergen dispersion—some genes that encode pollen production work in a heat-dependent manner; thus, increasing temperature can promote the earlier initiation of flowering and enhanced allergenicity of aeroallergens (Ju et al., 1998; Levetin and Van de Water, 2008). Similarly, low RH can promote the suspension of airborne pollutants ($PM_{2.5}$ and PM_{10}) and airborne pollen levels (Wyon et al., 2002; Qiu et al., 2019). As previously discussed, RH can also impact PM size, known as the hygroscopic effect (PM can absorb airborne moisture in settings of high RH and inflate in size) (Huang et al., 2020). These confounding factors must be taken into consideration when examining the reported relationships between RH and ocular disease.

Second, population demographics vary across climate regions and may play a role in environmental susceptibility; this may impact comparisons across geographically diverse studies. For example, heat sensitivity is heightened in elderly women, patients with decreased mobility or dementia, those on medications that affect thermoregulation (diuretics or anticholinergics), and those with disorders that compromise thermoregulation (obesity, hypertension, pulmonary disease, and diabetes) (Kovats and Hajat, 2008; Kenny et al., 2010). In addition, individual differences in the ability to adapt to one's environment may

drive geographic differences (Hori et al., 1977). A Japanese study found that men in hot subtropical zones who later moved to colder temperate zones showed signs of superior heat acclimation than those who spent their lives in the temperate region, including less skinfold thickness (e.g., upper arm: 5.3 ± 2.3 mm vs. 7.7 ± 3.2 mm; $p < 0.001$) and more effective sweating with less salt wasting (0.022 ± 0.004 mEq/L vs. 0.029 ± 0.008 mEq/L; $p < 0.05$) (Hori et al., 1978). Several biologic modifications underlie climate adaptation, including a heat-dependent shearing mechanism for controlling blood flow (Carter et al., 2014), improved fluid balance and sweating mechanics (Périard et al., 2015), and changes in thermal behavior (e.g., brown adipose plasticity and metabolic enzyme activity) (Lee et al., 2014; Ning et al., 2016), and it is not known how these factors impact tear metrics, corneal epithelial cells, and corneal nerves. These factors may confound study results and account for variability across studies, along with other factors such as DED definitions and variance in methods for capturing environmental exposures.

2.4 Indoor environment

The indoor environment is also an important potential contributor to DED (Mandell et al., 2020). Ocular irritation is a frequently reported complaint of office workers, with studies suggesting that beyond indoor meteorological exposures, activities like work-related tasks (concentration causing decreased blink rate) and behavioral factors (contact lenses, eye make-up, medications, and smoking) may also impact ocular health (Wolkoff et al., 2003; Rozanova et al., 2009).

2.4.1 Indoor meteorological factors

DED has been associated with indoor temperature, RH, and air pollution (organic and inorganic). In one American study, 396 office workers working on two floors of the same building had ocular pain assessed weekly via a questionnaire (scale 0–25)—a 1°C decrease in temperature was associated with an increased severity of dryness, itching, and irritation [OR = −1.11 (per unit decrease in temperature), 95% CI = −1.76 to −0.47; $p < 0.005$] (Mendell et al., 2002). Next, similar to outdoor studies, low RH indoors has also been implicated in DED. In a Finnish study, 290 office workers located in two wings of the same building were crossed over between high humidity conditions (30%–40% RH) and "natural" conditions (20%–30%) for 3 weeks each (6 weeks total). Daily ocular pain symptoms (Likert 0–3) were worse on average while working in the low-RH conditions (0.39 vs. 0.35; $p < 0.05$) (Reinikainen et al., 1992). Similar findings were noted in a geographically diverse population—a study of 44 individuals in New Zealand had subjects work with and without a desktop humidifier (which increased RH by 5.4% ± 5.0%). This study found that 36% of participants noted improved ocular comfort scores while working with a humidifier, as compared to 5% in the non-humidifier group; $p < 0.001$) (Wang et al., 2017).

Studies focusing on at-home air PM have aligned with findings focusing on outdoor air pollution. Specifically, an American study of 97 individuals found that a 1 unit increase in $PM_{2.5}$ was associated with increased OSDI (β = 0.59, 95% CI = 0.58 to 2.59; $p = 0.002$) and reduced tear production (Schirmer's: β = −0.67, 95% CI = 0.75 to −0.03; $p = 0.04$) (Huang et al., 2020). In addition to these factors, building-related factors may also relate to DED—an American study evaluated the short-term effects of 88 subjects working in an older building (with a higher concentration of airborne PM (24,436 particles ≥0.5 μm/ft^3) as compared to 102 subjects working in a newer building (12,313 particles ≥0.5 μm/ft^3).

Like with outdoor air, few studies have examined indoor air bioaerosols and how they relate to ocular health. One American VA-based study examined the relationship with ocular health in 157 individuals seen at a VA eye clinic between October 2017 and October 2019. This study examined microbial presence in indoor air via bioaerosol concentrations (CFUs). Positive associations were noted between indoor air microbial load and the amount of corneal epithelial disruption (OR = 28.07, 95% CI = 1.8 to 443.8; $p < 0.05$) as well as with meibomian dropout (OR = 39.6, 95% CI = 1.8 to 875.2; $p < 0.05$). As expected, inter-meteorological relationships were noted; a 1% increase in RH was associated with a 3% increase in CFUs (OR = 0.03, 95% CI = 0.01 to 0.04; $p < 0.001$) (Rock et al., 2021).

Short-term exposures have also been studied as they relate to DED. An American study questioned 88 individuals as they left an older building (with a higher concentration of airborne PM (24,436 particles ≥0.5 μm/ft^3) as compared to 102 subjects who left a newer building (12,313 particles ≥0.5 μm/ft^3). When adjusting for other variables (e.g., building and time interaction), there was a 1% increase in the odds of reporting worsening DED symptoms per hour spent in the older versus newer building (OR = 1.01; 95% CI = 1.00 to 1.02; $p < 0.05$). In multivariate analyses, subjects working in the older building for longer periods (upwards of 3 h) were more likely to report pain (OR 3.89, 95% CI = 1.21 to 12.5; $p < 0.05$) than those working in the newer building (Idarraga et al., 2020).

Overall, the literature supports findings that are similar to what has been noted with respect to outdoor exposures, as various indoor exposures have been found to relate to various aspects of DED.

2.4.2 Behavioral factors

2.4.2.1 Smoking

Smoking is a behavioral factor that has been connected to DED, among other ocular conditions, including macular degeneration, glaucoma, and cataracts (Makrynioti et al., 2020). Smoke exposure can lead to tear film instability, secondary to a direct irritant action, through free-radical formation or the promotion of lipid peroxidation at the tear film (Sahai and Malik, 2005; Sayin et al., 2014). Studying this question with a focus on e-cigarettes, an American study examined 49 e-cigarette flavoring liquids and analyzed ROS production (via electron paramagnetic resonance (EPR)) as well as synthetic lipid peroxidation *in vitro* (analyzed for secondary lipid oxidation products using a thiobarbituric acid reactive substances (TBARS) assay kit). The study found that 43% of the e-cigarette flavorants analyzed led to a significant increase in free-radical production as compared to a flavor-free liquid (PG:GLY) (specific data not provided; $p < 0.05$ each). In addition, the effects of these liquids on lipid peroxidation were also measured *in vitro*, and significant increases in lipid peroxidation were noted for several flavorants, especially those that contained linalool (4 mg/mL), piperonal (1.6 mg/mL), and citral (4 mg/mL) (257%, 197%, and 205% increase in peroxidation rate vs. PG:GLY, respectively;

specific data not provided; $p < 0.05$) (Bitzer et al., 2018). For reference, similar lipid peroxidase abnormalities have been noted in non-smokers with DED (95), suggesting that similar downstream mechanisms of DED can be caused by a variety of insults.

Epidemiological studies have been mixed with respect to the impact of smoking on ocular health. Some studies have found positive relationships between smoking and DED—in a Turkish study of 49 smokers and 53 non-smokers, tear stability (TBUT: 8.24 ± 2.39 s vs.11.15 ± 1.94 s; $p < 0.0001$) and tear production (Schirmer's 13.30 ± 4.63 vs. 15.45 ± 4.11; $p = 0.02$) were both decreased in the smoking group. However, the values were still within normal ranges in both groups (Sayin et al., 2014). Supporting these findings, the Beaver Dam study of 3,583 individuals found that DED symptoms were present in 534 patients (14.4%) and that both past (OR = 1.22, 95% CI = 0.97 to 1.52; $p < 0.05$) and current smoking status (OR = 1.82, 95% CI = 1.36 to 2.46; $p < 0.05$) acted as risk factors for symptom presence (Moss et al., 2000). Other studies, however, have not found smoking to be a risk factor for DED—a meta-analysis of 10 studies (two cohort and eight cross-sectional studies) reported no relationship between DED diagnosis and a smoking history, when considering the impact of age and gender (OR = 1.16, 95% CI = 0.83 to 1.64; $p = 0.38$). However, the same study presented a subsequent sensitivity analysis in which only general (non-hospital) populations were included, and in this sub-analysis, the association became significant (OR = 1.50, 95% CI = 1.08 to 2.09; $p = 0.02$) (Xu et al., 2016).

In summary, the effects of smoking on DED are not entirely understood. While studies have demonstrated the direct negative effects of smoking on ocular health *in vitro*, results have been mixed when examined on the epidemiological level. Of growing interest are the health effects of other forms of smoking, such as vaping, for which preliminary studies have also demonstrated toxic effects on ocular health (Isa et al., 2019; Martheswaran et al., 2021).

2.4.2.2 Video display units

The impact of office work has been studied with respect to ocular disease (Huang et al., 2020), with the focus centered on the use of video display units (VDUs; e.g., computer screens) and reading tasks suggesting altered blink rates due to these tasks negatively affecting ocular health (Wolkoff, 2020). A Saudi study demonstrated a time-dependent positive association between ocular discomfort scores and visual tasks—in this study, 40 healthy men who read from a book and an electronic tablet for 15 min each found that the blink rate decreased significantly under both reading conditions (19.74 ± 9.12 blinks/min at baseline to 11.35 ± 0.20 and 14.93 ± 10.90 blinks/min for book and a tablet; $p < 0.05$ each). Concurrently, ocular discomfort scores [via a visual analog scale (VAS)] increased significantly from baseline values at all time intervals (5, 10, and 15 min) during both forms of reading. While still being explored, studies suggest that prolonged VDU use has a negative impact on ocular health.

2.5 Molecular mechanisms of injury

As previously stated, several exposures have been linked to ocular surface disorders like DED. However, the mechanisms that link a specific environmental insult to a specific facet of DED have not been fully elucidated. Some potential mechanisms include hyperactivation of pro-inflammatory cytokines and reactive oxygen species (ROS) (Zheng et al., 2014; Dogru et al., 2018; Ma et al., 2021), pathological apoptosis of epithelia (corneal and conjunctival) (Yeh et al., 2003; Stern et al., 2004), impaired activation of protective autophagy mechanisms (Wang et al., 2019; Liu et al., 2020), and tear film unit glandular dysfunction [e.g., lacrimal and meibomian dysfunction as a result of immune cell infiltration (Hikichi et al., 1993) and hyperkeratinization] (Jester et al., 1981; Yu et al., 2021).

Only a few studies have examined the molecular mechanisms that underlie the impact of adverse ambient conditions (RH, temperature) on the eye, with most focusing on animal or *in vitro* human cell models. One Canadian study examined tear cytokine levels after an incident of desiccating stress in volunteers with known DED ($n = 8$, diagnostic criteria not provided) and healthy controls ($n = 8$)—individuals sat in an environmental chamber with a controlled temperature (23°C ± 3°C), relative humidity (10% ± 3%), and air velocity (3–5 ft/s) for 180 min. Basal tears were collected before and after exposure to the low-RH environment, and tears were analyzed for cytokines (via V-plex assay). Individuals with DED had higher baseline IL-2 levels than controls (1.11 ± 0.83 pg/mL vs. 0.45 ± 0.37 pg/mL; $p < 0.05$). Post-exposure, IL-2 levels significantly increased in the DED group compared to baseline (1.57 ± 0.91 pg/mL vs. 1.11 ± 0.83 pg/mL; $p < 0.05$). On the other hand, no significant changes were noted in the control group after exposure (0.45 ± 0.37 pg/mL vs. 0.47 ± 0.15 pg/mL, $p > 0.05$) (Subbaraman et al., 2014). In summary, preliminary findings suggest that inflammatory mediators may link desiccating stress to tear abnormalities, with individuals differentially impacted based on baseline disease status.

A larger body of literature has focused on the molecular consequences of air pollution (PM and reactive gases). One study exposed mice to PM_{10} (50 µL PM_{10} eye drop four times daily for 14 days to the right eye). Expression of pro-inflammatory molecules in the cornea (TNF-α; NF-κB) increased when compared to non-exposed eyes (specific data not provided; $p < 0.05$ for each). Furthermore, an increased level of apoptosis was noted in the corneal superficial and basal epithelia in the PM_{10}-treated group (specific data not provided; $p < 0.05$ for each) (Li et al., 2017). Other *in vitro* human (Tau et al., 2013) and animal model studies (Li et al., 2019) have similarly reported increased tear cytokines after exposure to air pollutants. Another noted mechanism is corneal epithelial oxidative stress— an *in vitro* Chinese study that studied the effects of air pollution (up to 320 µg/100 µL of PM) on human corneal epithelial cells found a dose–response relationship between PM concentration and oxidative stress (via 8-hydroxy-2' -deoxyguanosine (8OHdG): 214 ± 6.50 pg/mL with 5 µg/100 µL of PM vs. 400 ± 38.8 pg/mL with 80 µg/100 µL of PM; $p < 0.005$) (Xiang et al., 2016). Finally, altered cell autophagy has also been noted due to PM—an *in vitro* study found that human corneal epithelial cells exposed to $PM_{2.5}$ (50 µg/mL) had changed to autophagy; increased autophagosome formation was noted via immunofluorescence of epithelial cell LC3B (an autophagy-associated marker; ~80%

of total cells expressing autophagy post exposure vs. ~45% in non-exposed; $p < 0.01$). Interestingly, this effect did not occur linearly. Western blot analysis showed that the expression of LC3B decreased during the first 4 h of exposure and then slowly returned to the baseline before increasing with longer exposure periods (Fu et al., 2017). This suggests a time-dependent role in autophagy that requires further study.

Other studies have focused on molecular mechanisms related to reactive gas exposure, like O_3. One animal study exposed mice to O_3 (0.5 or 2.0 ppm of O_3 for 3 h in a whole-body exposure chamber) and noted conjunctival goblet cell damage on IVCM and a dose-dependent increase in tear cytokines (via BD cytometric bead array) post exposure as compared to baseline. Specifically, IL-1β, IL-6, IL-17, interferon (IFN)-γ, and NF-κB translocation and transcriptional activity levels all significantly increased at 1 week and 4 weeks after exposure in both experimental groups (specific data not provided; $p < 0.05$ for each) (Lee et al., 2013). In summary, several molecular pathways of injury have been attributed to toxic exposure to air pollution and reactive gases, including proinflammatory cytokine release, corneal oxidative stress, and alteration in normal apoptosis and autophagy mechanisms.

Molecular mechanisms have also been studied for smoking. One rat model examined corneal health after cigarette smoke exposure via a smoking chamber (six daily episodes, each 3 h long to 300 mL of x 5 days). Immunohistochemical analysis reported increased oxidative stress in the corneal epithelium and lacrimal glands after exposure (via 8OhdG: specific data not provided; $p < 0.05$ for each) (Higuchi et al., 2011). This has also been investigated in humans—a Japanese study exposed 12 healthy individuals to smoking in a controlled chamber for 5 min. Increased tear inflammatory cytokines, most notably IL-6, were reported at both 5 min and 24 h post exposure compared to pre-exposure (specific data not provided; $p < 0.05$ for each). In addition, tear abnormalities were noted in the form of increased tear evaporation (post: 3.34 ± 2.04 (10^{-7}) g/cm^2/s vs. pre: 1.84 ± 1.19 (10^{-7}) g/cm^2/s; $p < 0.05$) (Rummenie et al., 2008). The authors hypothesized that the change in evaporation was related to lipid layer peroxidation and damage, as this has been observed after cigarette smoking in other human studies (Choi et al., 2016; Bitzer et al., 2018).

Shared molecular pathways have also been found that link ocular allergy and DED (Proud et al., 1990; Albrecht and Dittrich, 2018). Mucin layer dysfunction has been implicated in both ocular allergy and DED independently (Davidson and Kuonen, 2004; Rabensteiner et al., 2019). The mucus layer, adjacent to the corneal epithelium, functions as part of the tear film to lubricate and protect the cornea, anchor the aqueous layer to the corneal epithelium, and modulate shearing forces, and dysfunction in this layer has been demonstrated in patients with known DED. In particular, studies have reported reduced or altered expression of mucins in the bulbar and tarsal conjunctiva of individuals with DED (Pflugfelder et al., 1997; Danjo et al., 1998). Demonstrating this finding in ocular allergy, a Japanese study that examined 18 individuals with atopic keratoconjunctivitis and 14 controls found alterations in corneal epithelium mucin transcription in atopic eyes. Specifically, increased MUC16 expression (501 copies/ng vs. 143 copies/ng in controls; $p = 0.001$) and decreased MUC5AC expression (311 copies/ng vs. 1,006 copies/ng in controls; $p = 0.001$) were reported (Dogru et al., 2008). Overall, this suggests pathologic changes to mucus layer protein expression in a similar manner to those observed in individuals with DED.

In summary, the research has implicated several molecular mechanisms, including inflammation, oxidative stress, and altered apoptosis and autophagy, as underlying causes that may explain the association between toxic environmental and behavioral exposures and risk for ocular diseases like DED. Given the lack of studies examining these relationships, especially with respect to variables such as temperature and allergen exposure, further research is needed to fully understand these relationships.

2.6 Mitigation strategies

Several mitigation strategies exist that can target outdoor and indoor environmental conditions, with varying levels of difficulty and cost. For patients with severe or refractory disease, environmental modulation should be considered. Understandably, mitigation strategies for the outdoor environment are difficult. With regards to direct contact exposures (e.g., pollutants and aeroallergens), providers have recommended frequent hand washing, wearing wrap-around glasses or goggles, the use of pollen screens, and tracking local forecasted levels to mitigate outdoor exposures (Bergmann et al., 2021).

Mitigation strategies for the indoor environment are more plausible, given that the space is smaller and thus more controllable. Options include maintaining temperature and humidity in the "Goldilocks" zone (with the use of air conditioning and humidifiers). According to EPA guidelines, the optimal indoor RH should be set between 30% and 50%. According to the American Society of Heating, Refrigerating, and Air-Conditioning Engineers, the indoor temperature should be set between 20°C and 25°C (Abdul-Wahab et al., 2015). Managing indoor sources of pollution is another important strategy. Steps to reduce indoor PM levels include replacing filters on central heating and cooling systems, installing air purifiers, and avoiding unvented stoves and fireplaces (Mandell et al., 2020). In addition to this, removing sources of mold growth (paper, sheetrock (drywall), and carpet) is also a possible strategy (Vance et al., 2016). While not studied directly in DED, similar environmental controls have been found to be beneficial in 937 children with atopic asthma. In a US-based trial, caretakers in the intervention group were asked to perform mitigation behaviors that were tailored to each child's skin-test-sensitization results for 1 year. These included high-efficiency air purifiers, allergen-impermeable covers on mattresses and pillows, and specific allergy interventions such as pest control for children with cockroach allergies. In the control group, no interventions were undertaken. Families were contacted every 2 months and asked about the number of days with symptoms such as wheezing, chest tightness, cough, disturbed sleep, or decreased playtime due to asthma in the last 2 weeks before the phone interview. The group that underwent interventions had fewer active symptom days than controls (3.39 ± 0.12 days vs. 4.20 ±

TABLE 1 Summary of hypothesized mechanisms underlying toxic environmental exposures that may predispose to DED.

Environmental exposure	Hypothesized mechanism
Temperature	Altered tear film lipid layer and tear film thickness (Nagymihályi et al., 2004)
Relative humidity (RH)	Altered tear film lipid layer and tear evaporation and increased tear inflammatory cytokines (Abusharha and Pearce, 2013; Peng et al., 2014; Subbaraman et al., 2014)
Air pollution and particulate matter (PM)	Direct surface irritant (larger molecules), corneal oxidative stress (smaller molecules), increased tear inflammatory cytokines, increased corneal epithelial apoptosis, and altered cell autophagy (Xiang et al., 2016; Fu et al., 2017; Li et al., 2017; Mandell et al., 2020)
Reactive gases	Corneal oxidative stress and increased tear inflammatory cytokines (Lee et al., 2013; Mandell et al., 2020)
Aeroallergens	Corneal epithelial disruption, corneal oxidative stress (IgE-mediated), and altered glandular mucins (Dogru et al., 2008; Rabensteiner et al., 2019)
Smoke exposure	Direct surface irritant, altered tear film lipid layer, excessive and reflexive tearing, corneal oxidative stress, and increased tear inflammatory cytokines (Sahai and Malik., 2005; Rummenie et al., 2008; Higuchi et al., 2011; Sayin et al., 2014)
Bioaerosols	Corneal epithelial disruption and promotion of Meibomian gland disease (Rock et al.,2021)

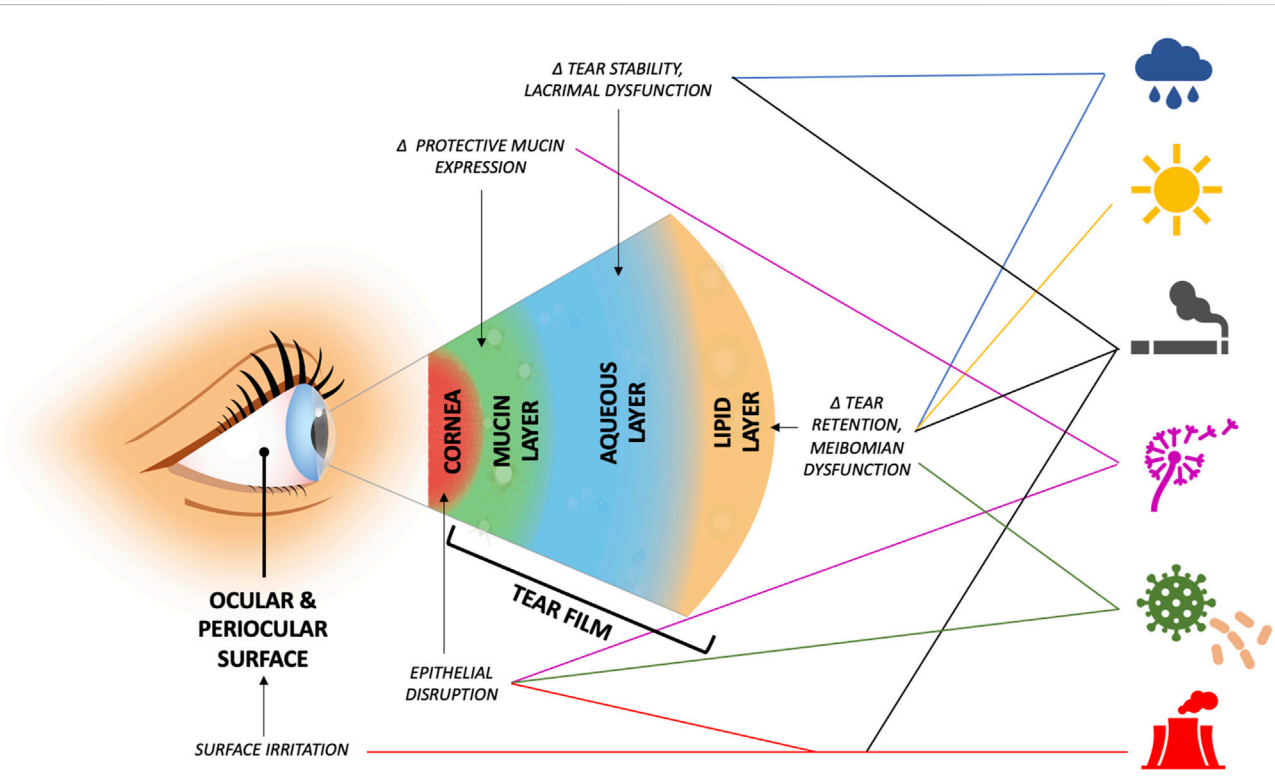

FIGURE 1
Suggested areas of dysfunction and possible underlying mechanisms that predispose to ocular surface disease by environmental exposure (exposures, top to bottom, by icon: relative humidity, ambient temperature, smoking, aeroallergens, bioaerosols, air pollutants, and reactive gases). Δ = alteration in.

0.12 in a 14-day period; $p < 0.001$) (Morgan et al., 2004). Similar approaches can be considered for DED.

3 Conclusion

Our Review highlights that environmental and behavioral exposures can impact the risk of DED diagnosis, symptoms, and signs, both in individuals with pre-existing DED and in healthy individuals. The studies summarized in this article suggest positive relationships between DED and weather extremes and air pollution, including PM, gases, allergens, and bioaerosols. In addition, links to behavioral factors like smoking have been reported, albeit with inconsistency in findings across studies. Data suggest that these environmental components may contribute to aspects of DED through a variety of molecular

mechanisms (Table 1). The pathophysiologic mechanisms that underlie the noted associations require further study to elucidate causal pathways, but several theories have been included in the Review (Figure 1). Given these findings, we suggest mitigation factors should be considered in appropriate patients (Alves et al., 2014); indoor factors such as air filters to minimize pollutant and allergen levels or tighter control of indoor RH and temperature may be the most cost-effective solutions for those most at risk. In the meantime, these associations can be incorporated into clinical practice by discussing exposure avoidance and/or mitigation for susceptible patients (Rozanova et al., 2009).

Author contributions

Conceptualization, AG; supervision, NK and AG; writing—original draft, SP; writing—review and editing, RM, NK, and AG. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Bisphenol A exposure triggers endoplasmic reticulum stress pathway leading to ocular axial elongation in mice

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Background: Ocular axial elongation is one of the features of myopia progression. Endoplasmic reticulum (ER) stress-associated scleral remodeling plays an important role in ocular axial elongation. Bisphenol A (BPA) is one of the most common environmental pollutants and is known to affect various human organs through ER stress. However, whether BPA exerts an effect on scleral remodeling remains unknown. The purpose of this study was to determine the effect of BPA on the development of myopia and scleral ER stress.

Methods: BPA was administered by intraperitoneal injection. 4-PBA was administered as an endoplasmic reticulum stress inhibitor by eye drops. Refraction and axial length were measured by refractometer and SD-OCT system. Western blot was performed to detect the expression level of ER stress-related proteins.

Results: BPA-administered mice exhibit axial elongation and myopic refractive shift with endoplasmic reticulum stress in the sclera. BPA administration activated scleral PERK and ATF6 pathways, and 4-PBA eye drops attenuated ER stress response and suppressed myopia progression.

Conclusion: BPA controlled axial elongation during myopia development in a mouse model by inducing scleral ER stress and activation of the PERK/ATF6 pathway. 4-PBA eye drops as ER stress inhibitor suppressed BPA-induced myopia development.

KEYWORDS

bisphenol A, endoplasmic reticulum stress, ATF6, PERK, sclera, myopia

1. Introduction

The incidence of myopia is rapidly increasing worldwide, particularly in East Asia (1). The refractive status is determined by the balance of the refractive power of the cornea and lens as well as the axial length of the eye, which is the result of an uncoordinated contribution of ocular components to the overall ocular structure (2). Among these factors, axial length (AL), one of the main determinants of myopia, has received extensive attention in related studies (3).

The sclera plays an important role in controlling the shape and size of the eyeball (4). Previous studies have shown that scleral remodeling is regulated by multiple factors such as genetics and the environment (4). The matrix remodeling that occurs in the sclera during

myopia development results in changes in biomechanical properties, which are critical for the increase in axial length that promotes myopia (5). However, the mechanism underlying scleral remodeling during myopia remains to be further elucidated. Recent studies have demonstrated that endoplasmic reticulum (ER) stress plays an important role in scleral remodeling in a form-deprivation myopia model in guinea pigs and a lens-induced myopia model in mice (6, 7). Activating transcription factor 6 (ATF6) is an ER transmembrane transcription factor with a mechanism for sensing ER stress and responding via translocation to the Golgi apparatus (8). Protein kinase RNA (PKR)-like ER kinase (PERK), mediated by phosphorylation of eukaryotic translation initiation factor 2 (eIF2α), is the major protein responsible for attenuated mRNA translation under ER stress (9). The canonical ER stress sensor proteins ATF6 and PERK are activated under ER stress to regulate the sclera and axial length (6). Moreover, 4-phenylbutyric acid (4-PBA), classified as a chemical chaperone, has been recognized as an inhibitor of ER stress (10). Numerous studies have substantiated the effectiveness of 4-PBA in mitigating ER stress across diverse cellular contexts, resulting in enhanced cell viability and functionality (11, 12). Notably, 4-PBA has been validated as an inhibitor capable of impeding myopia progression through its ability to diminish ER stress within the scleral tissue (6).

Bisphenol A (4,4-isopropylidenediphenol, BPA) is a common organic compound widely used in the production of various plastics and resins. Because of its wide range of uses, BPA has become one of the most widely produced industrial compounds worldwide (13). BPA has been detected as a potential health risk factor in a range of aquatic systems, wildlife, and humans as a potential health risk factor (14). In the ocular environment, BPA exposure can exacerbate hypertensive oculopathy in rat models (15). In addition, BPA-induced ER stress is associated with various pathological processes in the liver, nervous, and reproductive system (16–20). At the same time, the relationship between environmental pollution and myopia is receiving attention (21). However, substantive research on whether BPA affects ER stress in the ocular tissues, especially the sclera, is still lacking.

In this study, we explored the effects of BPA on the development of eye axial length in mice and investigated the mechanism of BPA exposure on the sclera from the perspective of ER stress.

2. Materials and methods

2.1. Materials

BPA was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Oil of corn (23–0320-5) purchased from Sigma was used as a vehicle. 4-Phenylbutyric acid (4-PBA) was purchased from Cayman Chemical (MI, USA; Catalog #11323). Relevant antibody sources and dilution multiple are listed in Table 1.

2.2. Animal administration

Male C57BL6J mice were housed in standard transparent cages in a temperature (24 ± 2°C) and humidity (40–60%) controlled clean room under a 12-h light–dark cycle. Animals had free access to a standard rodent diet and water throughout the experimental period.

TABLE 1 The list of antibodies for western blot.

Name	Dilution ratio	Company	Catalog
ATF6	1:1000	Bio Academina	73–505
p-IRE1α	1:1000	GeneTex	GTX132808
IRE1α	1:1000	Cell Signaling Technologies	#3294
p-eIF2α	1:1000	Cell Signaling Technologies	#3398
eIF2α	1:1000	Cell Signaling Technologies	#5324
β-actin	1:5000	Cell Signaling Technologies	#3700

All animal experiments in this study were approved by the Animal Experimental Committee of Keio University and adhered to the Institutional Guidelines on Animal Experimentation at Keio University, ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines for the use of animals in research.

The mice (3 weeks old) were randomly divided into two groups to detect whether BPA could induce ER stress in the sclera: the BPA and control groups. In the BPA group, 100 mg/kg BPA was administered daily for 14 days by intraperitoneal (IP) injection, and the mode of administration and dose were determined based on previous studies (22, 23). Mice in the control group were injected with the same volume of vehicle.

In the 4-PBA inhibition of endoplasmic reticulum stress experiments, since data from previous study showed that 4-PBA eye drops alone did not significantly affect axial length and refraction (6), mice were randomly divided into three groups: control, BPA, and BPA + 4-PBA. Mice in the BPA group received 100 mg/kg BPA and PBS eye drops once daily for 14 days. BPA + 4-PBA group was injected 100 mg/kg BPA and 4-PBA (2% solution) eye drops, once daily for 14 days. The control group received the same volume of vehicle and PBS eye drops.

2.3. Ocular biometric measurements

Refractions were obtained using a refractometer (Steinberis Transfer Center, Tübingen, Germany) subsequent to the induction of general anesthesia in mice through intraperitoneal injection of midazolam (40 μg/100 μL; Sandoz, Tokyo, Japan), medetomidine (7.5 μg/100 μL; Orion, Espoo, Finland), and butorphanol tartrate (50 μg/100 μL; Meiji Seika Pharma, Tokyo, Japan). The measurement of axial length (AL) was conducted utilizing a spectral domain-optical coherence tomography (SD-OCT) system (Envisu R4310, Leica), specifically designed for mice, in accordance with established methodologies outlined in prior study (24).

2.4. Western blot

After anesthesia as described above, mice were euthanized by cervical dislocation followed by enucleation of eyes for further tissue isolation. Sclera samples were homogenized in RIPA buffer (50 mM HEPES (pH 7.5), 150 mM NaCl, 1% NP-40, 50 mM NaF, 10 mM β-glycerophosphate, 5 mM benzamidine, 0.1% sodium deoxycholate 1 mM EDTA, 1 mM Na3VO4, and 1 mM PMSF)

containing Halt protease inhibitor cocktail (ThermoFisher Scientific, USA). The protein concentration was measured using a bicinchoninic acid (BCA) protein assay and adjusted with Laemmli sample buffer (Nacalai Tesque). Extracted protein samples were resolved by SDS-PAGE, then transferred to PVDF membranes (Merck Millipore, MA, USA), blocked with Blocking One (Nacalai Tesque, Tokyo, Japan). After that, the membrane was incubated overnight at 4°C with IRE1 alpha, IRE1, phosphor-eIF2α, eIF2α, ATF6 and β-actin antibodies at 4°C. The corresponding secondary antibody (1:10000) was incubated with the membrane at room temperature for 1 h. The SuperSignal West Femto Maximum Substrate (Thermo Fisher Scientific) was used for visualization. SDS-PAGE was performed on 10% acrylamide gels using protein size markers (MagicMark XP Western Protein Standard, Thermo Fisher Scientific).

2.5. Statistical analysis

Independent sample Student's two-tailed *t*-test and analysis of variance (ANOVA) with Fisher's least significant difference (LSD) *post hoc* test were performed using GraphPad Prism 9 to determine the statistical significance of the comparisons. Image J (version 1.52v; NIH) was used for histogram analysis of the western blots. $p < 0.05$ was considered statistically significant.

3. Results

3.1. BPA induce ocular axial elongation

To determine whether BPA could induce ocular axial elongation and myopia development, BPA was administered for 2 weeks. Changes in axial length and refraction were detected using SD-OCT and refractometer systems between the oil- and BPA- administration groups. Mice of BPA administration group showed axial elongation ($p < 0.05$) compared with oil administration group (ΔAL means ± SD Control:0.163 ± 0.010, BPA:0.198 ± 0.028 mm) (Figure 1A). At the same time, a significant myopic shift ($p < 0.0001$) occurred compared to control eyes (ΔRF means ± SD Control:2.66 ± 0.86, BPA: −4.80 ± 1.73 D), which were typical features of myopia development (Figure 1B). In addition, we also analyzed the changes in corneal thickness and retinal thickness by OCT between the oil- and BPA-administration groups but failed to find significant differences (Supplementary Figures S1A,B).

3.2. BPA induce ER stress in sclera

The sclera is a key tissue that controls the axial length of the eye (4). Previous studies by our group demonstrated that ER stress occurs in the sclera and leads to matrix remodeling and axial elongation in myopia (6). To explore the effect of BPA on scleral ER stress, we measured the changes in the expression levels of scleral endoplasmic reticulum stress-related proteins in mice exposed to BPA (100 mg/kg/d, 14 days).

Scleral samples were collected to assess the expression of the ER stress-related proteins IRE1, eIF2, and ATF6 (Figure 2A). BPA administration group showed higher phosphorylation levels of eIF2, which is a downstream factor of the PERK pathway in the sclera (Figure 2B). Simultaneously, the ratio of cleaved activated ATF6 (ATF6-N) to full-length ATF6 (ATF6-P) was higher in the BPA group (Figure 2B). This is consistent with the conclusions of our previous study that the PERK and ATF6 pathways are involved in scleral remodeling and ocular axial elongation (6). However, there was no difference in the p-IRE1/IRE1 ratio between the control and BPA-treated groups (Figure 2B). This result is similar to previous reports in some organs, where BPA activated the PERK and ATF6 pathways but failed to affect the IRE1 pathway (25).

3.3. Attenuation of scleral ER stress to suppress BPA induced myopia

To determine whether scleral ER stress is one of the main factors in BPA-induced axial elongation and myopia development, 4-phenylbutyric acid (4-PBA) was used to attenuate scleral ER stress. During BPA administration, 4-PBA (2% solution in PBS) was administered as eye drops, and changes in axial length and refraction error were compared between the PBS eye drop administration group and the oil control group. Compared with the BPA + PBS group, the BPA + 4-PBA group showed a shorter change in the axial length of the eye (ΔAL means ± SE Control:0.161 ± 0.004, BPA + PBS:0.203 ± 0.009, BPA + 4-PBA:0.164 ± 0.006 mm) (Figure 3A). Similarly, 4-PBA-administered mice reduced the myopic shift in refraction (ΔRF means ± SE Control:2.30 ± 1.19, BPA + PBS: −5.51 ± 2.00, BPA + 4-PBA:0.34 ± 1.77 D) by 2 weeks (Figure 3B).

To further verify the effect of 4-PBA on scleral ER stress, the expression levels of PERK (assessed by eIF2) and ATF6 pathway-related ER stress markers were detected using western blotting (Figures 4A,B). BPA administration activated mouse scleral eIF2 phosphorylation and the ATF6 pathways in the BPA + PBS group. In contrast, the 4-PBA eye drop-treated group showed a lower ER stress response in the sclera. The western blotting results corresponded to the refraction and axial elongation results. BPA administration activated the PERK and ATF6 pathways, whereas 4-PBA eye drops attenuated ER stress and reduced myopic shifts in refractive and axial elongation.

4. Discussion

Substantial evidence suggests that environmental BPA may adversely affect human health (26–28). A recent study pointed out that BPA administration can affect the eye, but extensive research in this area is lacking (15). At the same time, East Asia, a major producer of BPA (29), is facing a myopia epidemic (30), which means that it will be interesting to further explore the effects of BPA on the eye. In the current study, we observed that BPA administration group showed axial elongation and a myopic refraction shift. Simultaneously, BPA induced ER stress in the sclera, especially via the PERK and ATF6 pathways.

The sclera plays an important role in controlling the size of the eyeball (31). Axial elongation, accompanied by scleral matrix remodeling, is a hallmark of myopia progression (32). It was recently reported that hypoxia, biomechanical stress, and ER stress may

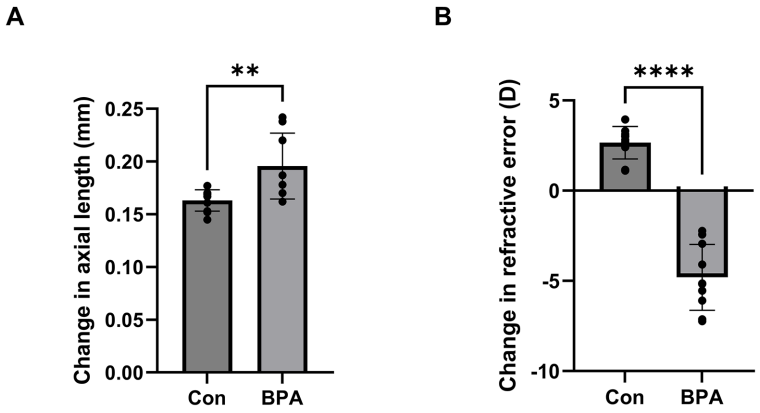

FIGURE 1
BPA induced axial elongation and myopic refraction shift. **(A)** Change in axial length during 2-week BPA administration in C57BL6J mice ($n = 10$). Con: control group with corn oil administration; BPA: BPA group, BPA was administered 100 mg/kg BPA daily. Student's two-tailed t-test, $^{**}p < 0.01$. The values are presented as mean ± SD. **(B)** Change in refractive error during 2-week BPA administration in C57BL6J mice ($n = 10$). Con: control group with corn oil administration; BPA: BPA group, BPA was administered 100 mg/kg BPA daily. Student's two-tailed t-test, $^{****}p < 0.0001$. The values are presented as mean ± SD.

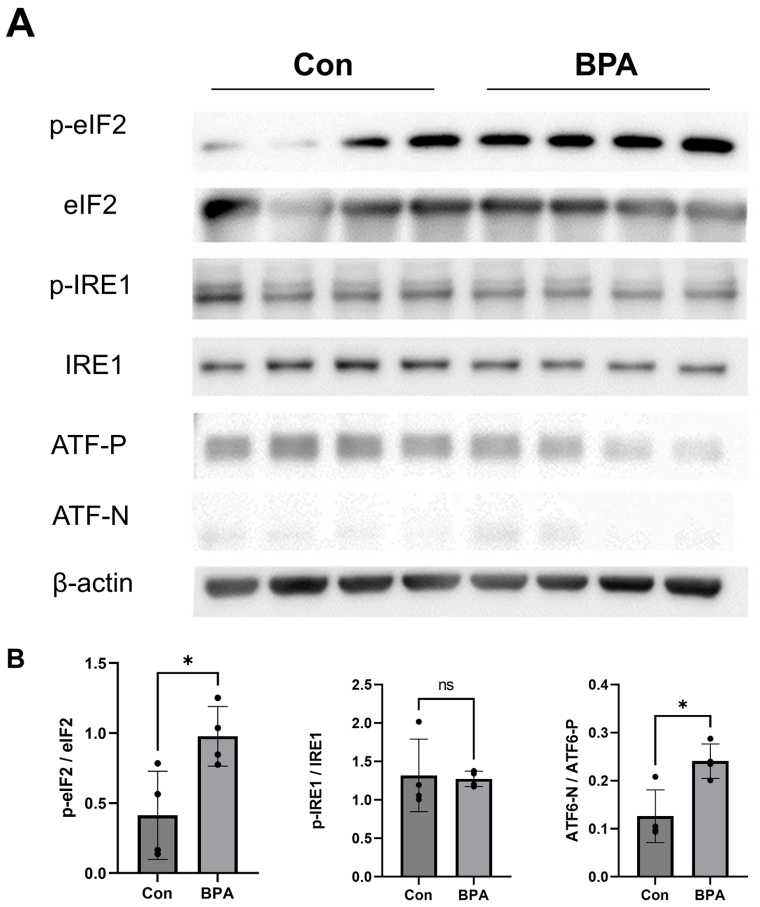

FIGURE 2
ER sensor protein activation by BPA administration. **(A)** Western blot results showed ER sensor protein activation (phosphorylation levels of IRE1, PERK, eIF2α, and the ATF6 precursor and cleaved form of ATF6). Con: control group with corn oil administration; BPA: BPA group, BPA was administered 100 mg/kg BPA daily. **(B)** Densitometric quantification of the blot in **(A)** using ImageJ. Con: control group with corn oil administration (Blue); BPA: BPA group, BPA was administered 100 mg/kg BPA daily (Red). Student's two-tailed t-test, $^{*}p < 0.05$, NS, Not Significant. The values are presented as mean ± SD.

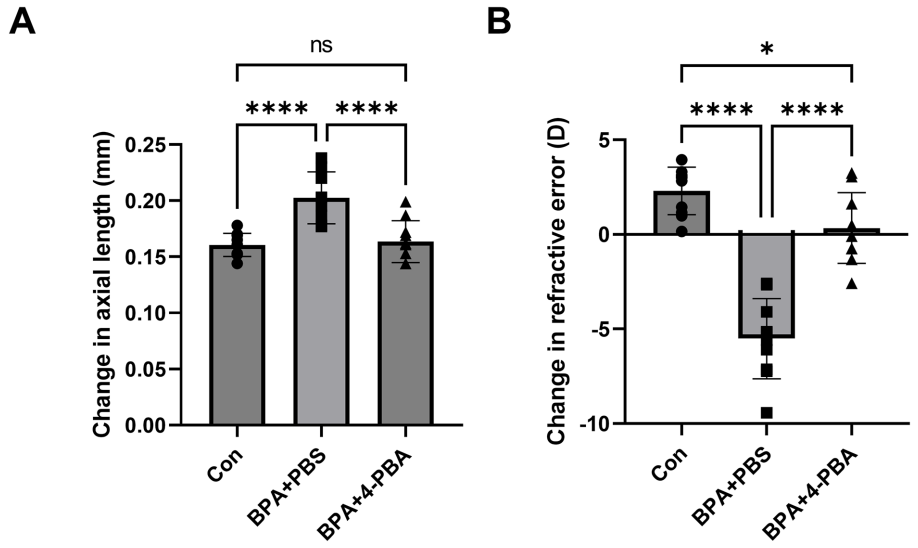

FIGURE 3
Effect of ER stress inhibitors on BPA-induced myopia development. **(A)** Change in axial length during 2-week BPA administration with 4-PBA eye drops in C57BL6J mice ($n = 10$). Con: control group with corn oil administration, PBS eye drops; BPA + PBS: group with BPA administration, PBS eye drops. BPA + 4-PBA: group with BPA administration, 4-PBA eye drops (2% solution in PBS). One-way ANOVA with Fisher's LSD *post hoc* test, ****$p < 0.0001$, NS: Not Significant. The values are presented as mean ± SD. **(B)** Change in refractive error during 2-week BPA administration with 4-PBA eye drops in C57BL6J mice ($n = 10$). Con: control group with corn oil administration, PBS eye drops; BPA + PBS: group with BPA administration, PBS eye drops. BPA + 4-PBA: group with BPA administration, 4-PBA eye drops (2% solution in PBS). One-way ANOVA with Fisher's LSD *post hoc* test, *$p < 0.05$, ****$p < 0.0001$. The values are presented as mean ± SD.

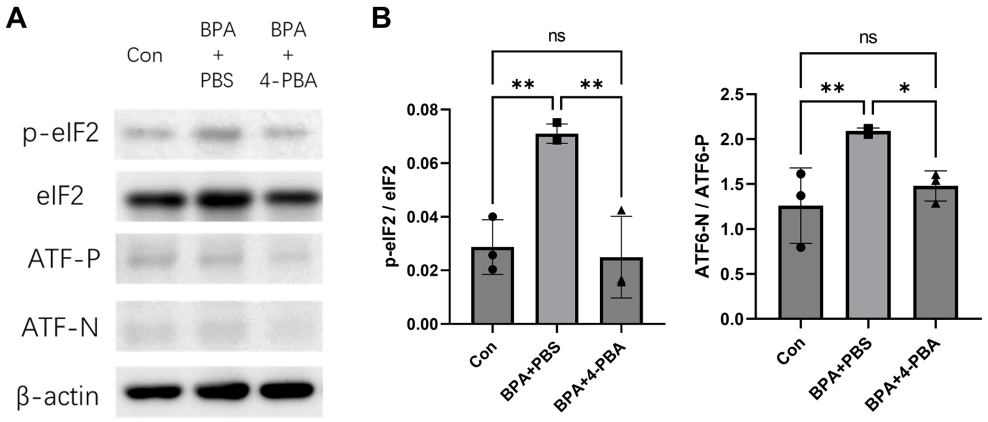

FIGURE 4
Effect of ER stress inhibitors on BPA-induced scleral ER stress. **(A)** Western blot showed effect of 4-PBA on BPA-induced scleral ER stress (phosphorylation levels of IRE1, PERK, eIF2α, and the ATF6 precursor and cleaved form of ATF6). Con: control group with corn oil administration, PBS eye drops; BPA + PBS: group with BPA administration, PBS eye drops. BPA + 4-PBA: group with BPA administration, 4-PBA eye drops (2% solution in PBS). **(B)** Densitometric quantification of the blot in **(A)** using ImageJ. Con: control group with corn oil administration, PBS eye drops; BPA + PBS: group with BPA administration, PBS eye drops. BPA + 4-PBA: group with BPA administration, 4-PBA eye drops (2% solution in PBS). One-way ANOVA with Fisher's LSD *post hoc* test, *$p < 0.05$, **$p < 0.01$, NS, Not Significant. The values are presented as mean ± SD.

be related to scleral remodeling, indicating that there may be multiple factors involved in the regulation of the sclera during the process of myopia development (33, 34). Furthermore, our previous research illustrated that endoplasmic reticulum stress within the sclera could wield a substantial influence over the expression of ECM proteins. This eventuality transpires through the activation of both the PERK and ATF6 pathways, culminating in the subsequent restructuring of scleral collagen (6). BPA affects extracellular matrix remodeling in various organs; however, its effect on the sclera has not been reported (35–37). Our results confirm that BPA may affect scleral remodeling. Similarly, BPA exposure reduced the repair function of myofibroblasts and their ability to successfully remodel after myocardial infarction (35). Together, these findings highlight the importance of scleral remodeling as a potential research target in myopia.

Accumulation of unfolded proteins in the ER activates ER stress sensor proteins such as PERK, ATF6, and IRE1 (38). ER stress is associated with various physiological and pathological conditions, including matrix remodeling and fibrosis (39–42). ER stress promotes

nuclear pulposus cell apoptosis and disc degeneration by affecting extracellular matrix homeostasis (39). During pulmonary fibrosis, ER stress can affect profibrotic effector pathways, including apoptosis, differentiation, and inflammatory signaling (40). In Schmid metaphyseal chondrodysplasia, ER stress occurs in chondrocytes and activates the PERK, ATF6, and IRE1 pathways, whereas IRE1 is not involved in the short-bone-length phenotype (42). Simultaneously, scleral matrix remodeling is recognized as an important factor in myopia development (4, 31). Given that BPA can induce ER stress in multiple organs (16, 18, 19, 43), it would be valuable to investigate the association between BPA, scleral ER stress, and myopia.

In our experimental mouse model, BPA administration upregulated the expression of ER stress-related proteins in the sclera, particularly in the PERK and ATF6 pathways (Figure 5). According to previous report, lens-induced myopia caused ER stress in the sclera rather than the retina in mouse models, and the induction of scleral ER stress was sufficient to induce changes in eye axial length (6). Consistent with our previous report (6), myopia development was associated with scleral ER stress, as myopia development was not induced in the control group (administered corn oil), whereas BPA induced the upregulation of ER stress-associated proteins, axial elongation, and myopic refractive change. These results suggested a cause-and-effect relationship between ER stress and BPA-induced myopia in mice. Furthermore, BPA-induced progression of myopia was attenuated by the ER stress inhibitor 4-PBA. This inhibitory effect was demonstrated by changes in the expression of ER stress-related proteins, reduction in axial growth, and refractive changes in mice. However, it is worth noting that there are differences in the patterns of human exposure to BPA and in animal models. The United States Environmental Protection Agency (EPA) has established a reference dose (RfD) of 0.05 mg/kg body weight (BW)/day for BPA in humans, which is derived from adverse effects observed in rats exposed to 1,000 mg/kg BPA (44). Future additional experiments would be advantageous in order to investigate the potential long-term impacts of BPA on the sclera.

Although existing results point to ER stress as the main pathway for BPA-induced myopia, it must be pointed out that there may be other pathways that play a role in this process. Several research studies have indicated a connection between the adverse effects caused by BPA and the dysregulation of autophagy (45). In the process of BPA's effect on myocardial remodeling, BPA-induced inflammation is considered a potential factor and MMP-2 is considered a response molecule to inflammation (35). In myopia research, it has also been suggested that inflammation may play a role in sclera remodeling (46). However, another study demonstrated that although the upregulation of scleral MMP-2 induces a myopic refractive shift, no meaningful change in ocular axial length is observed (47). In addition, some studies have indicated that BPA directly regulate the expression of MMP-9 to mediate extracellular matrix remodeling (48). The role of MMP-9 in the myopic sclera requires further study. On the other hand, BPA has been shown to have estrogen-like effects (49). Although the expression of estrogen receptor alpha protein was not detected in human male and female sclera samples in previous reports (50), it would be interesting to compare the effects of BPA on the eyes of mice of different sexes in the future.

Moreover, a noteworthy aspect to consider pertains to the exposure pathway of BPA to the human body, particularly the ocular tissue. It is imperative to underscore that while our study used intraperitoneal administration for experimental precision, the actual exposure of ocular tissues to BPA predominantly occurs from contact lenses and containers of eye drops (51, 52). In forthcoming research endeavors, the adoption of low-dose topical administration via eye drops would be instrumental in more faithfully emulating the genuine routes of BPA exposure encountered in real-life context.

In conclusion, our study demonstrated that BPA administration induces scleral endoplasmic reticulum stress and results in axial elongation of the mouse eye. 4-PBA inhibited the progression of BPA-induced myopia. Furthermore, the PERK and ATF6 axes were the main pathways activated during BPA-induced ER stress.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

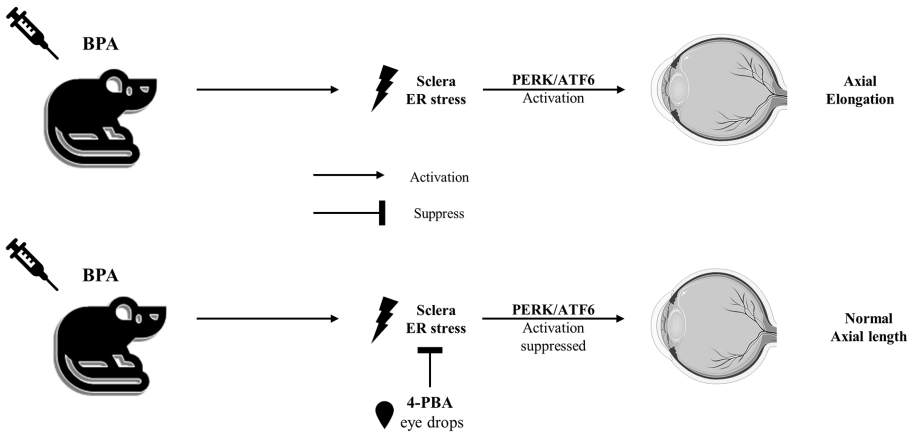

FIGURE 5
The sketch map of the pathway mediated by BPA to induce axial elongation and suppressed by 4-PBA.

Ethics statement

The animal study was approved by the Animal Experimental Committee of Keio University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JC: Data curation, Formal analysis, Investigation, Writing – original draft. S-iI: Data curation, Investigation, Methodology, Project administration, Writing – original draft. LK: Data curation, Methodology, Writing – review & editing. KN: Supervision, Writing – review & editing. KT: Funding acquisition, Supervision, Writing – review & editing. TK: Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

KT reports his position as CEO of Tsubota Laboratory, Inc., Tokyo, Japan, a company producing myopia-related devices.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1255121/full#supplementary-material

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Ophthalmic manifestations and management considerations for emerging chemical threats

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Chemical agents have been utilized for centuries in warfare and pose a health threat to civilians and military personnel during armed conflict. Despite treaties and regulations against their use, chemical agent exposure remains a threat and measures to understand their effects and countermeasures for systemic and organ-specific health are needed. Many of these agents have ocular complications, both acute and chronic. This mini-review focuses on key chemical agents including vesicants (mustards, lewisite), nerve agents (sarin, VX), knockdown gasses (hydrogen cyanide), and caustics (hydrofluoric acid). Their ophthalmic manifestations and appropriate treatment are emphasized. Acute interventions include removal of the source and meticulous decontamination, as well as normalization of pH to 7.2–7.4 if alteration of the ocular pH is observed. Besides vigorous lavage, acute therapies may include topical corticosteroids and non-steroid anti-inflammatory therapies. Appropriate personal protective equipment (PPE) and strict donning and doffing protocols to avoid healthcare provider exposure are also paramount in the acute setting. For more severe disease, corneal transplantation, amniotic membrane graft, and limbal stem cell transplantation may be needed. Orbital surgery may be required in patients in whom cicatricial changes of the ocular surface have developed, leading to eyelid malposition. Multidisciplinary care teams are often required to handle the full spectrum of findings and consequences associated with emerging chemical threats.

KEYWORDS

chemical threats, sulfur mustard, lewisite, sarin, VX, cyanide, hydrofluoric acid

1 Introduction

Chemical agents have been used in warfare as early as 600 BCE when the Athenian military tainted the water supply of a sieged city. In the modern era, the first large-scale chemical weapons were used during World War I at the Second Battle of Ypres where chlorine gas resulted in 6,000–7,000 casualties (Fitzgerald, 2008; Mayor, 2003). As chemicals can easily immobilize troops with relatively low costs and effort compared to arms-based tactics, there is a risk for use by adversaries who do not adhere to the multiple treaties limiting their use, such as the Geneva Protocol and Chemical Weapons Convention. Recently, chemical warfare was used in 2018 by the Syrian Air Force causing nearly 70 deaths (Omar, 2020).

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TABLE 1 Summary of chemical agents, systemic and ocular findings and management.

Agent	Odor and color	Systemic effects	Ocular effects	Management[a]
Vesicants				
Sulfur mustard	Odorless, garlic, onion, mustard	Pulmonary: Acute rhinitis, pharyngitis, tracheitis, bronchitis; dyspnea, pulmonary edema, alveolar hemorrhage, chronic bronchitis, bronchial asthma, recurrent respiratory infections	Acute: Conjunctival injection, keratitis, corneal edema, uveitis, blepharospasm, corneal neovascularization, corneal perforation	Sodium thiosulfate
		Skin: Erythema, bullae, ulceration, scar formation		Pulmonary: humidified oxygen, N-acetylcysteine, rehydration, intensive respiratory support
	Colorless, may be yellow-brown	Lethal dose in 50% of the population (LD50) estimates: 100 mg/kg. dermal; 0.7 mg/kg oral	Chronic: persistent conjunctival irritation and photophobia, corneal opacities, ulceration, band keratopathy, corneal melting with neovascularization	Ocular: mydriatics, sunglasses, petroleum jelly
				Consideration for limbal stem cell therapy or corneal keratoplasty[b]
Lewisite	Odor of geraniums	Respiratory: rapid burning, pain, irritation, pneumonitis, respiratory failure, severe pulmonary edema, development of malignant lesions	Acute: Immediate pain and irritation, vesication of corneal epithelium, full-thickness keratocytosis, corneal neovascularization, uveitis, miosis	Chelating agent: British Anti-Lewisite (2,3-dimercapto-1-propanol)
		Skin: instantaneous erythema and burning, edema and bullae formation, necrosis, development of malignant lesions		
	If pure, colorless. If impure, amber to black color	"Lewisite Shock"—increased vascular permeability, hypotension, multi-organ failure	Chronic: corneal opacification, corneal perforation, blindness	
		LD50 estimate: 40 mg/kg dermal		
Nerve agents				
Sarin	Odorless, colorless	Systemic: Diarrhea, micturition, bronchospasm, bradypnea, increased respiratory secretions, bradycardia, dysrhythmia	Acute: Lacrimation, miosis, ciliary muscle spasm, blurred vision, myopia, ocular pain, headaches, blunted light reflex	Atropine followed by pralidoxime infusion
		Neurologic: confusion, altered mental status, central apnea, seizure. Long term can have insomnia, depression, anxiety, impaired memory		
VX	Odorless, amber when liquid	Sarin LD50 estimate: 100–500 mg dermal	Chronic: No known long-term sequelae	
		VX LD50 estimates:10 mg dermal; 25–30 mg inhalation		
Knockdown gases				
Hydrogen	Bitter almond odor	Systemic: tachycardia and tachypnea early, arrhythmias, bradycardia, hypotension, apnea late manifestations	Acute: mydriasis, decreased visual acuity due to optic neuritis	Rescue breaths contraindicated
Cyanide	Colorless gas, colorless or pale blue liquid	Neurologic: headache, confusion, dizziness, seizures, coma	Chronic: optic disc atrophy, retrobulbar visual tract lesions	Hydroxocobalamin preferred over sodium nitrate and thiosulfate combination therapy
		LD50 estimates: 100 mg/kg dermal; 1.52 mg/kg oral; 1.0 mg/kg oral		
Caustics				
Hydrofluoric	Pungent irritating odor	Skin: Pain (classically out of proportion to exam), ulceration and necrosis, potential involvement of underlying bones and tendons	Acute: immediate or delayed pain, conjunctivitis, edema, erosion, sloughing, ulceration of corneal epithelium	Consider 1% calcium ocular irrigation[b], calcium gluconate gel for dermal burns
	Colorless	Respiratory: pain, inflammation of respiratory mucosa, ulceration, nasal septal perforation, laryngitis, tracheitis, bronchitis. Pulmonary edema and hemorrhage, pneumothorax		

(Continued on following page)

TABLE 1 (*Continued*) **Summary of chemical agents, systemic and ocular findings and management.**

Agent	Odor and color	Systemic effects	Ocular effects	Management[a]
Acid		Gastrointestinal: nausea, vomiting, severe pain, melena, hematemesis due to ulceration, perforation	Chronic: Corneal opacifications, visual acuity impairment, photophobia, globe perforation, glaucoma, uveitis, keratitis sicca	Hexafluorine shown to be effective in small case series[b]
		Cardiovascular: fatal arrhythmias		
		LD50 estimate: 20 mg/kg oral		

[a]The most important immediate consideration for every agent is decontamination with clothing removal, cleaning the contaminated skin, ocular rinsing, etc.
[b]Variable results in the literature for treatment.

The Centers for Disease Control and Prevention categorize chemical agents into the following groups: vesicants (blister agents), nerve agents, choking/lung agents, caustics, blood agents, incapacitating agents, metals, riot control agents, toxic alcohols, and biotoxins (CDC, 2022). These agents may be titrated depending on the level of attempted damage. In addition to their ability to temporarily disarm opposing forces or cause fatality at high concentrations, many of these agents have significant long-term effects. The potential for chronic effects underscores the importance of understanding their consequences, including systemic and organ-specific findings, as well as the appropriate management paradigms for front-line healthcare providers.

This focused review covers vesicants (mustards, lewisite), nerve agents (sarin, VX), knockdown gasses (hydrogen cyanide), and caustics (hydrofluoric acid). Their ophthalmic manifestations and appropriate treatment are emphasized. A brief summary of each agent discussed can be found in Table 1.

2 Sulfur mustard

Sulfur mustard, also called mustard gas or its military designation, HD, is a vesicant. Approximately 77% of the gas injuries during World War I were due to sulfur mustard (Ganesan, 2010). More recently, the Iran-Iraq war saw its widespread use (Smith and Dunn, 1991). Although chemical damage begins minutes after contact, manifestations of toxicity appear after a latency period, lasting up to 12 h with exposures under 60 mg min/m^3 or under 3 h with exposures over 60 mg min/m (Institute of Medicine Committee on the Survey of the Health Effects of Mustard Gas and Lewisite, 1993; Gates and Moore, 1946; Mandel and Gibson, 1917; Balali-Mood and Hefazi, 2005). The latency period is also dependent on the ambient temperature, with hot, humid environments decreasing the latency period significantly. Within the respiratory system, acute exposure leads to acute rhinopharyngeotracheobronchitis and vacuolization of respiratory epithelium, resulting in dyspnea and alveolar hemorrhage (Devereaux et al., 2002; Khateri et al., 2003). Skin manifestations range from pain and erythema to deep bullae, which can ulcerate (Poursaleh et al., 2012). The most common chronic pulmonary complication is chronic bronchitis, seen nearly half of exposures, as well as recurrent respiratory infections and bronchial asthma (Emad and Rezaian, 1997).

2.1 Ocular complications

The acute ocular effects range in severity from conjunctival injection to corneal edema, corneal opacities, keratitis, uveitis, and blepharospasm (Balali-Mood and Hefazi, 2005). Given the corneal epithelium's high metabolic rate, the eyes are ten times more sensitive to sulfur mustard injury than other primary target organs (Institute of Medicine Committee on the Survey of the Health Effects of Mustard Gas and Lewisite, 1993). In addition, sulfur mustard is lipophilic, which increase absorption through the tear film (Solberg et al., 1997). Acute ocular symptoms typically occur after exposures of at least 50 mg min/m^3 (Goverman et al., 2014). The chronic effects of exposure appear to be related to the extent of initial exposure, route of contact, whether removed from exposure and treated, and individual factors, such as age, sex, and health status (Amini et al., 2020). Up to 83% of patients report chronic ocular symptoms, most commonly persistent conjunctival irritation and photophobia (Khateri et al., 2003; Namazi et al., 2009). However, more severe chronic symptoms include moderate corneal opacities and ulceration, corneal edema, band keratopathy, and corneal melting with neovascularization are seen in 10% of exposures (Namazi et al., 2009). The constellation of chronic corneal findings is termed *mustard gas keratopathy*, in which the extent of corneal damage may lead to months of hospitalization or blindness (Daryabari et al., 2022). About 0.5% of patients with severe sulfur mustard injuries later develop a delayed, recurrent keratopathy which can happen 8–40 years after initial injury (Institute of Medicine Committee on the Survey of the Health Effects of Mustard Gas and Lewisite, 1993; Solberg et al., 1997).

2.2 Therapeutic considerations

Immediate decontamination is the most important initial management. Please refer to the "Therapeutic Approach to Chemical Exposures" section for decontamination and ocular rinsing procedures. Skin absorption occurs in 2 min, so effective decontamination within those 2 min can prevent the effects of sulfur mustard. Additionally, sodium thiosulfate can be used for systemic effects (Etemad et al., 2019). Humified oxygen, N-acetylcysteine, rehydration, and more invasive respiratory support as needed are the mainstay of treatment for pulmonary symptoms.

For ocular symptoms, mydriatics can be used for pain and ciliary muscle spasms, dark sunglasses for photophobia, and

petroleum jelly or antibiotic ophthalmic ointment for the prevention of lid adhesions (Panahi et al., 2017). Skin lesions should be kept clean to prevent secondary infections. For cicatricial eyelid changes, corrective surgeries such as ectropion repair can be done.

3 Lewisite

Lewisite is an arsenic-based chemical that was once a primary agent but is now used as an adjunct to increase the environmental persistence of sulfur mustard (McNutt and Hamilton, 2015). Unlike sulfur mustard, lewisite symptoms appear within minutes of exposure, making it a less effective agent (Gates et al., 1946). The acute symptoms are similar to that of sulfur mustard, with instantaneous erythema and burning of the skin with later edema and bullae formation that is maximal at 36–48 h (Institute of Medicine Committee on the Survey of the Health Effects of Mustard Gas and Lewisite, 1993). Ulceration and necrosis can occur in skin with higher levels of exposure. There are reports of malignant lesions appearing in the areas of previous exposure in both the skin and respiratory tract (Doi et al., 2011). In the respiratory tract, acute exposure leads to rapid burning, pain, and irritation, with more severe exposures leading to pneumonitis, respiratory failure, and severe pulmonary edema (Manzoor et al., 2020). Lewisite can be lethal with acute toxicity due to dermal absorption and systemic distribution, referred to as *lewisite shock*, which manifests as a result of increased vascular permeability and subsequent third-spacing with damage to the biliary tree, liver, gallbladder, and lungs (Chauhan et al., 2008). Multiorgan failure including renal and liver failure can lead to death (Srivastava et al., 2018).

3.1 Ocular complications

The acute ocular effects of lewisite include immediate eye pain, irritation, lacrimation, blepharospasm, and chemosis that peaks 4–6 h after exposure (Institute of Medicine Committee on the Survey of the Health Effects of Mustard Gas and Lewisite, 1993). With high doses of vapor exposure, vesication of corneal epithelium, full-thickness keratocytosis, and neovascularization can occur. Lewisite has also been shown to cause severe uveitis and miosis due to penetration into the eye (Institute of Medicine Committee on the Survey of the Health Effects of Mustard Gas and Lewisite, 1993). The chronic effects of ocular exposure include corneal opacification, corneal perforation, and blindness (Tewari-Singh et al., 2016). Severe eyelid blistering and ulceration can lead to scarring and lid malposition.

3.2 Therapeutic considerations

The most crucial acute treatment is removal from the contaminated area in addition to decontamination measures (CDC, 2023). Unlike sulfur mustard, lewisite has a specific chelating antidote for lewisite, 2,3-dimercapto-1-propanol (British Anti-Lewisite), that has been shown to reduce systemic injury from lewisite exposure (Vilensky and Redman, 2003). However, ophthalmic formulations are not currently available. Eyelid wounds from lewisite are treated similarly to those from sulfur mustard: hygiene, monitoring for and prevention of secondary infections, and surgical treatment of ocular surface scarring and eyelid malposition.

4 Nerve agents–sarin and VX

Nerve agents are irreversible acetylcholinesterase inhibitors, similar to pesticides, leading to cholinergic hyperactivity (Mukherjee and Gupta, 2020). These agents are sub-classified into two main classes, the G-agents and the V-agents. The G-agents, such as sarin, are more volatile, making them less stable and less effective than the V-agents, such as VX (Radilov et al., 2009). Given its low volatility, VX has a long environmental persistence, making it a more lethal agent. Recently, sarin attacks were noted in Syria in 2013, and a VX attack occurred in Malaysia in 2017. The latter of which resulted in the death of Kim Jong-Nam, the brother of Kim Jong-Un (Chai et al., 2017; Rosman et al., 2014). Typically, exposure is through a liquid or vapor, with dermal exposure the most dangerous. The lethal dose of inhaled VX is 2.5–3 times higher compared to dermal exposure (Rosman et al., 2014).

Acute manifestations of systemic exposure are dose-dependent and involve nearly every organ system. Defecation, micturition, salivation, diaphoresis, and paralysis can occur (Holstege et al., 1997). In the respiratory tract, increased secretions and bronchoconstriction lead to wheezing and dyspnea, eventually resulting in respiratory failure and death. Initial tachycardia followed by bradycardia and dysrhythmias can occur (Moshiri et al., 2012). Prolonged or severe exposures can result in nervous system manifestations, including confusion, altered mental status, central apnea, and seizures resulting in status epilepticus (Figueiredo, 2018). Those who survive the initial toxidrome can have insomnia, depression, anxiety, and impaired memory.

4.1 Ocular complications

The ocular effects of the nerve agent toxidromes are the most sensitive manifestations. Miosis and lacrimation manifest nearly immediately following exposure (McNutt et al., 2020). Interestingly, miosis occurs only with ocular absorption but not with percutaneous exposure (Lukey et al., 2007). Miosis occurs at much lower concentrations than the lethal dose; for example, 3 mg min/m^3 of sarin (lethal dose 100 mg min/m^3) and 0.04 mg min/m^3 of VX (lethal dose 50 mg min/m^3) cause miosis (Lukey et al., 2007).

Excessive muscarinic stimulation results in ciliary muscle contraction and spasm, leading to blurred vision and myopia with associated ocular pain, headaches, and nausea (Gore, 2020). Eventually, muscarinic desensitization results in a blunted pupillary light reflex. The ocular effects typically resolve completely within weeks (Gore, 2020). There is debate whether these lingering effects

are due to a lack of acetylcholinesterase activity or inflammatory irritation of the iris.

4.2 Therapeutic considerations

Besides decontamination protocols, nerve agent toxicity is based on pesticide poisoning treatment regimens. Systemic atropine followed by pralidoxime is the therapy of choice (Chai et al., 2017). There is debate as to the appropriate dosage of atropine for prevention of mydriasis and lack of accommodation. There are also drugs that have been tested in animals with better CNS penetration than pralidoxime, however, they are not currently recommended in the management of acute toxicity (Chambers, 2016).

5 Knockdown gases—hydrogen cyanide

Hydrogen cyanide has been used as a chemical warfare agent, most notably during World War I and the Iran-Iraq War (Sauer and Keim, 2001; Mégarbane et al., 2003). Given its volatility and rapidly effective reversal agents, large quantities of the gas are needed to be an effective. Although not always present, exposure to cyanide vapor is classically associated with a bitter almond odor and a "cherry-red" skin discoloration (Parker-Cote et al., 2018). Cyanide affects aerobic cellular respiration; thus, symptoms are seen in systems with high metabolic rates. Early neurologic side effects include headache, confusion, and dizziness with seizures and coma in more severe exposures (Alqahtani et al., 2020). As a result of poor tissue oxygenation, acute tachycardia and tachypnea also occur. Later findings include arrhythmias, bradycardia, hypotension, and apnea (Fortin et al., 2010).

5.1 Ocular complications

Acute ocular complications of hydrogen cyanide exposure are scarcely reported in the literature due to few survivors receiving an ophthalmologic exam. Acute exposure has been associated with mydriasis and delayed, chronic, severely decreased visual acuity with bilateral optic disc atrophy on exam secondary to optic neuritis (Pentore et al., 1996). A case of bilateral vision loss with a normal fundoscopic exam has been reported as well (Chen et al., 2011). In this case, the physical exam was normal 5 months pre-exposure, but the patient developed visual changes shortly after intoxication. There was no apparent visual pathway lesion on magnetic resonance imaging (MRI) and normal physical examination, optical coherence tomography, retinal nerve fiber layer testing, and color vision testing. The only abnormal finding was visual evoked potentials, which indicated a likely posterior visual pathway lesion (Houston and Hendrickson, 2005).

5.2 Therapeutic considerations

Please see the "Therapeutic Approach to Chemical Exposures" section for detailed instructions on decontamination protocols. Rescue breaths are contraindicated in these patients due to the risk of exposure to the provider (Bryson, 1996). Several antidotes exist for acute cyanide toxicity, but hydroxocobalamin is preferred over sodium nitrite and sodium thiosulfate combination management (Hall et al., 2007).

6 Caustics—hydrofluoric acid

Hydrofluoric acid (HFA) is a highly corrosive chemical commonly encountered in occupational settings, such as glass etching and industrial and pharmacologic applications (Bajraktarova-Valjakova et al., 2018). It has not been frequently used in warfare or terroristic acts. However, it could potentially be a dangerous chemical weapon, given its unique ability to penetrate deeper into tissue and cause more extensive damage than other acids (McKee et al., 2014). Exposure to HFA can be through vapor inhalation, vapor contact, liquid burns, or ingestion.

With dermal exposure to a >50% concentrated solution, symptoms include intense, immediate pain; pain may not appear until up to 8 h after exposure with less concentrated solutions (Zhang et al., 2016). Intense pain out of proportion to the exam is the hallmark of dermal exposure (McKee et al., 2014). Ulceration and necrosis follow with potential involvement of the underlying tendons and bones. Full-thickness skin necrosis has been noted 1 hour following exposure (Dennerlein et al., 2016).

When exposure occurs via the inhalational route, immediate respiratory tract pain, inflammation, and bleeding occur, and ulceration or septal perforation if severe (Bajraktarova-Valjakova et al., 2018). Laryngitis, laryngotracheitis, and tracheobronchitis can occur and lead to cough, dyspnea, stridor, and wheezing. Pulmonary edema and hemorrhage occur in severe cases. An eventual perforation of the lower airway can lead to pneumothorax.

With ingestion, burns to the oropharynx, esophagus, and gastric mucosa occur rapidly (Balali-Mood and Hefazi, 2005). Nausea, vomiting, and severe pain are common symptoms. Melena, hematemesis, and potential perforation may occur (Bajraktarova-Valjakova et al., 2018). Systemically, fluoride ions in the bloodstream have a direct cardiotoxic effect. However, they also bind magnesium and calcium ions and raise potassium levels leading to a risk of potentially fatal arrhythmias (Vohra et al., 2008).

6.1 Ocular complications

Ocular contact, either through liquid or vapor, causes immediate pain, however, pain may be delayed with a low concentration exposure (Hatai et al., 1986). Conjunctivitis with edema and congestion follows pain with subsequent erosion, sloughing, and ulceration of the corneal epithelium (Hatai et al., 1986). Corneal opacification may follow and lead to long-term visual complications, including permanent visual acuity impairment, photophobia, globe perforation, glaucoma, uveitis, and keratitis sicca (Atley and Ridyard, 2015). Delay in treatment leads to worse long-term prognosis (MacKinnon, 1988).

6.2 Therapeutic considerations

Please see the "Therapeutic Approach to Chemical Exposures" section for detailed instructions on decontamination protocols and ocular rinsing. With dermal exposure, following copious irrigation, calcium is a first-line chelating agent as it can form inorganic salts with fluoride ions to prevent deep tissue penetration. Following water irrigation, 1% calcium gluconate irrigation may be done for 15–20 min using a Morgan Lens, but variable efficacy has been reported (Rubinfeld et al., 1992; Bentur et al., 1993; Mathieu et al., 2007). Severe necrosis may lead to exposure of the ocular surface, which will require lubrication of the ocular surface with frequent eye drops and ointments. Scarring of the eyelids may require later surgeries such as ectropion repair or more complex reconstructive surgeries such as skin grafts or flaps. Hexafluorine is another safe and effective chelating therapy that binds both free hydrogen and fluoride ions. A case series has shown no sequelae in patients treated with hexafluorine for HFA burns (Soderberg et al., 2004).

7 Therapeutic approach for chemical exposures

An important immediate consideration for every agent is decontamination with removal of affected clothing, cleaning the contaminated skin with neutral soap and water, and ocular rinsing for eye exposures. Contaminated clothing should be removed with shears to avoid inadvertent exposure caused by pulling clothing over the face (Balali-Mood and Hefazi, 2005). Ocular rinsing should be done with tap water, normal saline, or lactated Ringer's solution. It is preferred to use a Morgan Lens or eye irrigator and move the globe in every direction during irrigation. In exposures with alteration in ocular surface pH, irrigation should continue until the ocular surface pH has normalized to a range of 7.0–7.2.

Topical ocular steroids may be used to reduce chemosis and corneal epithelial edema, however, local steroids must be avoided if there is corneal epithelial defects, which may predispose patients to infectious keratitis (Rafati-Rahimzadeh et al., 2019). Administration of topical matrix metalloproteinase inhibitor (MMI) doxycycline has anti-inflammatory properties that can reduce acute and delayed ocular injuries (Kadar et al., 2009). Human amniotic membrane has anti-fibrotic, anti-angiogenic, and anti-inflammatory properties and can be useful for decreasing persistent inflammation and neovascularization (Alió et al., 2005). There has been success with limbal stem cell transplants and corneal keratoplasty for mustard gas keratitis (Javadi et al., 2007; Javadi et al., 2011). In patients with decreased visual acuity due to corneal opacification, penetrating keratoplasty (PKP), lamellar keratoplasty (LKP), or deep anterior lamellar keratoplasty (DALK) are commonly used (Baradaran-Rafii et al., 2011). In addition, limbal stem cell transplantation may be used in patients with persistent epithelial defects, focal corneal thinning and ulceration that do not respond to conservative treatments. Oculoplastics management of cicatricial conditions leading to eyelid malposition should be considered.

Moreover, healthcare provider contamination prevention is paramount while caring for patients. Specifically, proper personal protective equipment should be worn with appropriate training in donning and doffing protocols. These include fluid-impervious gowns, aprons, protective footwear, gloves, chemical-resistant glasses, face shields, and respirators to provide physical barriers to the hands, skin, clothing, eyes, nose, and mouth.

8 Conclusion

In this review, we provide a synthesis of the literature on ocular complications and the management of selected chemical agents. However, further investigation is needed to better understand these agents' acute and chronic complications, as well as appropriate local ophthalmologic and systemic management. Chemical warfare agents continue to remain a threat for military personnel and civilians, especially in areas with political and civil unrest. The ocular effects of these chemical agents are not nearly as well-known as many of their systemic effects. However, the early onset of ophthalmic symptoms requires an assessment of ocular structures in the event of any chemical exposure. Early recognition of the toxidromes is imperative to prevent acute and long-term disabling complications and ocular consequences. A better understanding of these agents will improve our ability to identify and treat both civilians and military personnel in the event of a chemical incident.

Author contributions

BM: Writing–original draft, Writing–review and editing. CR: Writing–review and editing. GJ: Writing–review and editing. RC: Writing–review and editing. BH: Writing–review and editing. MD: Writing–review and editing. SY: Conceptualization, Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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