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ROLE OF INTRAVENOUS LEVETIRACETAM IN ACUTE SEIZURE MANAGEMENT

Topic Editor
Batool F. Kirmani

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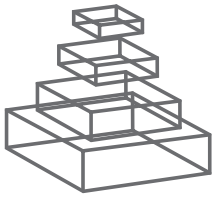

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ROLE OF INTRAVENOUS LEVETIRACETAM IN ACUTE SEIZURE MANAGEMENT

Topic Editor:
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Intractable epilepsy still remains the main issue despite new advances in medical and surgical treatment of epilepsy. Acute seizure management in a timely manner is crucial to prevent irreversible brain damage. Benzodiazepines still remain the first initial treatment to abort the seizure activity. The approval phenytoin, fosphenytoin, intravenous valproate, and rectal diazepam provided additional options. The approval of intravenous levetiracetam gave another option to physicians if and when the above treatment fails to control the seizure activity.

In this Ebook, we have included chapters from renowned researchers in the field of neurology and epilepsy who have covered the various aspects of these agents in detail including the properties, mechanism of action, pharmacology, neurobehavioral effects, and the roles of these agents in special populations including traumatic brain injury and brain tumor related epilepsy. These data further show that intravenous levetiracetam can be used in acute seizure management and in special circumstances.

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Role of intravenous levetiracetam in acute seizure management

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Keywords: levetiracetam, acute seizure management, anticonvulsants, treatment, epilepsy

Epilepsy is a chronic medical condition that still poses a challenge in terms of treatment. There are a variety of anticonvulsants available today, but these do not prevent frequent hospital admissions and emergency room visits. Benzodiazepines, however, remain the first line treatment in acute seizure management. The approval of phenytoin, fosphenytoin, intravenous valproate, and rectal diazepam has given more options to the physicians for acute management. The approval of intravenous levetiracetam has provided another option for physicians with patients who failed the other approved anticonvulsants. Intravenous levetiracetam is approved for patients 4 years and older as an alternative to oral treatment. There have been various case reports, case series, and retrospective studies showing the efficacy of intravenous levetiracetam both in status epilepticus (SE) and acute seizure exacerbation. These studies reported favorable response of intravenous levetiracetam in both adults and children. Data have even shown good results in neonates and preterm children. In this volume, we have included articles from renowned researchers in the field of neurology and epilepsy who have covered the various aspects of these agents in detail including the properties, mechanism of action, pharmacology, neurobehavioral effects, and the roles of these agents in special populations. These data further show that intravenous levetiracetam can be used in acute seizure management.

This book opens with a chapter by Jennifer L. DeWolfe and Jerzy P. Szaflarski (1) that discusses in detail the role of intravenous levetiracetam in the critical care setting. The literature review was conducted, which showed that intravenous levetiracetam is effective in terminating different types of seizures including SE, post-traumatic and tumor-related seizures, seizures due to stroke, and intraparenchymal hemorrhage. The pharmacokinetics of this agent in special populations including elderly, pregnant, and neurocritical patients is also discussed in this chapter. The authors concluded that there is still need for larger prospective trials, but based on current data, the drug appears to be safe and better tolerated in different subgroups of seizure population. The second chapter by Wright et al. (2) reviewed the current literature about the pharmacology and pharmacokinetics of intravenous levetiracetam and the safety profile of this drug in adults and children. The article also showed unique mechanism of action, linear pharmacokinetics, and no known drug–drug interactions with other anticonvulsants, which makes it a viable option for acute seizure management in both adults and children. The third chapter by Aceves et al. (3) further discusses in detail the current data regarding the safety and tolerability of intravenous levetiracetam in children and neonates in the management of SE and acute repetitive seizures. The authors also emphasize the need for a larger prospective multicenter trial to further define the roles of these anticonvulsants in this population subgroup.

Status epilepticus is a major neurological emergency that is associated with high morbidity and mortality. SE can cause significant neuronal injury and the patients who survive SE develop long term neurological sequelae including major cognitive issues. The fourth chapter is by Laxmikant S. Deshpande and Robert J. DeLorenzo who discuss the mechanisms of levetiracetam in the control of SE and epilepsy. (4) The authors in their review focused on the unique anticonvulsant properties of levetiracetam and concluded that the unconventional mechanism of action, favorable safety profile, and lack of sedating effects makes it a viable candidate to be used in the management of this neurological emergency. The fifth chapter by Hae Won Shin and Robin Davis is the review of levetiracetam as a first line treatment in SE in adult patients and the need for larger prospective trials in the future (5).

The sixth chapter by Fonkem et al. (6) and seventh chapter by Bernett et al. (7) focus on a special population subgroup – those with brain tumor-related seizures. Fonkem et al. (6) in his literature review article has shown that levetiracetam is an attractive option for brain tumor-related seizures because levetiracetam can increase the sensitivity of glioblastoma tumors to the chemotherapy drug temozolomide. Levetiracetam can also be used as prophylaxis in patients with brain tumors and in patients undergoing neurological surgery. Bernett et al. (7) summarize the limited data available, which show the potential risk of neurobehavioral side-effects with levetiracetam in brain tumor-related seizure patients and the need for future research.

The next four chapters focus on traumatic brain injury and the neuroprotective properties of Levetiracetam. The eighth chapter by Shetty et al. (8) discusses in detail the data available about the potential mechanism of epileptogenesis and neuroprotective properties of this agent, which are beneficial in treating seizures associated with neurological conditions like SE, stroke, and traumatic brain injury. The ninth chapter by Kovacs et al. (9) discusses the blast traumatic brain injury models, neuropathology, and implications for seizure risk. In this review, the authors reviewed the pathological results, which also included immunohistochemical and special staining approaches from recent preclinical explosive blast studies to better understand the mechanism by which

explosions cause brain injury. This is followed by chapter by Kirmani et al. (10), which discusses the role of intravenous levetiracetam in severe traumatic brain injury patients. The authors concluded, based on current literature review, that intravenous levetiracetam can be considered as a viable option in acute care settings if phenytoin is unavailable or the administration is not feasible due to side-effects.

The next chapter by Benge et al. (11) summarizes the limited current data available about the neurobehavioral effects of levetiracetam in traumatic brain injury patients and the need for future studies.

The last chapter is an original article by Rogers et al. (12) in which effects of levetiracetam on the mitochondrial membrane potential of neuronal and non-neuronal cells were examined *in vitro* to determine if levetiracetam influences metabolic processes in these cell types. These results suggested that both neuronal and non-neuronal anticonvulsant properties of levetiracetam involve control over energy metabolism.

This book provides a comprehensive review of the role of intravenous levetiracetam in acute seizure management and emphasizes the need of larger prospective trials to further define the role of this anticonvulsant.

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Levetiracetam use in the critical care setting

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Intravenous (IV) levetiracetam (LEV) is currently approved as an alternative or replacement therapy for patients unable to take the oral form of this antiepileptic drug (AED). The oral form has Food and Drug Administration (FDA) indications for adjunctive therapy in the treatment of partial onset epilepsy ages 1 month or more, myoclonic seizures associated with juvenile myoclonic epilepsy starting with the age of 12 and primary generalized tonic-clonic seizures in people 6 years and older. Since the initial introduction, oral and IV LEV has been evaluated in various studies conducted in the critical care setting for the treatment of status epilepticus, stroke-related seizures, seizures following subarachnoid or intracerebral hemorrhage, post-traumatic seizures, tumor-related seizures, and seizures in critically ill patients. Additionally, studies evaluating rapid infusion of IV LEV and therapeutic monitoring of serum LEV levels in different patient populations have been performed. In this review we present the current state of knowledge on LEV use in the critical care setting focusing on the IV uses and discuss future research needs.

Keywords: intravenous levetiracetam, status epilepticus, pediatric population, stroke-related seizures, post-traumatic seizures, loading dose, therapeutic monitoring

INTRODUCTION

Currently, intravenous (IV) levetiracetam (LEV) is approved only as an alternative or replacement therapy for patients unable to take the oral form of this antiepileptic drug (AED). The oral form of LEV is also approved for use in patients with multiple types of seizures and epilepsies. But, this AED has been increasingly used in the critical care setting (e.g., emergency rooms or intensive care units) due to its relative ease of use, positive outcomes, and the low side effects profile which are thought to be better than some of the other commonly used in this setting AEDs, e.g., phenytoin (PHT) (1, 2). When it was introduced to the market, LEV was marketed as an AED with a novel structure and mechanism of action – its main mechanism of action is modulating neurotransmitter release via binding to the synaptic vesicle protein 2A and, thus, via inhibiting calcium release from intracellular stores. Other mechanisms of action include opposition of the negative modulation of gammabutyric acid (GABA-) and glycine-gated currents, inhibition of the neuronal synchronization and of the N-type calcium channels (3). Oral LEV is rapidly and almost completely absorbed with plasma peak concentration reached within 1 h of intake but food can delay and reduce the peak concentration without an effect on bioavailability (4). In ICU patients who have received LEV for seizure prophylaxis (500 mg every 12 h) the clearance of LEV was faster when compared to the similar values obtain in healthy controls and patients in status epilepticus (SE); Monte Carlo simulation determined the most optimal LEV doses in these patients to achieve appropriate serum concentration should be either 1,000 mg every 8 h or 1,500–2,000 mg every 12 h (5). In patients with or without preexisting epilepsy who presented with SE and who were taking between none and several concomitant AEDs the pharmacokinetic data of IV infusion were comparable to the previously published values derived from healthy volunteers (6) while doses of IV LEV that were antiepileptogenic in animal models of epilepsy (55 mg/kg/day) administered to patients with traumatic brain injury (TBI) resulted in comparable pharmacokinetics (PK) in children, adults, and elderly with similar results observed between days 3 and 30 of treatment (delay in T_{max} in elderly was observed but this was of unclear clinical significance) (7). One study in patients with subarachnoid hemorrhage (SAH) compared the plasma concentrations of LEV while receiving IV or parenteral forms for seizure prevention – when switched to parenteral form the levels decreased to 70% of the IV levels but complications in response to this change were not observed (8). Finally, LEV is known to suppress seizures in the animal models of epilepsy and pretreatment with LEV can delay or altogether prevent the development of kindled seizures (9–12). Thus, the overall very favorable clinical and pharmacokinetic profiles make LEV a desirable treatment option for the use in the critical care setting. The goal for this invited commentary was to review the available literature focusing on the use of LEV in the critical care setting and to provide recommendations for future research.

MATERIALS AND METHODS

An extensive literature search was performed evaluating studies for IV LEV use in critical setting for the management of SE in adults, stroke-related seizures, TBI, SAH, intracranial hemorrhage, seizure prophylaxis in patients undergoing surgery for brain tumors and its use in neonates, and children and blood levels and therapeutic monitoring.

RESULTS

SPECIAL PATIENT POPULATIONS

Status epilepticus

The initial reports of the use of LEV in the setting of SE utilized oral doses administered via feeding tube in patients ages 16–91 years. One study reported complete seizure control in all patients within

12–96 h of the initial LEV administration and the other study reported good outcomes in 11/13 episodes of SE (13, 14). Since then and since the IV form of LEV became available in 2006, this AED has been frequently favored in the critical care setting over other AEDs because of the simplicity of administration, linear PK, lack of significant cardiovascular side effects and lack of interactions with other medications (1, 3). This includes the use of IV LEV for the treatment of all types of seizures and SE. Four open-label prospective clinical studies evaluated use of IV LEV in adults with convulsive SE and found IV LEV to be effective in terminating SE with minimal side effects (6, 15–17). In the first study, Fattouch et al. used LEV as first-line therapy to demonstrate resolution or significant reduction in SE and seizures in 8/9 elderly patients who had no seizure recurrence within 24 h and who did not report any adverse events (16). The study by Misra et al. randomized 79 patients with seizures lasting >5 min to an initial therapy with IV LEV 20 mg/kg over 15 min or IV lorazepam (LZP) 0.1 mg/kg over 2–4 min with switch-over in case of lack of efficacy (17). This study demonstrated similar efficacy for the treatment of SE between LEV (29/38; 76.3%) vs. LZP (31/41; 75.6%); after switch-over 88.9% were controlled with LZP vs. 70% with LEV. The 24-h seizure-free rate was 23/29 for LEV and 21/31 for LZP. However, LZP patients experienced a higher need of artificial ventilation (17). In another study, Uges et al. determined that IV LEV added to standard SE treatment [IV clonazepam and/or rectal diazepam followed as needed by PHT or valproic acid (VPA)], was feasible and safe (6). Finally, in the study by Eue et al. 43 patients with SE were treated with IV LEV 1,000 or 2,000 mg after treatment with benzodiazepines was deemed to be ineffective. IV LEV was well tolerated and terminated SE in 19/43 patients; LEV was more effective in simple focal, complex focal, and myoclonic SE than in non-convulsive, subtle, or secondarily generalized SE (0/8) (15).

Several retrospective studies of IV LEV for the treatment of various forms of SE were conducted in 236 adults (18–22). For example, one study found that LEV was effective in controlling SE in 57.5% of patients with higher chance of seizure control if used as initial therapy or add-on to benzodiazepines (BZD; 78.5%) than as an add-on to treatment (BZD plus PHT, VPA, or both; 46.1%) (18). A study by Alvarez et al. compared benzodiazepines plus second-line treatment with PHT, VPA, or LEV to find LEV to be less effective in controlling SE than VPA at 51.7 vs. 74.6% but there were no differences in outcomes at discharge between the three groups (19). Another study by Möddel et al. found that IV LEV (bolus or continuous infusion) resolved refractory SE in 69% of 36 patients; higher incidence of failures was associated with doses >3,000 mg/day, lack of bolus, treatment initiated >48 h after diagnosis, non-convulsive SE with coma, periodic lateralized epileptiform transients, acute cerebral lesion, and intubation narcosis (21). Overall, these studies used variable doses of LEV between 1,000 and 9,000 mg/day with or without initial bolus. Of importance, these studies reported low numbers of patients with side effects due to LEV which typically included nausea and vomiting (20, 21), elevated liver enzymes (20), and transient thrombocytopenia (22). The reported mortality was 17% (responders 4%, non-responders 45%) (21). The message from these prospective and retrospective data collections appears to be fairly clear – the efficacy of IV LEV for the management of SE appears to be between 48 and 94% (probably closer to 50%) with better efficacy reported with early LEV initiation and with pre-treatment with BDZ as seen in studies of other AEDs in animal and human SE (23, 24).

Stroke-related seizures

The American Stroke Association's guidelines for early management of adults with ischemic stroke state that prophylactic use of AEDs in patients with stroke who have not had seizures is not recommended (Class III, Level of Evidence C); but, if seizures after stroke occur, treatment should follow the guidelines for the management of seizures in other neurological conditions (Class I, Level of Evidence B) (25). Overall, three studies reported on treating 98 patients with post-stroke seizures with LEV (ages 57–89 years) (26–28). In one prospective study, 82.4% of patients were seizure-free on LEV doses ranging from 1,000 to 2,000 mg/day (27). Another study, reported on the treatment of early and late seizures in the setting of ischemic or hemorrhagic stroke and found that in ∼90% of patients seizures were controlled (26/29) with LEV dosed at 1,000–2,000 mg/day (28). Finally, Belcastro et al. treated 35 post-stroke seizure patients with LEV to report seizure freedom of 77.1% (26). Additional retrospective studies evaluated the efficacy of LEV in a total of 92 patients with early or late post-stroke seizures in doses of up to 3,000 mg/day (29–31). In either monotherapy or adjunctive therapy, in the majority of patients seizures were controlled. While the incidence of early and late seizures in patients with stroke (ischemic or hemorrhagic) is fairly high, reaching in some studies 10% or more (32, 33), and many calls made for the development of randomized controlled trials for seizure treatment or seizure prevention in these patients, such studies have not been conducted to date (34).

Post-traumatic seizures

According to the published guidelines, the prophylactic use of PHT may reduce early post-traumatic seizures (within 7 days; Class I) but this or other AEDs are not recommended for preventing late post-traumatic seizures (>7 days of injury; Class I) (35, 36). One open-label, non-randomized phase II study compared prophylactic LEV for 30 days ($N = 66$) to no AED use (observation; $N = 60$) in 86 adults and 40 children following TBI (37). Patients with early presentation (within 8 h of TBI) received LEV while patients presenting >8 h after TBI did not receive LEV. The severity of TBI was higher in the LEV-treated group ($p = 0.03$). This study reported seizure incidence of 10.9% in the treated group (more severe TBI group) vs. 20% in the observation group at 2 years but the difference was not significant ($p = 0.18$) (37). Two-year follow-up of the pediatric group ($N = 40$) revealed that only one patient developed late seizures/epilepsy (defined as seizures after the initial 7 days period) (38).

Several prospective studies of seizure prevention in adults following TBI focused on the use of LEV up to the dose of 4,000 mg/day. Szaflarski et al. in a prospective, single-blinded, randomized clinical trial compared LEV to PHT within 24 h of TBI or SAH in 52 patients (39). While there were no differences in seizure or mortality outcomes between the groups, patients dosed with LEV had better outcomes including lower Disability Rating Scale (DRS) scores at 3 months and higher Glasgow

Outcomes Scale at 6 months when compared to patients treated with PHT. In this study, seizure incidence was based on the results of video/EEG monitoring conducted for up to 72 h after the initial dose of AED was administered which is considered standard in the setting of severe TBI (40). In another prospective non-randomized and not blinded study, Inaba et al. evaluated 813 patients with blunt TBI who were treated prophylactically with LEV ($N = 406$) or PHT ($N = 407$) and then monitored for the development for clinical seizures (no EEG monitoring) within 7 days (41). Further, patient distribution in the treatment arms was unbalanced with each center following local practice patterns and one of the centers preferentially utilizing LEV and the other PHT. Results demonstrated no differences in mortality (5.4 vs. 3.7%, $p = 0.236$), seizure rate (1.5 vs. 1.5%, $p = 0.997$), or adverse drug reactions (7.9 vs. 10.3%, p = 0.227) between the two groups. Jones et al. prospectively evaluated 32 patients who had received LEV for seizure prevention in the setting of severe TBI and compared them to 41 patients treated with PHT (42). While only some patients in each group received EEG, increased "seizure tendency" on EEG was observed in patients who have received LEV when compared to PHT ($p = 0.003$); seizure incidence between groups was similar ($p = 0.556$). In another report, 6/7 patients with post-traumatic epilepsy became seizure-free after initiation of add-on therapy with LEV but only a relatively short (10–16 months) follow-up period was reported (43). Adverse outcomes reported in these studies included headache, somnolence, memory impairment, irritability, dizziness, depression, and ataxia with some of the studies reporting higher incidence of adverse outcomes in patients receiving PHT (39, 41, 42).

Approximately 30% of the use of LEV in the critical care setting is for seizure prophylaxis in patients with TBI (1) but the data to support such use are incomplete. Randomized and double-blinded studies are needed to address this unmet need and to provide unambiguous data regarding the short- and long-term outcomes (seizures/epilepsy, cognitive, quality of life, etc.) in patients with TBI.

Seizures following subarachnoid or intracerebral hemorrhage

The published guidelines recommend prophylactic anticonvulsant use in the immediate post-hemorrhagic period in patients with aneurysmal SAH (Class IIb, Level of Evidence B) but discourage routine long-term use of anticonvulsants (Class III, Level of Evidence B) (44, 45). Prospective studies in this population include one that compared IV LEV ($N = 18$) to IV VPA ($N = 17$) and demonstrated no difference in seizure occurrence between the groups and no adverse effects in the group using LEV (8). In a convenience sample of 442 consecutive patients with SAH ($N = 297$ treated before ICU protocol change with IV PHT load followed by 14 days of PHT treatment with doses adjusted based on the presence of low levels or seizures and $N = 145$ treated with IV LEV 500 mg twice daily without loading dose for 3 days after protocol change) Murphy-Human et al. found no difference in early seizures, mortality rate, and intensive care unit or total hospital stay in patients with SAH. There was an increased likelihood of late seizures ($\geq$3 days post-SAH) and in-hospital seizures in the LEV group. However, the significant differences in treatment pattern between AEDs in this study (lack of loading and much shorter treatment with LEV) make the comparison of efficacy for seizure prevention between the groups difficult which the authors recognize as a shortcoming (46). A prospective observational study in patients with intracerebral hemorrhage (ICH) found similar risk of seizures between patients who had received PHT ($N = 28$) and LEV ($N = 18$) for seizure prevention ($p > 0.1$) but patients treated with PHT fared overall worse with increased risk of poor outcome ($p = 0.02$) and more adverse events of treatment; (47) these results have confirmed their previous findings of poorer outcomes in patients with SAH treated for seizure prophylaxis with PHT (48).

A retrospective study of the prophylactic use of PHT ($N = 25$; loading dose 15–20 mg/kg with later adjustments of the dose) or LEV ($N = 60$; dose 500–2,000 mg/day) in patients with ICH ($N = 40$), SAH ($N = 26$) or subdural hemorrhage (SDH; $N = 19$) found patients treated with LEV to have higher Glasgow Coma Scale (GCS) scores at discharge, lower seizure incidence, and higher percentage discharge home when compared to the PHT group (2). Trend toward better cognitive outcomes in the LEV group was also observed ($p = 0.08$). Shah and Husain retrospectively evaluated 176 patients with post-aneurysmal SAH who received prophylactic treatment with PHT (loading dose 20 mg/kg and maintenance dose 5–7 mg/kg/day) who were later transitioned to LEV (1,500 mg twice daily) due to adverse events including elevated transaminases, thrombocytopenia, rash, unexplained fever, mental status decline, or gastrointestinal (GI) disturbance; all but one patient switched to LEV with GI disturbance and three patients with mental status abnormalities had subsequent improvement or resolution of symptoms at discharge or by the first follow-up visit (14–41 days following discharge). Adverse events occurred more frequently in the PHT group and there were no clinical seizures in the LEV group (49).

Tumor-related seizures

It should not be a surprise to note LEV being used in the setting of seizure prevention or seizure treatment in patients with central nervous system (CNS) malignancies – several early studies reported positive experiences in this setting (50, 51). The main reason for this switch in practice pattern is the fact that the newer AEDs (including LEV) do not interfere with the metabolism of chemotherapeutics and, thus, do not negatively affect their efficacy (52). Overall, seizures/epilepsy is common in patients with brain malignancies ranging from ~10% in patients with CNS lymphomas and up to 100% in dysembryoplastic tumors (53). Generally, initiation of therapy with an AED is warranted in patients who had at least one seizure in the setting of a brain tumor but whether an AED should be initiated in patients with brain tumors who have not experienced a seizure is less clear. Depending on type of tumor, age, location, etc., patients diagnosed with CNS malignancies have 20–45% chance of developing seizures (53). Some authorities suggest the use of LEV or gabapentin as first-line therapy for the treatment of seizures in patients with brain tumors (54). One of the first LEV studies in this population enrolled 26 patients with gliomas – LEV was used as an add-on therapy from 2,000 to 4,000 mg/day to achieve seizure reduction of >50% in 65% of the patients (4/20 previously refractory patients became seizure-free) (51). A prospective

observational study enrolled 30 patients with brain tumors and epilepsy who were treated with LEV administered for 4 weeks prior to and for 4 weeks following respective procedure ($N = 25$ for the post-surgical group) (55). Initial doses were 1,000 mg/day with dose escalation in case of seizures up to 3,000 mg/day. Of the 25 patients, 88% were seizure-free at 48 h and 84% were seizure-free at 4 weeks following surgery (55). Another prospective open-label study evaluated treatment with LEV monotherapy for the first post-resection month in 17 patients with brain tumors who had >1 seizure within 1 month prior to surgery (56). Postoperatively, all patients received IV LEV for 48 h at 500 mg BID or pre-surgery dose, then titrated up by 500 mg/day to goal 3,000 mg/day as tolerated. There was a >50% reduction in seizures in 11/12 patients who completed the study. Lim et al. conducted a prospective, open-label study of transition from monotherapy with PHT to monotherapy with LEV in 29 patients for postoperative control of glioma-related seizures (1/3 continued on PHT while 2/3 transitioned to LEV) (57). At 6 months after surgery, 87% (13/15) of patients on LEV and 75% (6/8) of patients on PHT were seizure-free. Both groups had similar incidence of excessive sleepiness, sleeping difficulty, and lack of energy or strength, although increased incoordination in PHT group and increased slurred speech in LEV group.

Finally, Milligan and colleagues performed a retrospective analysis on the incidence of early seizures and postoperative epilepsy in 315 adults following supratentorial surgery who received prophylactic monotherapy LEV (500–3,000 mg/day) vs. monotherapy PHT (200–800 mg/day). Ninety-nine patients had a primary brain tumor and in those patients, early seizures occurred in 2.3% on LEV and 3.6% on PHT. Fifty-five of the 99 patients were followed >12 months and 5/11 on LEV and 24/44 on PHT developed epilepsy. Thirty-eight patients on PHT vs. one patient on LEV discontinued AED treatment due to side effects ($p = 0.03$) (58). Another retrospective study evaluated prophylactic use of LEV (1,000–3,000 mg/day) in 78 patients with supratentorial brain tumors. Preoperative seizure incidence was 38.5% and postoperative seizures occurred in 2.6% (2/78) patients with 91% of patients being seizure-free at the end of the mean follow-up to 10.5 months (59). Finally, Hildebrand et al. reported on the use of various AEDs in the setting of brain tumors including LEV to find epilepsy in 80% of their patients; the typical dose was 1,000–3,000 mg/day but the treatment of LEV was not compared specifically to other AEDs (60).

While substantial body of evidence is available regarding the treatment of seizures in the setting of brain tumors or supratentorial surgery for the management of brain tumors and some have advocated the use of LEV in this setting after the data by Milligan et al. were published (58, 61), careful prospective studies are needed to assess the use of LEV as a preventive AED in this setting, to evaluate complex interaction between surgery, chemotherapy, and AEDs and, finally, whether LEV should be the preferred AED in this setting instead of PHT or VPA (53, 54).

Geriatric population

There were no observed safety differences between 347 geriatric patients (age ≥ 65) and younger patients treated with LEV for seizures, although the number of the elderly patients enrolled in the controlled trials of epilepsy is insufficient to determine the effectiveness of LEV in this population [package insert (62)]. Nevertheless, geriatric patients have been enrolled in many of the retrospective and prospective studies of LEV including studies that used IV doses of LEV. For example, Uges et al. analyzed safety and PK of IV infusion of LEV in patients with SE ages 44–75 years of age (median 60 years) to show PK values in the studied group similar to norms obtained from healthy (and younger) volunteers (6). Another study by Klein et al. showed that T_{max} was longer in subjects older than 65 years of age when compared to children and young adults at the initiation of the therapy and at 30 days (7). In the elderly LEV appears to be safe and associated with a relatively low level of adverse events. In part, this is related to lack of significant drug–drug interactions. Overall, PK studies and safety/efficacy studies of LEV in the elderly are needed as the incidence and prevalence of epilepsy, and thus the use of AEDs in this population are increasing.

The use of IV LEV in the pediatric population

When initially approved by the FDA, IV LEV was not indicated for use in children less than 16 years of age. Since then, prospective studies using IV LEV to treat acute seizures in neonates and children have assessed the safety and efficacy of LEV use in these age groups (63, 64). Ramantani et al. conducted a prospective feasibility study in 38 newborns with LEV applied as first-line treatment for EEG-confirmed seizures (64). In this study the initial IV dose was 10 mg/kg with gradual increase up to 45–60 mg/kg over 7 days; 30/38 infants were seizure-free at the end of the evaluation period (22 had to receive additional doses of phenobarbital). Another study evaluated a single dose of IV LEV 50 mg/kg infused over 15 min in 30 children (mean age 6.3 years; range 6 months to 14.8 years) diagnosed with epilepsy (29/30) or a single seizure related to a brain lesion. The mean blood level 10 min after infusion was 83.3 mcg/mL (47–128 mcg/mL); administration of LEV was associated with a subsequent reduction of all seizure types for up to 24-h after the infusion (63). Adverse events in both studies included sleepiness and/or fatigue, drowsiness with titration, and thrombocytopenia with concurrent VPA use. Further studies utilizing IV LEV for the treatment of acute seizures in 189 pediatric patients (1 day to 18 years) in eight retrospective case series and two case reports resulted an improved clinical seizure control; 118/189 reported concurrent EEG monitoring which demonstrated improved electrographic seizure control (65–74).

METHODS OF INFUSION AND MONITORING

Rapid infusion

Intravenous LEV is supplied in a concentrated form that needs to be diluted in compatible diluent prior to administration. While the IV formulation is reported to be bioequivalent to the oral formulation and doses should be interchangeable some differences in bioavailability between the IV and parenteral doses have been reported (8). There are also some age-related differences in PK (7) but it is unclear whether these differences are of clinical significance. In one study, Wheless et al. assessed rapid infusion (over 5–6 min) of 20, 40, and 60 mg/kg ($N = 15$ per group) of IV LEV in children and adults (4–32 years of age). Maximum

plasma concentration peaked 15 min after infusion. The infusion was well tolerated with minimal side effects including nonpruritic rash ($N = 1$) and infusion site pain ($N = 2$); there were no electrocardiographic changes reported (75).

Therapeutic monitoring of serum LEV levels in different patient populations

Although therapeutic serum concentration ranges and a schedule for blood level monitoring for LEV have not been established, monitoring is recommended, e.g., from pregnancy through the postpartum period due to physiologic changes leading to gradual decreases in LEV plasma levels with the advancement of the pregnancy (62). One prospective study in 30 epilepsy patients on >2 AEDs, including LEV in doses ranging from 2,000 to 3,000 mg/day, defined the therapeutic LEV plasma range of 10–40 mcg/mL (76). Eighteen patients were either seizure-free ($N = 5$) or had >50% seizure reduction ($N = 13$). The majority of patients had an associated therapeutic LEV range in the low-therapeutic range. In patients with LEV level within the low-therapeutic range adjustments in dose produced either further therapeutic response or allowed for the patients to be weaned from one of the other AEDs without any ill effects. Another study in 297 inpatients using LEV in doses 250–7,000 mg/day demonstrated serum concentrations 1.5–48.2 mcg/mL with the level to dose ratio (LDR) lower in LEV monotherapy compared to concurrent use of enzyme inducing AEDs (77). In this study, the median LDR was significantly lower when patients were co-medicated with enzyme inducer (e.g., PHT, carbamazepine, or oxcarbazepine) when compared to LEV monotherapy whereas the LDR of patients co-medicated with VPA or lamotrigine did not differ significantly from the LDR of LEV of patients on LEV monotherapy ($p > 0.05$); children had lower LEV concentrations than adults on the same dose per body weight (77).

In a pooled analysis of LEV levels in 1,023 patients enrolled in four Phase III double-blind trials and during which patients receiving one to three concomitant AEDs were treated with LEV ($N = 672$) or placebo ($N = 351$) as adjunctive therapy to treat seizures (78–81), LEV concentrations were normalized to a dose of 1 mg/kg twice daily with mean plasma concentration at 1 h ranging between 1.74 and 2.27 μg/mL at 1,000–4,000 mg/day and a mean plasma LEV level concentration of 2.09 μg/mL (95% CI 1.99, 2.19) (82). The mean plasma LEV concentration at 12 h ranged from 0.7 to 0.88 μg/mL at 1,000–4,000 mg/day and a mean plasma LEV level concentration of 0.82 μg/mL (95% CI 0.19, 0.85). LEV concentrations were lower (<25% on average) in patients using concurrent enzyme inducing AEDs and moderately higher in patients using concurrent VPA (12 h post-dose). Two retrospective studies in 73 adults with epilepsy reported LEV doses ranging from 1,000 to 4,000 mg/day with therapeutic plasma concentrations between 6 and 65 μg/mL (83, 84). Adverse events leading to LEV discontinuation included behavioral changes ($N = 3$), gait disturbance ($N = 1$), and depression ($N = 1$) (83, 84). Two retrospective studies in pediatric patients reported LEV doses in 93 children ranging from 12.7 to 84 mg/kg/day with blood levels in responders ranging from 5 to 60 μg/mL (85, 86). None of these pediatric and adult studies reported dose – level – seizure response relationship.

Neurocritical care patients

In a prospective open-label, steady-state pharmacokinetic study 12 adults admitted to the neurocritical care unit with SAH, SDH, or TBI were treated prophylactically with IV LEV (5). Doses of 1,000 mg every 8 h and 1500–2000 mg every 12 h were most likely to achieve trough levels between 6 and 20 μg/mL than doses of 500 mg twice daily; these critically ill patients demonstrated faster systemic clearance and shorter terminal elimination half-life compared to previously published data on healthy volunteers and adults in SE. Another prospective single-center registry in 35 critically ill patients with aneurysmal SAH reported decreased LEV plasma concentrations after transition from IV to parenteral dose with concurrent decrease in bioavailability by ∼30% (8).

SUMMARY

Intravenous LEV is a safe and effective treatment for acute seizures and SE and has fewer side effects than some traditional first-line agents. The evidence suggests that early treatment and use in focal and myoclonic SE may be more effective than in secondarily generalized SE. However, large controlled and blinded studies are needed to answer these questions. Most studies in patients with SAH or ICH demonstrated no difference in early seizures or mortality with prophylactic use of LEV when compared to other AEDs. Only one prospective study suggested increased rate of late seizures in patients on LEV compared to PHT, however IV formulation was changed to enteral formulation and it is unclear how that may have affected the outcome; the treatment with LEV in this study was overall shorter and the LEV dose substantially lower than the dose of the comparator – PHT which may have affected the results. Several studies in patients with SAH, ICH, or TBI found decreased side effects in patients on LEV vs. PHT or VPA. There was no difference in rate of post-traumatic seizures or mortality in patients with TBI whether treated with LEV or PHT, however reduced disability scores at 3 months and higher Glasgow Outcomes Scale scores at 6 months in patients on LEV suggest a potential neuroprotective effect of LEV which is in agreement with animal studies. IV LEV has proven to be effective and safe for use in treating acute seizures in children of all ages from premature neonates to teenagers. Rapid infusion of IV LEV over 5 min in children and adults is safe and well tolerated. Therapeutic LEV monitoring is important to perform in some patient populations, especially in those who are critically ill, but the relationship between the dose – level – seizure response has not been established. Neurocritical care patients may have increased clearance with a shorter half-life compared to patients who are healthy or in SE patients.

FUTURE DIRECTIONS

The above presented data collected prospectively or retrospectively support further studies of the use of LEV in the setting of CNS emergencies whether for seizure prevention/treatment or for assessing the short- and long-term cognitive and societal outcomes (e.g., employment, quality of life, etc.). Further, randomized and double-blind studies of acute seizures and SE across ages appear to be warranted. Long-term neurological functional and disability status outcomes after administering IV LEV within 24 h of TBI should be performed to confirm the neuroprotective effects of LEV

observed in animal studies. Further studies should be performed to evaluate effective doses of LEV in critically ill patients and determine optimal schedule for therapeutic monitoring. Although some studies suggest IV LEV is safe and tolerable in geriatric patients, larger prospective studies are needed to determine the efficacy in this population, including potentially decreased renal function.

KEY CONCEPTS

1. Intravenous LEV is effective in terminating many types of seizures and SE, including convulsive SE and partial SE and is well tolerated with minimal side effects unlike some typical first and second-line agents.
2. In patients with TBI or intracranial hemorrhage long-term prophylaxis with LEV vs. PHT may not alter the incidence of seizures or mortality, however, patients treated with LEV may have better long-term outcomes.
3. In prospective and retrospective studies IV LEV appears to be a safe and effective treatment for acute seizures in premature and term newborns, school-aged children, and teenagers.
4. IV LEV can be infused rapidly over 5 min with maximal peak concentration in 15 min without significant clinical or electrocardiographic side effects in children and adults.
5. Critical care patients may have faster systemic clearance and shorter terminal elimination half-life of IV LEV compared to previously published data on healthy volunteers and adults in SE. Conversion to enteral formulation in these patients may result in lower plasma concentrations. Serum level monitoring should be considered in critically ill patients though the clinical importance of the monitoring is not clear.

AUTHOR CONTRIBUTIONS

Jennifer L. DeWolfe and Jerzy P. Szaflarski are original authors of this manuscript.

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Clinical pharmacology and pharmacokinetics of levetiracetam

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Status epilepticus and acute repetitive seizures still pose a management challenge despite the recent advances in the field of epilepsy. Parenteral formulations of old anticonvulsants are still a cornerstone in acute seizure management and are approved by the FDA. Intravenous levetiracetam (IV LEV), a second generation anticonvulsant, is approved by the FDA as an adjunctive treatment in patients 16 years or older when oral administration is not available. Data have shown that it has a unique mechanism of action, linear pharmacokinetics and no known drug interactions with other anticonvulsants. In this paper, we will review the current literature about the pharmacology and pharmacokinetics of IV LEV and the safety profile of this new anticonvulsant in acute seizure management of both adults and children.

Keywords: levetiracetam, pharmacokinetics, epilepsy

INTRODUCTION

Intravenous levetiracetam (IV LEV) is a second generation antiepileptic currently approved by the FDA as an adjunctive treatment in patients 16 years of age and older as an anticonvulsant when oral therapy is not tolerated (1). The intravenous formulation was approved in 2006. The cornerstone of therapy remains the older intravenous antiepileptic drugs (AEDs) such as benzodiazepines, phenytoin, and phenobarbital, all of which have unwanted interactions and side effects. Increasing use in clinical practice for the management of acute seizures warrants a review of the pharmacology, pharmacokinetics, and safety profile of this drug.

PHARMACOLOGY

Levetiracetam is an (*S*)-enantiomer of the ethyl analog of piracetam, in the class of nootropic drugs which are considered to be "pharmacologically safe" (2, 3). It is structurally unrelated to any other antiepileptic class and has a novel mechanism of action. Although the precise mechanism is unknown, in animal models it has been shown to bind to synaptic vesicle protein SV2A. This protein has been related to modulation of synaptic vesicle exocytosis and neurotransmitter release. Animal models show that the affinity for SV2A is associated with protection against seizures making it an important target for new AEDs (4). *In vitro* studies demonstrated oppositional activity to negative modulators of gamma-aminobutyric acid (GABA)-gated currents despite lack of binding affinity to GABA receptors (5, 6).

INTERNATIONAL LICENSING INDICATIONS FOR LEVETIRACETAM

Levetiracetam is approved for partial seizures. The parenteral form is approved as an alternative for treatment of partial seizures if oral form is not feasible.

Dosage and directions: PO/IV-Adjunctive therapy for partial seizures – the initial dose is 500 mg twice daily with gradual upward titration to a maximum of 3 g/day.

The dose is the same for monotherapy and for partial seizures with and without secondary generation (1).

Minimum: 500 mg twice daily
Maximum: 3 g daily

PHARMACOKINETICS

Levetiracetam is rapidly and almost completely absorbed (96%) after oral administration. Normal time to maximum concentration (C_{max}) is delayed from 1 to 1.5 h when administered with food, but the extent of absorption is not affected. C_{max} is decreased by 20% as well. There is little protein binding (<10%), therefore it does not compete with other drugs for binding sites (7). The

volume of distribution ranges from 0.5 to 0.7 l/kg in adults and 0.6–0.9 l/kg in premature infants and children (2, 8). Ramael and colleagues evaluated the single-dose bioavailability of an intravenous (IV) LEV relative to oral tablets and IV relative to placebo pharmacokinetics and multiple-dose tolerability in 18 healthy subjects. There were nine white females and nine white males. The first phase of the study was a single-dose, randomized, open-label, 2-way crossover comparison of bioavailability of a 15-min infusion of LEV 1,500 mg and three 500 mg oral tablets. After this, subjects entered the second phase, which was a multiple-dose, randomized, double-blind, placebo-controlled, parallel-group tolerability, and pharmacokinetic study. They received nine successive doses of LEV 1,500 mg IV or placebo every 12 h. Plasma LEV concentrations were measured and the researchers compared the bioavailabilities. The IV infusion and oral tablet had a similar C_{max} (50.5 and 47.7 μg/ml, respectively) and AUC (392.4 and 427.9 μg h/ml, respectively) after a single dose. The IV and oral formulations were bioequivalent, indicated by the findings that geometric mean IV/oral ratios were 92.2 (90% CI, 89.0–95.6) for AUC and 103.7 (90% CI, 91.6–117.4) for C_{max}. Within 48 h steady state was reached after multiple twice-daily infusions. The incidence of treatment-emergent adverse events was 89% (16/18) for the IV formulation and 72% (13/18) for the oral tablets during the single-dose phase. During the multiple-dose phase the incidence of treatment-emergent adverse events was 67% (8/12) in the IV LEV group and 33% (2/6) in the placebo group. Somnolence (33 vs. 17% placebo) and postural dizziness (25 vs. 0% placebo) were the most common adverse events with IV LEV in the multiple-dose phase (9).

Ramael and colleagues also performed a phase I, randomized, single-blind placebo-controlled study to evaluate the safety and tolerability of LEV administered intravenously at higher doses and/or at a faster infusion rate than proposed. Forty-eight healthy subjects (three male and three female patients per dose) were randomized to receive IV infusion single-ascending doses of LEV, administered at different dosages vs. placebo (1,500, 2,000, 2,500 mg over 5 min; 2,000, 3,000, 4,000 mg over 15 min). Healthy subjects were aged 18–55 years of age, had a body mass index (BMI) of 19–28, had to be in good physical and mental health, and were excluded for any disorder that could alter the pharmacokinetics or be a risk factor to not tolerating the drug (e.g., allergies, previous intolerance of pyrrolidone derivatives or its injectable diluents).

Adverse events most commonly reported were dizziness (52.8%), somnolence (33.3%), fatigue (11.1%), and headache (8.3%). There was no clear relation with IV dose level or infusion rate, and were consistent with the oral formulation safety profile. Each dose level and for both IV infusion rates had similar safety profiles. The pharmacokinetics of LEV administered by IV infusion were comparable across dose groups and infusion rates. The geometric means for 4,000 mg administered over 15 min and 2,500 mg infused over 5 min were maximum plasma concentration, respectively 145 (24.6%) and 94.3 (36.2%). Area under the plasma concentration-time curves were 1,239 (19.2%) and 585 (9.6%) μg/h/ml, and terminal half-lives were 8.0 (14.5%) and 7.0 (12.7%) h. Thus, Ramael and colleagues surmised that the pharmacokinetic profile was consistent with oral LEV and that the higher rates than those proposed of LEV IV administration dosages and infusion rates were tolerated in healthy subjects (10).

In a crossover study conducted by Leppik and colleagues, five women and five men were given intramuscular (IM) and IV LEV. All subjects were healthy and ranged in age from 21 to 59 years old (mean 35.0 years). Subjects were randomized so that half of them first received the IM injection followed 2 weeks later with IV administration. The administration of IM LEV was double-blinded to fully assess tolerability. To determine absolute bioavailability, the IV administration was unblinded. IM LEV was determined to be well tolerated due to no observation of inflammation or tissue break down and the pain scores at 1 min after IM LEV (29 mm for women and 18 mm for men, both with significant subject variability) returning to a baseline 15 min after IM injection. Compared to IV, IM LEV was completely absorbed. The mean bioavailability was 1.08 ± 0.19 (0.94–1.12) with CI of 97–118%. Within 2 h after IM injection 85% of C_{max} was reached. Within 0.75–4 h (median = 2 h) of IM administration maximum concentration occurred. Two hour post-dose LEV concentrations were similar for both IV and IM doses. The study concluded that 5 ml (500 mg) IM LEV is well tolerated and its bioavailability is equivalent to an IV injection (11).

Wheless and colleagues evaluated the safety of a rapid loading dose of IV LEV in a prospective, open-label, single-center study conducted from February 2007 to August 2008. Patients had a confirmed diagnosis of generalized epilepsy or partial-onset seizures and received an AED prior to IV LEV. A total of 45 study patients, aged 4–32 years, were divided into three equal dosing groups of 20, 40, and 60 mg/kg. A single loading dose of IV LEV was administered using a flow control pump which aided in timing of infusion accuracy. The 20 and 40 mg/kg group doses were administered as a 5-min infusion, and the 60 mg/kg dose was administered as a 6-min infusion. Baseline hematology and serum chemistries were collected upon hospital admission. During the infusion, safety assessments, and electrocardiograms (EKGs) were performed. The serum LEV concentrations were 14–189 μg/ml after infusion. There were no EKG abnormalities, no local infusion site redness or tenderness, and no changes in blood pressure. The mean dose administered in the 5- and 6-min infusion groups were 26.1 and 51.3 mg/kg. The 5- and 6-min infusion groups had a comparable volume of distribution (l/kg), with a mean of 0.40 and 0.42, respectively. Within 15 min of the end of the infusion of IV LEV, 95% (38/40 patients) had achieved maximum plasma drug concentrations. The researchers concluded a rapid infusion can safely achieve high serum levels of parenteral LEV (12).

HEPATIC IMPAIRMENT

No dosage adjustment is needed for patients with hepatic impairment. However in patients with severe hepatic impairment, Child-Pugh C, total body clearance was half that of normal subjects. Decreased renal clearance was attributed for most of the decrease (1). In an open-label, parallel-group, single-dose pharmacokinetic study, Brockmoller and colleagues studied the pharmacokinetics of LEV and its metabolite UCB L057 in patients with liver cirrhosis. Five healthy subjects and patients with Child-Pugh class A ($n = 5$), B ($n = 6$), or C ($n = 5$) alcohol-induced cirrhosis received a single-dose of LEV. Biochemical liver function parameters were

measured and correlated with the pharmacokinetics of LEV. Three dynamic liver function tests characterized liver function during the screening phase with the caffeine test, lidocaine test, and D-sorbitol as a probe for liver blood flow. After these tests were used to determine the baseline function, a 1,000 mg dose of LEV was administered to measure the pharmacokinetics of LEV and UCB L057. Validated gas chromatographic assays measured plasma and urine levels of LEV.

A deterioration of liver function was revealed by dynamic liver function tests. The healthy subjects and class A or B cirrhosis did not differ in their pharmacokinetics of LEV or UCB L057. A statistically significant 57% reduction was found in LEV total clearance in patients with class C cirrhosis ($p < 0.001$). The Child-Pugh class C vs. control of the geometric mean ratio of the area under the plasma concentration-time curve (AUC) for LEV was 2.41. The geometric mean of the half-life ratio was 2.27. The conclusion was that patients with mild to moderate liver impairment do not require a dose adjustment of LEV. However, half of the commonly recommended dose should be given initially to patients with severe cirrhosis (13).

RENAL IMPAIRMENT

The majority (66%) of LEV is eliminated renally as unchanged drug primarily by glomerular filtration with some subsequent tubular reabsorption (7). Mean half-life in infants and young children of 5.3 h is slightly shorter than the half-life in older children of 6 h. Adults have a reported half-life of 6–8 h and reach steady state concentrations in 2 days (7, 8). Total body clearance in infants <6 months is 1.23 ml/min/kg, >6 months is 1.57 ml/min/kg, and in adults approximately 1 ml/min/kg (9). Renal clearance in children is 0.8 ml/min/kg, 0.6 ml/min/kg in adults, and 0.5 ml/min/kg in the elderly (2). Renal failure can be expected to prolong the half-life to approximately 25 h (7). Doses in patients with altered renal function should be reduced (7). Nearly 50% of the drug can be removed by a 4 h dialysis session (1). The half-life of LEV was found to be 2.5 h longer in the elderly population most likely due to their decreased renal function (1).

PREGNANCY

The pharmacokinetic profile of AEDs is known to be affected by pregnancy, leading to concerns of seizure control, and fetal drug exposure. Tomson and colleagues studied the pharmacokinetics of LEV during pregnancy, delivery, lactation, and the neonatal period. In this prospective study, 14 women with epilepsy treated with LEV during pregnancy, and lactation during 15 pregnancies, were included to determine the LEV concentration in plasma and breast milk. The women's ages ranged from 21 to 37 years old. During pregnancy, LEV was used as monotherapy in six patients and used as combination therapy in nine patients. Each trimester and after delivery, maternal plasma samples were collected. Maternal blood samples were collected at delivery, from the umbilical cord, and 2 days after delivery, blood samples were obtained from the newborns. Breast milk and plasma were collected from 11 mothers and their suckling infants after birth to determine LEV concentration.

The maternal/umbilical cord plasma concentration ratios ranged from 0.56 to 2.0 with a mean of 1.15. Neonatal plasma LEV concentration had an estimated half-life of 18 h. The mean milk/maternal plasma concentration ratio was 1.05 (range 0.78–1.55). The LEV dose in infants was estimated to 2.4 mg/kg/day. Breastfed newborn plasma concentrations were 13% of the maternal plasma levels. When compared with the baseline concentrations outside of pregnancy, during the third trimester maternal plasma concentrations were only 40% of baseline. There appears to be enhanced elimination of LEV in pregnancy. The resulting significant decline in plasma concentration indicates that therapeutic monitoring in pregnancy may be valuable (14).

CHILDREN AND NEONATES

Weinstock and colleagues assessed tolerability, safety, and pharmacokinetics of IV LEV in 52 children with epilepsy in a prospective, single-arm, multicenter study. Eligible children were aged 1 month to <4 years and 4–16 years with epilepsy requiring short-term in-hospital IV LEV administration. Children with difficult venous access, EKG abnormalities, ketogenic diet, felbamate exposure within 18 months, and status epilepticus in the previous 3 months were excluded. On study day 1, LEV pharmacokinetic assessments were performed from blood and saliva at 3–10 min intervals after the start of the infusion, at the end of the infusion, and up to 12 h post-infusion. The study completion rate of 16 of the 19 patients in the 1 month to <4 year group and 33/33 in the 4–16 years group. The seizures types in the 1 month to <4 year group vs. the 4–16 years group were partial onset 15/19 vs. 25/33, generalized onset 6/19 vs. 12/33, and unclassified 2/19 vs. 10/33. Sixty-three percent of patients had mild to moderate treatment-emergent adverse events. These were most frequently pyrexia and dry mouth. The LEV plasma and saliva concentration ranges were at expected levels based on the administered dose. The researchers concluded that IV LEV in the acute setting was overall well tolerated in children 1 month to 16 years (15).

Merhar and colleagues enrolled neonates in a prospective study to determine the pharmacokinetics of LEV. Eighteen neonates admitted to the neonatal intensive care unit were enrolled who were ≤30 days of age and ≥32 weeks gestational age with seizures treated with LEV from October 2008 to May 2010. Neonates with birth weights <2,000 g and creatinine levels ≥2.9 mg/dl were excluded. Before the first dose of LEV was administered, blood draws were taken. A dose of at least 20 mg/kg of phenobarbital was given to all subjects prior to receiving LEV. Fifty-four total measurements of LEV blood levels were obtained at time points during the entire dosing interval and concentrations were quantified by a liquid chromatography–electrospray tandem mass spectrometry assay. The pharmacokinetic analyses were performed with non-linear mixed effects modeling. The initial loading doses of LEV were between 14.4 and 39.9 mg/kg. The model prediction of the median maximum drug concentration was 39.8 mg/l (14.8–91.9 mg/l). One hour after a 30 mg/kg dose was the highest measured concentration at 87.6 mg/l.

When compared with older children and adults, neonates were found to have a lower clearance (neonates = 1.21 ml/min/kg, adults = 0.96 ml/min/kg), higher volume of distribution (neonates = 0.89 l/kg, adults = 0.5–0.7 l/kg), and longer half-life (neonates = 8.9 h, adults = 6–8 h). LEV was well tolerated in this population. The only adverse effect observed was mild somnolence 24 h after LEV administration. Thus, Merhar and colleagues concluded that

the pharmacokinetics of LEV in neonates differed from children and adults (16).

Ng and colleagues assessed the safety of IV LEV in 30 children (average 6.3 years, 0.5–14.8 years) with seizures in a prospective study from July 2007 to October 2008. Enrollment criteria included hospitalized in patients treated for seizures who were LEV naïve or had not received LEV 3 days prior to administration. Exclusion criteria were unstable patients (including status epilepticus), prior LEV allergy, or patients >15 years old. Subjects received a single-dose of IV LEV 50 mg/kg, up to a maximal dose 2,500 mg, over 15 min. Ten minutes after the infusion, a blood level of LEV was drawn. Then the patients continued IV LEV or oral LEV as tolerated. Seizure types, duration, frequency, and seizure outcomes were evaluated via hospital chart review. The 50 mg/kg LEV dose was well tolerated by all patients and was a safe, appropriate loading dose. There were no observations of serious adverse reactions, although sleepiness, fatigue, and restlessness were noted. 10 min after the infusion a blood level of LEV was performed. LEV levels ranged from 47 to 128 μg/ml with a mean of 83.3 μg/ml. All seizure types had an apparent decrease in seizure frequency from 24 h before compared to 24 h after the infusion. Ng and colleagues reported 15.4% (4/26) had no seizures before or after the 24 h infusion. In addition, 57.5% (15/26) of patients who were having seizures within 24 h before the infusion became seizure free. The authors also reported that 38.5% (10/26) had more than 50% reduction in seizures (17).

Glauser and colleagues also conducted a multicenter, open label, single-dose pharmacokinetic study to assess LEV and its major metabolite L057 in infants and young children who were diagnosed with epilepsy. Thirteen subjects were enrolled in the study with the age range between 2.3 and 46.2 months. One patient was excluded because of a medical condition. The subjects received a dose of 20 mg/kg administered as 10% solution followed by evaluation for 24 h to assess the pharmacokinetics. The samples were collected predose and at 1, 2, 4, 9, 12, 16, and 24 h. The half-life of LEV was found to be 5.3 ± 1.3 h and clearance was 1.46 ± 0.42 ml/min/kg. No serious side-effects were reported. The authors concluded that the mean half-life of the drug was shorter and clearance was much more rapid as compared to previously reported adult data. The authors suggested that larger doses of the drug, which are corrected for body weight, should be administered to infants and young children (18).

DRUG INTERACTIONS

Otoul and colleagues studied 187 children aged 4–16 years with epilepsy being treated with adjunctive LEV to determine whether plasma concentration of carbamazepine, valproic acid, topiramate, and lamotrigine were affected. There were 95 males and 92 females with 94 subjects randomized to receive adjunctive treatment with LEV and 93 subjects receiving placebo. Data from a randomized placebo-controlled phase III trial in children receiving concomitant AEDs and adjunctive LEV were used to perform these retrospective analyses. During an initial 4-week titration period, LEV was increased in 20 mg/kg/day increments to a target dose of 60 mg/kg/day. Then a 10-week period of treatment remaining at 60 mg/kg/day was evaluated. Study patients were evaluated if they had at least 2 weeks of a constant dose of LEV/placebo, unchanged AED dose for 2 weeks, and a maximum of two concomitant AEDs was allowed. Blood samples were taken at each study visit for trough AED levels and LEV levels, including two to three baseline period visits and five visits over the evaluation period.

At baseline and during LEV treatment the geometric mean concentrations were carbamazepine 8.4 μg/ml vs. 8.1 μg/ml (coefficient of variation, CV = 30%; $n = 35$), valproic acid 83.8 vs. 82.5 μg/ml (CV = 38%; $n = 23$), topiramate 7.3 vs. 7.2 μg/ml (CV = 82%; $n = 28$), and lamotrigine 8.2 vs. 7.7 μg/ml (CV = 62%; $n = 22$). The mean concentration ratios (LEV/baseline and their 90% confidence intervals for each AED were unaffected when combined with LEV administration. When LEV was compared to placebo, no differences were observed. The researchers concluded that in children with epilepsy LEV does not affect plasma concentrations of carbamazepine, valproic acid, topiramate, or lamotrigine (19).

Freitas-Lima and colleagues assessed whether LEV elimination was influenced by enzyme inducing antiepileptic drugs (EIAEDs). Study subjects were healthy besides being diagnosed with epilepsy and were aged 18–65 years. Patients were excluded if they were pregnant. The 15 subjects included in the EIAED group were stable for at least 1 month of treatment with carbamazepine, phenytoin, or phenobarbital alone or in combination. The 15 subjects in the control group were matched patients not receiving AEDs. Subjects on valproate or other drugs influencing drug metabolism were excluded. At baseline and at frequent intervals, serum and urine LEV levels were measured after a single oral 1,000 mg dose. High performance liquid chromatography (HPLC) determined plasma LEV concentrations. There were no reports of adverse effects related to LEV doses. When compared to controls, the EIAED group showed significantly lower AUC values, shorter half-life ($p = 0.02$) and a higher LEV oral clearance ($p = 0.01$), with respective magnitude differences of 21, 16, and 26%. The conclusion was that this interaction could have clinical significance for some patients even though the magnitude of the effect was relatively modest (20).

EFFICACY AND SAFETY

Intravenous levetiracetam is an effective AED for seizure control. In an observational, multicenter retrospective study, LEV's efficacy was found to be dependent on the timing of administration. Forty patients were included and in approximately half (57%) of the patients, IV LEV was effective in a mean time of 14 h. In 26 of the patients, IV LEV was used as add-on treatment with an efficacy of 46.1%. As early treatment (either pretreatment with benzodiazepines or nothing) in 14 of the patients, IV LEV showed an efficacy of 78.5% leading to the conclusion that it is more effective as a first line agent and that it is more difficult to treat refractory status epilepticus (21). The use of IV LEV in neonates resulted in favorable efficacy and tolerability as described by Abend and colleagues (22), Khan and colleagues (23), and Michaelides et al (24) in a variety of seizure etiologies. Furwentsches and colleagues (25) and Ramantani and colleagues (26) have also demonstrated LEV efficacy in prospective studies in which LEV was used as a first line treatment. Li et al (27) also demonstrated that LEV is a safe and effective treatment for infants and children in an observational, prospective study. Kirmani and

colleagues (28), Goraya and colleagues (29), and Gallentine and colleagues (30) also showed the efficacy of LEV in acute seizure management in children. In another study, Khan and colleagues showed the efficacy of IV LEV in preterm neonate seizure management (31). The literature shows that a dose range from 10 to 70 mg/kg can be used effectively in children (17, 22–33). However, IV LEV is not approved for status epilepticus because no randomized larger multicenter trials were done to evaluate the efficacy in status epilepticus.

Levetiracetam can be used for both partial and generalized epilepsies but limitations in terms of seizure types have been published in the form of case reports and retrospective case series. LEV has been reported to be associated with aggravation of myoclonus in children and with adolescents with juvenile myoclonic epilepsies (34, 35). Caraballo and colleagues reported data which revealed LEV induced worsening of seizures during continuous spikes and waves during slow sleep in children with refractory epilepsies (36). Similar data about increased frequency of absence seizures with LEV have also been reported in the literature (37).

ADVERSE EFFECTS

The most common side-effects of LEV are neurobehavioral, including fatigue, nervousness, generalized weakness, irritability, agitation, emotional lability, depression, mood swings, vertigo, anxiety, unsteadiness, seizures, memory loss, confusion, increased reflexes, paresthesias, aggression, cognitive decline, and increased risk of suicide (1, 38). Other common side-effects include hypersensitivity reactions, infections, myalgias, rhinitis, and anorexia (1). Neurobehavioral side-effects are the main cause of discontinuing the medications in most instances (38). There are several case reports and case series which report acute onset of psychosis with the initiation of LEV (39–41). Increased risk of suicide has also been reported in patients on LEV therapy (42, 43).

TAKE AWAY POINTS

1. Levetiracetam is a novel AED which is approved as adjunctive therapy for partial-onset seizures both in adults and children 1 month and older.
2. The metabolism of LEV has no effect on the cytochrome P450 enzyme system so it is favorable in terms of no drug–drug interactions.
3. No dose adjustment is needed in hepatic impairment but dose needs to be adjusted in patients with renal impaired.
4. The dose used in double-blind placebo-controlled trials is 1,000–3,000 mg/day. No tolerance was observed and efficacy was maintained in long term studies.
5. The drug seems to be well-tolerated in pregnancy and teratogenic potential is less than first generation antiepileptics. The anticonvulsant levels seem to decline toward the latter part of pregnancy requiring close monitoring of the drug levels.
6. The most common side-effects are somnolence, dizziness, and asthenia. The other reported side-effects are irritability, agitation, aggressive behavior, and anger.
7. The intravenous formulation is approved for patients 16 years or older if oral administration of the drug is not feasible. However, the off – label use in adults and children for acute seizure management yielded favorable results. Further studies are needed to prove the efficacy of this drug for acute seizure management.

CONCLUSION

The literature shows that LEV has a novel mechanism of action and unique pharmacokinetic profile to be used as a desirable antiepileptic choice in an acute inpatient setting. Our conclusions, on the other hand, are based on existing data which include case reports, case series, retrospective studies, and some prospective trials. However, there are limitations in that there are neurobehavioral side-effects of the drug. We believe that there is a need for larger, prospective, multicenter, randomized double comparative blind trials in order to further clarify the role of this anticonvulsant in acute seizure management.

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Efficacy and tolerability of intravenous levetiracetam in children

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Intractable epilepsy in children poses a serious medical challenge. Acute repetitive seizures and status epilepticus leads to frequent emergency room visits and hospital admissions. Delay of treatment may lead to resistance to the first-line anticonvulsant therapies. It has been shown that these children continue to remain intractable even after acute seizure management with approved Food and Drug Administration (FDA) agents. Intravenous levetiracetam, a second-generation anticonvulsant was approved by the FDA in 2006 in patients 16 years and older as an alternative when oral treatment is not an option. Data have been published showing that intravenous levetiracetam is safe and efficacious, and can be used in an acute inpatient setting. This current review will discuss the recent data about the safety and tolerability of intravenous levetiracetam in children and neonates, and emphasize the need for a larger prospective multicenter trial to prove the efficacy of this agent in acute seizure management.

Keywords: epilepsy, intractable epilepsy, seizures, status epilepticus, intravenous levetiracetam, children

INTRODUCTION

The pediatric epilepsy population is frequently admitted to the hospital because of status epilepticus or acute repetitive seizures. Status epilepticus is defined as continuous seizure activity lasting for 30 min or longer or intermittent seizures lasting for more than 30 min from which the patient does not regain consciousness (1). The other definition is a continuous seizure lasting at least 5 min, or two or more seizures without full recovery of consciousness between seizures lasting at least 5 min or more (2). Acute repetitive seizures are described as seizures that recur over a set period. These typically last for hours in children and up to 1 or 2 days in adults (3).

Acute management of seizures is crucial in the prevention of permanent neurological sequelae. Despite the use of benzodiazepines and first generation anticonvulsants, patients still remain refractory to treatment, emphasizing the need of newer anticonvulsants such as levetiracetam.

Levetiracetam is a pyrrolidine-derivative antiepileptic drug, which is chemically different from all other anticonvulsant agents. It has a novel mechanism of action, which does not involve inhibitory and excitatory neurotransmission. Levetiracetam works by binding SV2A, an integral membrane protein present on synaptic vesicles, preventing synaptic vesicle release (4). Thereby, impeding conduction across the synapse. The United States Food and Drug Administration (FDA) approved intravenous levetiracetam in August 2006 for patients above 16 years of age when oral treatment is not feasible.

Studies have demonstrated that intravenous levetiracetam has a favorable safety and pharmacokinetic profile as seen with oral levetiracetam in adult subjects (5, 6). Weinstock et al. conducted an Open-Label, Single-Arm, Multicenter, Safety, Tolerability, and Pharmacokinetic Study of Intravenous Levetiracetam in Children with Epilepsy. The age ranged from 1 month to 16 years of age. Fifty-two subjects were enrolled. No significant adverse events were reported and it was well tolerated in this population (7).

Levetiracetam has been found to be effective in certain experimental models of status epilepticus (8, 9). It also has been demonstrated that intravenous levetiracetam can be used as an alternative to oral dosing in patients, and data have been published about efficacy and safety in children of different age groups with epilepsy (10). In this review, we will discuss the literature that has displayed efficacy of intravenous levetiracetam in children and neonates.

EFFICACY IN NEONATES

Seizures affect approximately 1–5 out of 1000 newborns and 11 out of 1000 preterm neonates (11–13). The most common causes of neonatal seizures include hypoxic-ischemic encephalopathy, intracranial hemorrhages, infections of the central nervous system, cerebral infarctions, and metabolic disturbances (11, 12). Protection and prevention of significant adverse effects upon the developing brain is critical in neonatal period. Phenobarbital, which acts via GABAergic mechanisms, remains as the most frequently used antiepileptic drug for the treatment of neonatal seizures. Current research suggests that the GABA receptor may

be prone to deficient inhibition in the neonate (11). A muted or net excitatory affect from the binding of GABA in the neonatal brain may be due to an increased concentration of chloride in the immature brain's neurons (11). Additionally, evidence exists that phenobarbital causes neuronal apoptosis in animal models, and long term adverse neurodevelopmental effects related to phenobarbital have been demonstrated (14). Several other antiepileptic drugs are being researched and prescribed in children and neonates (14, 15). Levetiracetam is increasingly being used as an antiepileptic drug in the neonatal period, and is recognized as an antiepileptic drug with neuroprotective properties (14, 16). Koppelstäetter et al. reported on the use of levetiracetam in term and preterm neonates with rarely observed adverse effects in their analysis of surveys from neonatologists and pediatric neurologists (17). A study by Kilicdag et al. demonstrated a significant decrease in the number of apoptotic neuronal cells in a levetiracetam treated group of rat pups who underwent a hypoxic-ischemic brain injury (16).

In the only randomized control trial of antiepileptic drugs in neonates, Painter et al. demonstrated efficacy of seizure cessation in less than 50% of patients treated with phenobarbital and phenytoin. Phenobarbital and phenytoin were used to treat a variety of neonatal seizure etiologies in these studies, with the majority of patients having underlying hypoxic-ischemic encephalopathy (18). Retrospective studies have demonstrated that intravenous levetiracetam may be efficacious in the management of acute seizures in neonates when other medications have failed (19, 20).

Abend et al. conducted a retrospective cohort study of 23 neonates in the Newborn Infant Intensive Care Unit with electroclinical or electrographic-only confirmed seizures who received LEV. There were 12 female and 11 males with a mean gestational age of 38.7 ± 1.7 weeks. Patients were identified using the electronic pharmacy database over a 1-year period. IV LEV bolus doses of 10–20 mg/kg were given to neonates. Next, LEV was administered twice per day. LEV effectiveness was defined as a greater than 50% reduction in electrographic seizure within 24 h of the start of treatment. Neonatal seizure etiologies included eight hypoxic-ischemic encephalopathy, four presumed genetic/metabolic disorders, three brain malformations, three central nervous system infections, two strokes, two cryptogenic seizures, and one tumor. Seizure types included focal or multifocal clonic or tonic (18/23), subtle seizures (5/23), and desaturation/apnea (2/23). LEV was started at a mean age of 41 weeks. The mean initial dose was 16 ± 6 mg/kg and the mean maximum dose was 45 ± 19 mg/kg/day. There were no reported or detected respiratory or cardiovascular adverse effects. Greater than 50% seizure reduction within 24 h was considered to be effective and LEV was effective in 8 of 23 (35%). Seven of 23 patients had a complete seizure resolution (19).

Khan et al. performed a retrospective chart review of electronic medical records for neonates treated with IV LEV between January 2007 and December 2009. The researchers identified 22 neonates (0–28 days of age) born at term ($\geq$37 weeks) with neonatal seizures who received IV LEV. Loading doses were from 10 to 50 mg/kg (20 patients received a loading dose of 50 mg/kg). Seizure etiologies included hypoxic-ischemic encephalopathy (12/22, 55%), hemorrhage (2/22, 9%), viral meningoencephalitis (2/22, 9%), and one patient each with benign neonatal seizures, brain malformation, cryptogenic partial seizures, glucose transporter protein type 1 deficiency, infarction, and trauma (1/22, 5%). Nineteen of 22 patients (86%) demonstrated immediate seizure cessation within 1 h. After loading dose complete seizure cessation was achieved in 7 of 22 patients (32%), by 24 h in 14 of 22 (64%), by 48 h by 19 of 22 (86%), and by 72 h in all 22 (100%). The authors concluded that IV LEV can be used in neonates as monotherapy and adjunct therapy in acute seizure management (20).

Levetiracetam continues to be used in a variety of clinical situations and seizure etiologies in neonates. Shoemaker and Rotenberg reported on the successful use of levetiracetam in neonates with varying seizure etiologies (21). Hmaimess et al. showed the efficacy of levetiracetam in a neonate with intractable malignant migrating partial seizures (22). Ledet et al. also showed efficacy as a prophylactic antiseizure medication in a neonate with acute lymphoblastic leukemia (23).

Furwentsches et al. examined the use of LEV as monotherapy in the treatment of neonatal seizures in a prospective pilot feasibility study. The study sample size included six consecutive mature or premature newborns presenting with neonatal seizures. Exclusion criteria included seizures due to electrolytes or hypoglycemia, seizures who did respond to pyridoxine, and patients previously treated with other AEDs. LEV was administered orally over 3 days with each dose increasing 10 mg/kg/day up to 50 mg/(kg/day). The study endpoint was either if the patient needed additional AEDs after day 3 or 3 months of LEV treatment. Seizure semiology major symptoms include tonic and apnea (3/6 patients, 50%), oral automatisms, staring and apnea (2/6 patients, 33%), and clonic and apnea (1/6, 17%). The researchers did not observe any severe adverse effects, although one infant had mild sedation. Within 6 days, all six patients treated with oral LEV became seizure free. Three months later, after ongoing LEV monotherapy, five patients remained seizure free. The remaining patient did not respond to therapy and developed pharmacoresistant epilepsy (24).

IV LEV was used as a first-line treatment in Ramantani et al.'s prospective feasibility study. From 2006 to 2008, the study consisted of 38 consecutively admitted newborns with EEG-confirmed seizures and excluded seizures due to electrolyte deficiencies and pyridoxine dependency. Nineteen of 38 newborns were extremely premature at gestational age $<$28 weeks. Seizure semiology included subtle, focal clonic, multifocal clonic, focal tonic, generalized tonic, and myoclonic. Patients $<$28 weeks gestational age most commonly had subtle seizure semiology ($N = 12$, 63%), patients 28–36 weeks most commonly had multifocal clonic seizures ($N = 4$, 67%), and patients $\geq$37 weeks most commonly had focal clonic seizures ($N = 5$, 38%). Intravenous doses of LEV were started at 10 mg/kg and over the course of 3 days were increased to 30 mg/kg, and at the end of the week were further titrated to 45–60 mg/kg. When the infant's condition allowed, IV LEV was switched to oral administration. Up to two IV doses of phenobarbital (20 mg/kg) during LEV titration were tolerated for acute intervention. Acute interventions of one dose of phenobarbital was needed in 19 patients and three patients required two doses. At the conclusion of the first week, 30 infants treated with LEV were seizures free, while at the end of 4 weeks 27 infants remained seizure free. Seven infants received LEV for a duration up to 3 months, but in 19 infants LEV was discontinued

after 2–4 weeks. There was no report of severe adverse effects. Ramantani et al. concluded that the results indicated the safety and efficacy of LEV treatment in neonatal seizures (25).

Pharmacokinetic studies have established a benign safety profile for levetiracetam. Merhar et al. used initial loading doses of 15–40 mg/kg, and demonstrated that levetiracetam was well tolerated in 18 neonates with seizures. The only adverse event present was somnolence. The study also found linear kinetics, minimal protein binding, and no hepatic metabolism with levetiracetam use in neonates (26). At this time, there are no clear dosing recommendations available in the literature where doses range from 10 to 70 mg/kg (15, 19, 20, 26–29).

EFFICACY IN CHILDREN

There have been multiple case reports, case series, and retrospective studies reported in children which showed the efficacy and tolerability of intravenous levetiracetam in acute seizure management both as adjunctive and monotherapy.

Haberlandt et al. showed the use of levetiracetam in the treatment of two children with myoclonic status epilepticus (30). Alehan et al. contributed a case report to the literature supporting the use of levetiracetam in children with non-convulsive status epilepticus (31). Weber et al. showed efficacy of levetiracetam in a child with Angelman syndrome presenting in non-convulsive status epilepticus (32). Cilio et al. showed the termination of refractory status epilepticus in two patients with migrating partial seizures in infancy with intravenous levetiracetam (27).

Michaelides et al. performed a retrospective analysis of a pediatric population under the age of 14 on the use of IV formulation of LEV for in its first 9 months of availability in their institution. The researchers reviewed the paper and electronic medical records of 15 children $\leq$14 years of age (3 months–14 years) who received IV LEV in the first 9 months that it was available at the institution. Seizure etiologies included six patients had complex partial seizures, three had generalized tonic-clonic, two no seizure type only for prophylaxis, one myoclonic, one tonic, and one mixed. Over the 9 months there were 118 infusions performed in 15 patients. No adverse reactions were observed. Nine minor adverse reactions were observed during the rest of the hospitalization that were potentially related to the IV formulation: three of these were decreases in WBC count, and six were behavioral adverse effects. In LEV naïve patients ($N = 7$) the median starting dose was 8.8 mg/kg (range: 5.0–50), and all patients had a median maintenance dose of 30.4 mg/kg/day (5.0–92). There were six patients in a subgroup less than 4 years old (avg. = 1 year 4 months), who received the majority of the LEV infusions in the entire study population (82/118 total infusions). Of the 15 patients, five had very frequent seizures (two with status epilepticus) and three of these patients had a seizure frequency reduction of >50%. One patient had complete seizure resolution. Michaelides et al. concluded that IV LEV was very well tolerated in their pediatric population (28).

Goraya et al. retrospectively identified 10 patients (aged 3 weeks–19 years) through their hospital pharmacy records all patients who received intravenous levetiracetam. Forty percent of patients had received IV levetiracetam for acute repetitive seizures/status epilepticus, 30% presented on levetiracetam, 10% received IV LEV for seizure prophylaxis for a brain biopsy, 10% received it for severe thrombocytopenia, and 10% for an acute symptomatic seizure. The dosages of IV LEV used varied according to the indication for usage: replacement of oral LEV used a mg for mg substitution, for status epileptics 20–40 mg/kg/dose was used every 8 h for infants or 12 h for older children, and for maintenance treatment after biopsy a dose of 10–20 mg/kg every 12 h was used and was given as an infusion. Seventy-five percent of the patients who received IV LEV for control of status epilepticus (three of four) became seizure free and 25% (one of four) had a >50% reduction in seizure frequency. This neonatal patient became seizure free on IV LEV after an area of cortical dysplastic tissue that had been found was removed. None of the 10 patients had any adverse events noted during IV LEV usage and there was no discontinuation of IV LEV due to side effects (29).

Kirmani et al. retrospectively reviewed 32 pediatric patients from 2 months to 18 years of age. The sample size included 53.1% males and 46.8% females. Data were acquired from electronic medical records for patients admitted in the hospital with status epilepticus or acute exacerbation of seizure patients who received intravenous levetiracetam. The loading dose used was 25–50 mg/kg. Data analysis showed a favorable response to intravenous levetiracetam for all patients and seizures were aborted both clinically and electrographically. In 18 patients, intravenous levetiracetam was infused after fosphenytoin and lorazepam. No serious side effects were reported in all subjects. Fifteen patients were discharged on levetiracetam monotherapy and nine on adjunctive therapy. The study concluded that intravenous levetiracetam was found to be efficacious both in status epilepticus and acute exacerbation of seizures (33).

Abend et al. described a cohort of critically ill children who received intravenous levetiracetam for status epilepticus or acute repetitive seizures. All the subjects responded to intravenous levetiracetam resulting in either termination, temporary cessation, or reduction in ongoing seizure activity (34). Khurana et al. conducted a retrospective analysis at a single institution over a period of 3 years. Their group identified 81 patients, in which 18 of them received levetiracetam as monotherapy. Fourteen patients had partial epilepsy and four had generalized epilepsy. The dose range was 14–60 mg/kg, and duration of therapy ranged from 2 to 24 months. The study concluded that levetiracetam was found to be efficacious as monotherapy in the management of pediatric epilepsy (35).

Gallentine et al. conducted a retrospective analysis over a 7-year period. Eleven children had received levetiracetam for refractory status epilepticus with the age ranging from 2 days to 9 years. The patients were treated with two to seven anticonvulsants prior to infusion of levetiracetam. Levetiracetam was found to be efficacious in 45% of cases, resulting in resolution of refractory status epilepticus. The median latency to resolution of status epilepticus following initiation of levetiracetam was 1.5 days. The responding subjects received a median dose 40 mg/kg/day. No significant adverse effects were reported. The authors concluded that levetiracetam was safe to use as adjuvant therapy in children with refractory status epilepticus (36).

Pharmacokinetic studies have established a benign safety profile for levetiracetam. Li et al. demonstrated that levetiracetam

is a safe and effective treatment for infants and children in an observational, prospective study Li et al. prospectively analyzed 120 patients (39.3% female, 61.7% male) with epilepsy receiving mono or combination therapy with levetiracetam that were 14-year-old and younger. Therapy was started with a levetiracetam dose of 10 mg/kg/day and if seizures were poorly controlled titrated up with 10 mg/kg increments to a maximum daily dose of 60 mg/kg/day. They documented seizure type, seizure frequency, levetiracetam dose, and side effects. Li et al. found that the 83.0% of the study population had a seizure reduction of at least 50%, and 54.8% of patients were seizure free. There was a side effect incidence rate of 47.5%, and included somnolence, dysphoria, nervousness, dystrophy, somnipathy, astitia, and debilitation. There was a 3.3% patient withdrawal rate most commonly due to poor effect or intolerance of side effects. The researchers concluded that levetiracetam was safe and effective for epileptic children with multiple types of epileptic seizures (37).

CONCLUSION

Linear kinetics, minimal protein binding, and no hepatic metabolism with levetiracetam use in children and neonates, and favorable response in status epilepticus and repetitive seizures makes levetiracetam a suitable choice in acute seizure management in both children and neonates. However, larger prospective, randomized, blind comparison trials between phenobarbital, levetiracetam, and phenytoin would provide more information regarding the use of this newer agent in acute management of seizures in the pediatric population.

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Mechanisms of levetiracetam in the control of status epilepticus and epilepsy

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Status epilepticus (SE) is a major clinical emergency that is associated with high mortality and morbidity. SE causes significant neuronal injury and survivors are at a greater risk of developing acquired epilepsy and other neurological morbidities, including depression and cognitive deficits. Benzodiazepines and some anticonvulsant agents are drugs of choice for initial SE management. Despite their effectiveness, over 40% of SE cases are refractory to the initial treatment with two or more medications. Thus, there is an unmet need of developing newer anti-SE drugs. Levetiracetam (LEV) is a widely prescribed anti-epileptic drug that has been reported to be used in SE cases, especially in benzodiazepine-resistant SE or where phenytoin cannot be used due to allergic side-effects. Levetiracetam's non-classical anti-epileptic mechanisms of action, favorable pharmacokinetic profile, general lack of central depressant effects, and lower incidence of drug interactions contribute to its use in SE management. This review will focus on LEV's unique mechanism of action that makes it a viable candidate for SE treatment.

Keywords: levetiracetam, calcium homeostasis, status epilepticus, anti-epileptic, mechanisms

STATUS EPILEPTICUS: DEFINITION, CAUSES AND CONSEQUENCES

Status epilepticus (SE) is a neurological emergency associated with a significant morbidity and mortality (1). It is defined as continuous seizure activity lasting greater than 30 min or intermittent seizures without regaining consciousness lasting for 30 min or longer (2). An operational definition of SE has also been proposed that suggests any seizures lasting more than 5 min to be considered SE and immediate steps taken to stop it to limit further morbidity and mortality (3). SE affects approximately 200,000 people annually and accounts for as many as 55,000 deaths per year in the United States alone (1). The economic burden of SE is also high with SE patients having 30–60% higher reimbursements than patients admitted for other acute health problems, including acute myocardial infarction or congestive heart failure (4). SE can be caused by acute symptomatic processes such as metabolic disturbances (for example, electrolyte imbalance, renal failure, and sepsis), CNS infection, stroke, head trauma, drug toxicity, and hypoxia (5–7). Chronic symptomatic processes that cause SE include pre-existing epilepsy or the discontinuation of anti-epileptic drugs, chronic ethanol abuse and withdrawal, and remote processes such as CNS tumors or stroke (5–7). SE can be convulsive or non-convulsive, and under both situations SE can cause significant brain damage particularly in the limbic system (8, 9). SE patients are at a higher risk of developing acquired epilepsy (10, 11). About 12–30% of adults with a new diagnosis of epilepsy first present in SE (10, 11). Further, survivors of SE suffer from other neurological problems including depression, cognitive deficits, and suicidal ideations (12).

TREATMENT OF SE

It is extremely important to recognize and control SE since prolonged SE can quickly develop into refractory SE, which is very difficult to treat (13). In addition, prompt SE treatment is essential to prevent mortality and the progressive brain damage that produces neurological morbidities. Treatment of SE (14) begins with medical stabilization of the patient with an initial focus on respiratory and circulatory stabilization. Further evaluations are then made looking for underlying causes of SE (metabolic disturbances, infections, etc.) and treatments are provided to correct them. Following these emergency stabilizations of the patient's physiological status, treatment of SE is rapidly initiated using currently accepted first line drugs for stopping SE. This usually includes immediate treatment with benzodiazepines such as midazolam, diazepam, or lorazepam. The second-line of drugs to control SE include fosphenytoin, phenytoin, phenobarbital, and valproic acid. Despite the effectiveness of benzodiazepines and other anticonvulsant drugs in treating seizures, prolonged SE becomes refractory to treatment with currently available anticonvulsant agents treatment in over 40% of SE cases becoming refractory to the initial treatment with two or more medications (13). Clinical trials have shown that patients treated within 20 min of SE had better prognoses than those who did not respond within 20 min (15). However, epidemiological studies have shown that time to seizure treatment varies broadly with only about 41% of all patients receiving their first anti-epileptic drug within 30 min (16). In addition, termination of SE with benzodiazepines or phenytoin was effective in 80% of patients when administered within 30 min of seizure onset, but this effectiveness decreased to less than 40% when treatment

was initiated several hours after seizure onset (17). In such a scenario, the treatment options become extremely limited to drugs such as pentobarbital, midazolam, or propofol. Topiramate and ketamine are used as additive agents to benzodiazepines and first line drugs to control refractory SE (18). However drug interactions, side-effects, pharmacoresistance, CNS depression, all add to the medical complexity of treating SE effectively and highlight the need to develop additional agents to treat SE. Thus, there is an unmet need of developing newer anti-SE drugs.

LEV FOR THE TREATMENT OF SE

Levetiracetam (LEV) [(*S*)-α-ethyl-2-oxo-1-pyrrolidine acetamide] is a broad-spectrum anti-epileptic drug that was approved by the US Food and Drug Administration in 1999 and has quickly become one of the widely prescribed drugs for the treatment of partial and generalized epilepsy. While it is structurally unrelated to other anti-epileptic drugs, it is structurally related to nootropic agent piracetam. Levetiracetam is not considered a substrate for multi-drug transporters (19). The multi-drug transporter proteins are thought to be responsible for altering drug concentrations at the site of action by affecting drug uptake or increasing transport of drug cleaving enzymes. Increased expression of multi-drug transporter proteins is hypothesized to be a major mechanism for developing pharmacoresistance (20). This could explain the low probability of pharmacoresistance for LEV, despite daily chronic intake of the medication. In addition, minimal drug interactions, fewer side-effects, and broad-spectrum efficacy have all contributed to LEV's ever widening use for the treatment of seizures. These characteristics make LEV a strong candidate for second-line treatment of SE, especially in patients with refractory SE and where use of phenytoin is deemed inappropriate due to allergic side-effects (21). With the recent introduction of an intravenous preparation of LEV, there has been considerable interest in the use of LEV for the treatment of SE (22), although LEV is not approved for this indication. There are recent studies and review articles that discuss the use of LEV in the management of SE (18, 21, 23–28). The rest of this article will mainly focus on the molecular targets and unique mechanism of actions of LEV that makes it such an attractive drug candidate for not only the treatment of SE, but also other neurological disorders such as Huntington's chorea (29), Tardive dyskinesia (30), Tourette syndrome (31), anxiety disorders (32), traumatic brain injury and stroke (33), amongst others.

UNIQUE ANTICONVULSANT PROPERTY OF LEV

Currently, little is known regarding the mechanism underlying LEV's anti-epileptic action. The discovery of LEV's anticonvulsant activity is unique. It was devoid of anticonvulsant activity in the acute maximal electroshock seizure test and in the maximal chemoconvulsive seizure test in pre-clinical assays (34). However, a potent protection was observed against partial epileptic seizure activity induced by pilocarpine and kainic acid (34). It also exhibited anticonvulsant activity against kindled seizures and in the Strasbourg genetic absence epilepsy rats (35). Studies attempting to elucidate LEV's anticonvulsant action revealed a unique profile of mechanisms (36). Surprisingly, it did not exhibit the classical action in that LEV had no effect on voltage-dependent Na^+ channels, GABAergic transmission, or affinity for either GABAergic or glutamatergic receptors (37). These represent the most common mechanisms of action for the vast majority of anti-epileptic drugs. In light of these studies, multiple laboratories focused on elucidating the molecular mechanisms that make LEV a potent anti-epileptic and SE drug. The following sections highlight the unique properties of LEV as an anticonvulsant agent.

EFFECTS OF LEV ON NEUROTRANSMITTER RELEASE

Research has revealed several unique mechanisms for the anticonvulsant effects of LEV. Levetiracetam has been shown to affect GABA turnover in the striatum and decrease levels of the amino acid taurine, a low affinity agonist for $GABA_A$ receptors, in the hippocampus with no effect in other amino acids (38). In addition, LEV removed the Zn^{2+}-induced suppression of $GABA_A$-mediated presynaptic inhibition, resulting in a presynaptic decrease in glutamate mediated excitatory transmission (39). Other reports have also suggested that the mechanisms of the anti-epileptic and neuroprotective actions of LEV seem to be mediated, at least in part, through the combination of inhibitory effects on depolarization-induced and Ca^{2+}-induced Ca^{2+} release-associated neurotransmitter releases (40). Effects of LEV on Ca^{2+} channels have been widely studied (41, 42). Levetiracetam is also reported to modulate the presynaptic P/Q-type voltage-dependent calcium (Ca^{2+}) channel to reduce glutamate release in the dentate gyrus, the area of the hippocampus that regulates seizure activities (43). Similarly, LEV has been reported to inhibit neurotransmitter release via intracellular inhibition of presynaptic Ca^{2+} channels (44).

LEVETIRACETAM AND SV2A

Synaptic vesicle protein 2 (SV2) is a 12 trans-membrane integral protein present at all synaptic sites. It consists of three isoforms, 2A, 2B, and 2C. The SV2A isoform is most widely distributed, 2B is brain specific, and 2C is the minor brain isoform. SV2 proteins have been proposed to act as transporters of common constituent of the vesicles, such as Ca^{2+} or ATP (45). SV2A has also been shown to interact with the presynaptic protein synaptotagmin, which is considered the Ca^{2+} sensor for regulation of Ca^{2+}-dependent exocytosis of synaptic vesicles (46). SV2A is involved in controlling exocytosis of neurotransmitter-containing vesicles (47). SV2A is not essential for synaptic transmission, but SV2A knockout mice exhibit seizures (48). Thus, SV2A ligands could protect against seizures through effects on synaptic release mechanisms. Indeed, SV2 has been identified as the likely target for LEV. Studies have shown that the brain distribution of the LEV-binding site, as revealed by autoradiography, matches the equivalent distribution of SV2A as determined by immunocytochemistry (45, 49). Elegant studies have shown that SV2A is indeed the binding site for LEV in the brain (50, 51). Thus, LEV's interaction with SV2A is a leading mechanism of its anti-epileptic action.

LEVETIRACETAM AND Ca^{2+} SIGNALING

Ca^{2+} ions are major second messenger molecules that play a role in plethora of biological functions including neuronal excitability and synaptic plasticity (6, 52). Ca^{2+} levels are therefore tightly regulated to attain the high signal-to-noise ratio in cellular communications. Disturbances in Ca^{2+} homeostatic mechanisms resulting

in elevated intracellular Ca^{2+} levels have been reported in multiple neurological disorders including stroke, movement disorders, and seizure pathologies (6, 52). Incessant Ca^{2+} entry into the neurons via the NMDA receptors during SE and persistent leak of Ca^{2+} from intracellular Ca^{2+} stores have now been firmly established in SE induced epilepsy (6, 52). Laboratory research has shown that blocking the ryanodine receptor-mediated Ca^{2+} leak from endoplasmic reticulum using dantrolene lowers the elevated Ca^{2+} post SE and prevents the development of epileptiform discharges in hippocampal neurons (53). Interestingly, LEV reduced intraneuronal Ca^{2+} levels by inhibiting ryanodine and IP_3 receptor dependent Ca^{2+} release from endoplasmic reticulum (54). The ability of LEV to modulate the two major Ca^{2+}-induced Ca^{2+} release systems demonstrated an important molecular effect of this agent on a major second messenger system in neurons and could possibly contribute to its unique mechanism of action. In addition, LEV has also been shown to inhibit Ca^{2+} entry by blocking the L-type Ca^{2+} channels in hippocampal neurons of spontaneously epileptic rats (55). There are other studies that report no action of LEV on L-type Ca^{2+} channels, but LEV has been shown to be selective toward N-type Ca^{2+} channels' freshly isolated CA1 hippocampal neurons of rats (56). Thus, the effects on Ca^{2+} entry and release pathways are an important aspect of LEV's mechanism of action.

LEVETIRACETAM AND EPILEPTOGENESIS

The process by which healthy brain tissue is transformed by an injury into a hyperexcitable circuit of neurons giving rise to spontaneous seizures (acquired epilepsy) is called epileptogenesis (6). This transformation includes a myriad of neuronal plasticity changes including axonal sprouting, neuronal degeneration, neurogenesis, astrocytes activation, and changes in neurotransmitter release and their receptor response (6). Major second messenger systems that are activated after brain injury are suspected as initiating and sustaining these neuroplasticity changes that underlie epileptogenesis. Role of Ca^{2+} ions in epileptogenesis is well-established. Brain injury-induced protracted alterations in Ca^{2+} homeostasis are thought to trigger changes in protein transcription and gene expression that underlie abnormal synaptic plasticity changes expressed as seizure disorders and associated behavioral abnormality. Inhibition of Ca^{2+} elevations following SE are neuroprotective and produce an anti-epileptogenic effect (53, 57). Levetiracetam has been reported to limit epileptogenesis (58, 59). This effect could partly be attributed to LEV's effect on Ca^{2+} homeostasis, as discussed above. Thus, LEV significantly inhibited development of epileptic focus following kindling-induced epileptogenesis (59). Further, a significant inhibition of seizures even at 5 weeks following termination of LEV treatment was observed in spontaneously epileptic rats indicating that LEV possesses anti-epileptogenic properties (60). However, other studies have failed in observing LEV's anti-epileptogenic potential, for example 5-weeks of LEV treatment did not prevent development of seizures when administered 4 h after the onset of SE with seizure termination through diazepam (61). The ability of LEV to prevent development of seizures following SE makes it an important agent for the treatment of SE. Thus, LEV has important potential as an anti-epileptogenic agent that needs further elucidation.

CONCLUDING REMARKS

Levetiracetam is a unique anticonvulsant agent that has multiple mechanism of action that differentiates it from conventional anticonvulsant drugs. This makes it an ideal agent to add to the treatments for SE. Refractory SE is a major medical and neurological emergency associated with high morbidity and mortality. Levetiracetam offers a unique anticonvulsant treatment option to initiate for the treatment of refractory SE. Its low incidence of side-effects and sedative properties make it an ideal agent to consider in treating refractory SE. The availability of an intravenous preparation of LEV also facilitates its use in treating refractory SE. Further studies should confirm that LEV will also be a major first line drug for the treatment of SE, but at present it is not approved for this use. The unique anticonvulsant mechanisms of action of LEV make it an ideal agent to add to conventional anticonvulsant agents and to consider for the treatment of refractory SE and intractable seizure disorders.

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Review of levetiracetam as a first line treatment in status epilepticus in the adult patients – what do we know so far?

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With the advent of new antiepileptic drugs comes the potential for significant advances in the emergent management of status epilepticus. Traditional antiepileptic drugs possess side effect profiles that may limit their clinical utility or lead to increased patient morbidity or mortality. The relatively recent development of levetiracetam shows promise for effective control of acute status epilepticus in adults, but current objective data of its use as a first-line agent for control of status is quite limited. This paper serves to examine existing literature while considering levetiracetam as a first-line therapy in status in the adult patient population. Although existing studies are narrow in their scope, the present data lay a substantial foundation for further investigation of levetiracetam as a primary therapy in acute status epilepticus.

Keywords: levetiracetam, status epilepticus, seizure, first-line therapy for the status epilepticus, antiepileptic drug

INTRODUCTION

The emergent management of status epilepticus has been restricted to a small subset of antiepileptic drugs suitable for acute intravenous infusion. Of the three major traditional drugs used in first-line therapy for status, each carries a side effect profile that may ultimately lend to limited clinical utility in the critical patient. Lorazepam, phenytoin, and phenobarbital individually convey specific risks to the patient, among them respiratory depression, medication interaction due to cyp450 induction, and teratogenicity (1). Each of these known effects may complicate their use in an urgent setting and contribute to increased morbidity in patients presenting with status epilepticus (1).

Given its minimal known side effect profile, limited drug interactions, and availability as a rapid delivery IV formulation, levetiracetam, a relatively new AED by comparison, may be a viable, and practical option in the first-line management of status epilepticus (2, 3). Status epilepticus has been defined most commonly as seizure activity persisting for over 30 min, inclusive of tonic, absence, complex partial, convulsive, non-convulsive, and myoclonic seizure types, among others. The majority of data regarding the use of levetiracetam in status epilepticus has been applied to generalized seizure types, though there is growing interest in its application for a variety of epileptic activity (4).

TRADITIONAL AND EMERGING MANAGEMENT OF STATUS EPILEPTICUS

The traditional approach taken in the emergent management of status was described by Riviello et al. (5) in their survey of 120 physicians who were asked to manage hypothetical patients diagnosed with status epilepticus (5). When given the case of an adult male without a known underlying seizure disorder, the physician subjects overwhelmingly initiated benzodiazepine therapy first, choosing lorazepam followed by phenytoin as a secondary agent (5). Levetiracetam was not chosen until the physician was required to select a third agent, with it being selected with the same frequency as midazolam, propofol, or phenobarbital (5). Cook et al. (6) echoed this sentiment with a 150 patient retrospective study, again noting that in real clinical applications, physicians readily followed the first-line benzodiazepine trailed by phenytoin model (6). Although levetiracetam was not chosen until the patient required a third-line agent, Cook et al. (6) did note that 10% of patients received levetiracetam as a second-line agent in their evaluations (6). Additionally, levetiracetam was the second most-selected third-line agent. The authors postulate that this may be indicative of growing acceptance of levetiracetam as a promising therapy in status (6). Cook et al. (6) did call attention to 65.1% of patients in the study continuing levetiracetam therapy upon discharge home with a particular frequency in patients with no prior history of seizure (6). Taken together, this is suggestive of a slowly developing physician comfort with levetiracetam.

The reasoning behind the traditional management of status is perhaps most apparent in Brophy et al.'s (7) discussion of the Neurocritical Care Society's Status Epilepticus Guideline Writing Committee, which set forth parameters for a stepwise approach to the management of status epilepticus (7). This initial set of standards favored benzodiazepine therapy as a first-line modality citing the utility of the multiple administration routes and rapid infusion times common to many benzodiazepines, most notably lorazepam, and midazolam (7). The guidelines do offer the caveat of respiratory depression with benzodiazepine therapy with the potential need for intubation with repeated dosing (7). While the committee limited their recommendations for the emergent control of status epilepticus to benzodiazepines, levetiracetam was given a strong recommendation for urgent control

of status along with two other drugs, valproate sodium and phenytoin/fosphenytoin (7). Of these drugs, however, levetiracetam is the only agent not noted in the guidelines for having a serious potential side effect profile (7). Although levetiracetam currently is listed as having only Class IIA, Level C evidence supporting its use in emergent, urgent, and refractory status, its adoption as a strongly recommended therapy for urgent status epilepticus provides for reasonable interpretation that an expansion of available clinical evidence could lead to its eventual acceptance as a first-line therapy in the emergent management of status (7).

LEVETIRACETAM AS A FIRST-LINE AGENT

While levetiracetam is approved as an adjuvant agent for the management of status, data on its use as a first-line therapy is quite limited, as noted in the Neurocritical Care Society's Guidelines discussed previously. Review of available literature via PubMed search reveals fewer than 10 reports of levetiracetam used as a first-line agent in status, with only a single randomized pilot study and a limited number of case and retrospective reports available in current publication. Despite this, evaluating existing data as a whole opens the discussion for levetiracetam administration as a first-line approach in status epilepticus.

A clinical pilot performed by Misra et al. (8) provides the only direct comparison of levetiracetam and a traditional first-line agent, lorazepam, in available literature (8). A loading dose of lorazepam or levetiracetam was administered to each of 79 patients between the ages of 1 and 75 with convulsive or subtle convulsive status epilepticus (defined as two or more seizures) (8). While pediatric patients were included in the study, no formal breakdown of the age distribution of the patient population was included in the study report. It should be noted that the mean age of subjects receiving lorazepam was 38.9 years with a mean age of 39.2 for patients receiving levetiracetam. Levetiracetam infusion controlled status in 76.3% of patients with lorazepam achieving the same in 75.6% (8). Slightly improved 24-h seizure freedom was noted in patients in the levetiracetam group (8). Interestingly, there was more frequent need for artificial ventilation in patients receiving first-line lorazepam, though no significant difference in mortality between the two groups was ultimately illustrated (8). While this pilot was hindered by limited power due to subject recruiting, it stands as the only available direct comparison between a traditional first-line agent and levetiracetam (8).

In a review written by Zelano and Kumlien (9), levetiracetam was evaluated as a stage two therapy in status management in an analysis of 10 studies (9). The review included three prospective and seven retrospective studies, which comprised total 334 patients (9). Ultimately, the studies represented an efficacy range for levetiracetam of 44–94% (9). The authors' work further substantiates the notion of levetiracetam as an effective clinical option with applications to a broader range of patients, not just those without potential drug interactions, feared toxicity, or other foreseen adverse reactions to traditional therapies.

UTILITY IN PHARMACOLOGY AND TOLERABILITY

Levetiracetam's limited side effect profile makes it particularly appealing in older patient populations, where drug interaction and the potential for intubation leading to significant mortality is of great concern. Fattouch et al. explored this in a limited retrospective of nine patients, older than 65 years old with video EEG confirmed status epilepticus who received loading doses of 1500 mg IV levetiracetam as a first-line agent (10). Eight of nine cases responded to loading dose within 15–30 min of dosing with confirmation obtained via EEG, with only one of whom experienced a few isolated seizures in the days following dosing (10).

Additionally, Farooq et al. (11) reported two cases of patients, older than 80 years old who responded within 35 min to levetiracetam load (11). This limited report features the cases of a patient already receiving daily levetiracetam therapy who had missed a dose and another patient found to be therapeutic on phenytoin whose seizures ceased within a minute of levetiracetam administration (11).

Berning et al. (12) addressed the issue of first-line levetiracetam in patients in whom there was a high level of concern for the risks of coma induction and subsequent increased mortality (12). The group reported on 2 patients out of a larger group of 32 individuals in status who were treated exclusively with levetiracetam within 6 h of seizure onset and who responded to therapy between 6 and 18 h after administration of the agent and required no further therapy (12). Berning et al. (12) also incorporated patients categorized as "low first line therapy," specifically eight patients treated with low dose (2 mg) lorazepam or equivalent prior to administration of levetiracetam in lieu of "high first line" or "traditional first line therapies" (12). In this case, the patients who received either no traditional first-line drugs or only low dose benzodiazepines had lower change in morbidity when compared to more aggressive traditional first-line interventions (12).

LEVETIRACETAM IN THE CRITICALLY ILL

It is the potential for limited increase in mortality that makes levetiracetam particularly appealing for first-line use in critically ill patients, which was explored by Rüegg et al. (13). In their analysis, 12 patients received first-line levetiracetam for status epilepticus or seizure as part of a larger 50 patient study (13). Eleven of 12 patients became and remained seizure free after load with 4 of those patients becoming seizure free immediately after administration, with confirmation obtained via EEG (13). Importantly, the group described the need for a therapeutic ideal with properties of ability for rapid dosing with instantaneous onset and minimal toxicity, sedation, and interaction with the hypothesis that levetiracetam best fits such a description among commercially available AEDs (13).

Spencer et al. (14) pointed out the benefit of levetiracetam reaching peak steady state concentrations within an hour of administration; though with the caution that it has demonstrated a more rapid administration rate in a small subset of critically ill patients (14). Lyseng-Williamson (15) also described the clinical tolerability of levetiracetam, noting in her review that adverse effects of major concern in a critically ill patient, such as hypotension, arrhythmias, and cutaneous and hypersensitivity reactions, were not commonalities in the use of levetiracetam in its initial clinical trials (15). Instead, asthenia and somnolence were noted among its major side effects (15).

Nau et al. (16) served to further this data with a retrospective analysis of 51 patients, 18 of whom received a loading dose

of 1500 mg levetiracetam. Seventeen of those 18 patients received levetiracetam as first-line monotherapy (16). Of these patients, no drug interactions, cardiac arrhythmias, or adverse hemodynamic events were recorded, similarly suggesting that levetiracetam has the potential to be well tolerated in the critically ill (16). In the same vein, Rösche et al. (17) provided a comprehensive review of literature concerning the treatment of status epilepticus with levetiracetam, though not specifically as a first-line agent. The authors found an on-the-whole rate of levetiracetam terminating status in 53.7–58.1% of patients with the most frequent side effects of irritability and sedation (17).

PRACTICAL APPLICATIONS IN THE CURRENT THERAPEUTIC SEQUENCE

Liu et al. (18) served to further this concept with a literature based comparison of intravenous valproate sodium with other antiepileptic drugs by completing meta-analysis performed on five randomized control trials (18). No substantial difference was noted between valproate sodium and levetiracetam in time taken to control status, and similar responses for phenytoin and sodium valproate were found. These are suggestive of similar efficacies of all three agents (18). Of the three, however, the relatively benign side effect profile of levetiracetam makes it an appealing choice of agent given the agents' equivalent drug efficacies.

Aiguabella et al. (19) set out to determine the efficacy of intravenous levetiracetam as an add-on agent in the management of status in their observational retrospective study of 40 patients. These patients received initially the traditional regimen of a benzodiazepine followed by phenytoin or valproate (19). Ultimately, seizures were controlled in 57.7% of patients with a mean efficacy time of 14.4 h (19). Of the 40 patients, only 26 ultimately received the traditional regimen followed by levetiracetam given as a third-line agent. Those patients have a levetiracetam efficacy of only 46.1%. Interestingly, when levetiracetam was given early, with minimal or no pretreatment, as was done in the cases of 14 subjects, levetiracetam was found to have an efficacy of 78.5% (19). Ultimately, the authors postulate that the higher efficacy of levetiracetam early in therapy may be attributed to the idea that status epilepticus requiring multiple agents to reach control were likely to be more treatment refractory from the outset (19). That being said, Aiguabella et al.'s (19) work raises the notion that while other agents may also readily control status as an early therapy, levetiracetam may do so with at least equal efficacy with less morbidity.

DISCUSSION

While clinical studies of the use of levetiracetam are undoubtedly not yet sufficient to dictate large-scale changes to clinical practice, a substantial foundation for future clinical trials has been laid. Given multiple case reports of successful use of levetiracetam as an initial agent in the emergent treatment of status, there is certainly basis for the development of larger scale randomized controlled trials allowing for a direct, standardized comparison between traditional first-line agents and levetiracetam for emergent management of status epilepticus. The clinical utility of levetiracetam is most suggested by Misra et al.'s (8) pilot study as well as retrospective analyses of the use of levetiracetam in critically ill patients with seizures and status (8). Perhaps the greatest potential advantages to levetiracetam are its pharmacologic properties and restricted morbidity via minimal side effect and interaction profiles. Studies indicate that levetiracetam is an attractive option in patients who are unable to be treated with traditional first-line therapies. This may serve as a catalyst for the exploration of levetiracetam as a first-line medication. Although existing clinical evidence is inadequate, the recommendations of the Neurocritical Care Society for the management of status show a potential for an expanding role of levetiracetam in the management of status with its inclusion of recommended drugs that may be selected in the urgent management of status. In the same way, Cook et al. (6) foreshadow a shift in the traditional line of medical decision making in status with a small but not insignificant number of physicians choosing levetiracetam early on in the management of status (6). While a jump as extensive as levetiracetam approaching recognition as a first-line agent is still far beyond what current models and data support, the basis for more in-depth studies for its potential to function specifically as a first-line agent is formed with considerable feasibility by these underlying works.

Such a hypothesis exists that levetiracetam may well fit the description of an ideal agent as outlined by Reugg et al. (13). Lyseng and Spencer further support this notion of levetiracetam as a readily administered and rapidly absorbed drug with low frequency of high-risk adverse effects, and in fact, a benign side effect profile when compared directly to other traditional agents (14, 15). This then portends the argument that levetiracetam could 1 day usurp traditional antiepileptic agents in order of administration in status given the rapidity of its administration, absorption, efficacy noted as an early therapy, and minimal side effect profile. Perhaps, it is worthy of first-line administration, ahead of traditional agents. Accomplishing this cannot be done without further endeavors, however. No current randomized controlled clinical trial of levetiracetam used in the management of acute adult status epilepticus currently exists, though the potential yield is substantial. Aiguabella et al. (19) create an interesting scenario in their brief observation that difficult-to-control status is fundamentally what it purports to be challenging to manage (19). For example, a patient whose seizures prove complex to control will likely require multiple agents with repeated dosing in order to successfully stop the seizures. However, a patient with readily responsive seizures may respond initially to levetiracetam as well as traditional antiepileptic drugs, albeit with less risk of interactions, and side effects. It is worth questioning if adverse effects experienced in patients whose seizures ultimately responded to traditional first-line therapy but then experienced complications or side effects such as respiratory depression, could be avoided by a better-tolerated therapy, namely levetiracetam. In this manner, a wider range of patients could be potentially treated earlier, more safely, and just as effectively as with mainline drugs used in status.

Certainly no pharmacotherapy is without fault, and it remains both possible and likely that levetiracetam carries administration concerns not yet apparent to clinical practitioners. Just as our understanding of its clinical benefits are restricted by limited data, so too is our comprehension of its potential pitfalls. With the growing establishment of levetiracetam's clinical pedigree, as well as ever-increasing desire for an efficacious, accessible, and comparatively benign agent for the emergent treatment of status, levetiracetam may prove a worthy contender in the future of acute treatment of status epilepticus.

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The role of levetiracetam in treatment of seizures in brain tumor patients

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Levetiracetam, trade name Keppra, is a new second generation antiepileptic drug that is being increasingly used in brain tumor patients. In patients suffering with brain tumors, seizures are one of the leading neurologic complications being seen in more than 30% of patients. Unlike other antiepileptic drugs, levetiracetam is proposed to bind to a synaptic vesicle protein inhibiting calcium release. Brain tumor patients are frequently on chemotherapy or other drugs that induce cytochrome P450, causing significant drug interactions. However, levetiracetam does not induce the P450 system and does not exhibit any relevant drug interactions. Intravenous delivery is as bioavailable as the oral medication allowing it to be used in emergency situations. Levetiracetam is an attractive option for brain tumor patients suffering from seizures, but also can be used prophylactically in patients with brain tumors, or patients undergoing neurological surgery. Emerging studies have also demonstrated that levetiracetam can increase the sensitivity of Glioblastoma tumors to the chemotherapy drug temozolomide. Levetiracetam is a safe alternative to conventional antiepileptic drugs and an emerging tool for brain tumor patients combating seizures.

Keywords: intravenous levetiracetam, seizures, brain tumor patients, antiepileptic drugs, neurologic complications

INTRODUCTION

Individuals with brain tumors represent a very challenging patient population for clinicians. Not only must clinicians deal with and treat the primary tumor, but they must also manage the numerous accompanying sequelae. Seizures are commonly seen in brain tumor patients, with reports of 30% or more depending on the type of tumor (1). In fact, an epileptic seizure is the presenting symptom of a tumor in 30–50% of patients, and 10–30% of those patients will go on to develop recurrent seizures over the course of the disease (2, 3). The presence of seizures and convulsions has been shown to add substantial morbidity to these patients (4).

ROLE OF ANTICONVULSANTS IN ACUTE SEIZURE MANAGEMENT AND SEIZURE PROPHYLAXIS IN BRAIN TUMOR PATIENTS

Current consensus states that all patients with brain tumors should be treated with antiepileptic drugs. Currently, the first-line medications including phenytoin, carbamazepine, or valproate all have demonstrated major side-effects and dangerous drug interactions with commonly used chemotherapy and tumor medications. The most common side-effects seen with these highly used antiepileptic medications include cognitive impairment, bone-marrow suppression, liver dysfunction, and dermatological symptoms (5). Reinforcing the complexity of care in these cases are several studies demonstrating that side-effects are more frequent in patients with brain tumors compared with the overall epileptic population (3, 6). Tsai et al. conducted a retrospective chart review to assess the effect of valproic acid (VPA) on the outcome of patients with glioblastoma multiforme (GBM). The electronic medical records were queried from January 2004 to December 2006 and 102 patients newly diagnosed with GBM were found. Those patients were followed until January 2010 and over that time 87 patients died due to disease progression, 7 patients lived and were followed between 43.4 and 61.0 months, and 8 patients were lost to follow up. VPA was administered at a starting dose of 400 mg every 8 h in the form of a sodium salt. It was then adjusted as needed to serum level or seizure activity. Patients were analyzed in two groups: an "early" treatment group in which the patients began VPA treatment within 2 weeks of initial diagnosis and the "late" treatment group where the patients began treatment with VPA more than 2 weeks after initial diagnosis.

Seven patients on VPA therapy provided tissue samples from a second resection procedure for analysis of histone acetylation. When these samples were analyzed, there was an increase in acetylation in a small subset of patients. Eighty-five of 102 patients (83.3%) underwent radiotherapy, 61/102 patients (59.8%) underwent chemotherapy, and all patients had a neurosurgical procedure with the most common being total excision (47.1%). Thirty-three of 102 patients had VPA therapy and of those 16 patients (58.5 serum concentration) started therapy less than a week after diagnosis and 17 patients (52.4 serum concentration). The early treatment group had an average serum VPA level of 58.46 μg/ml (34.1–72.6) and the late group was 52.37 μg/ml (36.3–73.2). When the early treatment group was evaluated with univariate analysis there appeared to be a survival benefit conferred ($P = 0.035$). However, when a stratified analysis according to chemotherapy

was used, VPA therapy was not associated with a statistically significant difference ($P = 0.315$) in overall survival. Mild nausea and thrombocytopenia were the most commonly reported side effect (11/33 and 11/33, respectively). The authors concluded that VPA therapy did not affect patient survival significantly (7).

Drug interactions between antiepileptic drugs and commonly used tumor therapies can lead to inadequate control of the seizures or sub-therapeutic treatment of the tumor. Toxic effects have also been noted, leading to unnecessary morbidity. Many of the common antiepileptic drugs induce cytochrome P450 enzymes which cause faster metabolism and lower concentrations within plasma (5). Corticosteroids and many other chemotherapy drugs exhibit decreased effectiveness in the presence of enzyme activating antiepileptic drugs (8, 9). The reciprocal relationship is also seen as there are chemotherapeutic agents that induce enzymes of the P450 system, lowering concentrations of antiepileptic drugs (3, 10).

Within the past 10 years, several new antiepileptic drugs including lamotrigine, levetiracetam, oxcarbazepine, lacosamide, topiramate, and zonisamide have emerged that are without clinically relevant drug interactions (11–13). Data are limited for these newer agents and the main limitation in use is lack of intravenous formulation. Maschio et al. studied the effects of 12 months of oxcarbazepine (OXC) monotherapy on seizure control in patients with brain tumor-related epilepsy (BTRE) in a prospective, observational study. Eleven women and 14 men (mean age 49.7 years, range 25–75) with BTRE were enrolled between September 2007 and January 2009. Enrolled subjects had a histological diagnosis of meningiomas, primary grade I gliomas, low grade gliomas, anaplastic gliomas, or multiform glioblastoma. Epileptic patients were eligible if they had simple or complex seizures with or without secondary generalization, if they had greater than or equal to two seizures per month on no AEDs before referral to their center, or patients that had been treated at the maximum tolerated dosage with other AEDs. Twenty-four patients received monotherapy with OXC of which 17 patients were *de novo* and 7 patients rare on monotherapy with one AED. A baseline was established at the first visit of seizure frequency, neurological examination, Zung self-depression rating scale (ZSDRS), adverse events profile, and patients were given a seizure diary. During week one OXC began and any prior AED was tapered gradually over the first 3 weeks. The OXC dose started at 300 mg/day and was titrated every 4 days by 300 mg/day up to 2,100 mg/day over 4 weeks.

The mean follow up duration was 7.1 months (range 1–12 months). Five patients died as a result of tumor progression and 10 patients dropped out due to severe side-effects ($N = 6$), uncontrolled seizures ($N = 3$), and a lung complication ($N = 1$). Six patients (24%) had severe side-effects due to rash ($N = 4$), confusion ($N = 1$), and dizziness ($N = 1$) and one patient (4%) had a mild rash. Four patients had no systemic therapy, 1 had radiotherapy, 4 had chemotherapy, and 16 had both radiotherapy and chemotherapy. Patients received a mean OXC dosage of 1,230 mg/day (range 600–2,100 mg/day). Of the 10 patients who completed the 12 months of follow up, there was a significant seizure reduction ($P = 0.005$) in the mean weekly seizure number from 2.62 ± 6.35 at baseline to 0.13 ± 0.37 in the final follow up. There was a significant difference in the seizure freedom rate ($P = 0.002$) by the McNemar's test between the baseline and final follow up intent to treat population. Using logistic regression analysis, the authors found that the efficacy of OXC in seizure control was not affected by chemotherapy and radiotherapy ($P = 0.658$). The ZSDRS showed significantly increased mood in patients in the final follow up ($P = 0.011$). Thus, the researchers concluded that OXC was efficacious in controlling seizures. However, lack of intravenous formulation limits its use in acute cases. The other agent used is lamotrigine which is also not available in intravenous formulation (14).

Meyer et al. conducted a prospective study in order to determine the protein binding and distribution of LTG in serum, brain tissue, and brain tumor in three female and eight male subjects with brain tumors. From 1994 to 1996, 11 patients had neurosurgical operations for benign tumors ($N = 2$) and malignant tumors ($N = 9$). The subjects were aged 33–68 years (mean 56 years). Patients were enrolled if in the days immediately preceding neurosurgery they showed signs of seizures or if their preoperative EEG showed symptoms of epilepsy. For intraoperative and postoperative seizure control, subjects were administered LTG with PHT in seven patients or LTG with PHT and CBZ in three patients. Patients received a mean of 54.4 days of preoperative treatment (range 1 day–17 months) with LTG, and 2 h preoperatively were given a dose of 100–200 mg/day in addition to PHT or PHT and CBZ. After administration, serial blood samples were taken from 0.5 to 12.5 h, as well as an intraoperative blood sample and intraoperative removed tumor tissue ($N = 6$). HPLC was used to assay LTG concentrations and an ultrafiltration system was used to determine plasma binding of LTG.

The LTG concentration at the time of tumor sectioning was on average 3.7 μg/ml (range 1.1–9.8) in the serum, an average of 6.8 μg/g (range 1.0–14.9) in the brain tissue, and an average of 4.4 μg/g (range 2.0–8.3) in the tumor tissue. The resultant brain/serum ratio was 2.8 and the tumor/serum ratio was 1.9. The protein binding of LTG was determined to be a lipophile AED. The researchers concluded that LTG penetrates well into brain and tumor tissue and had a moderately high protein binding (15). It has been shown that pregabalin has been used as adjunctive therapy in this subgroup of patients although limited data are available, which is the case with most other newer agents (16).

Lacosamide is one of the newest agents which has the advantage of being available in both an oral and intravenous formulation. Few studies reported the efficacy as an adjunctive agent, and data are limited at this time.

Saria et al. conducted a retrospective chart review to study the use of lacosamide in patients with brain tumors. Seventy patients with primary brain tumors who received lacosamide for seizure control were identified by reviewing the medical records of five United States medical centers with brain tumor programs. Primary tumors included glioblastoma (40%), grade II gliomas (36%), and were followed by grade III anaplastic astrocytomas, anaplastic/atypical meningiomas, anaplastic ependymomas, and pleomorphic xanthoastrocytomas. Seventy-eight percent of patients ($N = 55$) had partial seizures and 17% had of patients ($N = 12$) had generalized seizures. Subjects had a mean age of 51 years of age. Eighty-four percent of patients had chemotherapy ($N = 59$) and 81% had radiation therapy ($N = 57$). The majority of patients

had a surgical procedure performed, including 63% had a craniotomy, 23% had a biopsy, and 14% had both. Most patients (83%) were on an additional AED as well as lacosamide, which was most commonly levetiracetam. The cause for the addition of lacosamide therapy was most commonly recurrent seizures (74%) or toxicity from another AED (23%).

A decrease in seizure frequency was found in 46 patients (66%) and seizure control remained unchanged in 21 patients (30%). A greater than 50% decrease in seizure frequency was achieved in 38 patients (83%). No medication toxicities were reported in 77% of patients ($N = 54$) receiving lacosamide. The most common toxicity reported was fatigue noted by four subjects (6%). The researchers concluded that in patients with brain tumors lacosamide was well tolerated and an active add-on AED (17).

Maschio et al. also conducted a case series of 14 patients with BTRE that had not obtained adequate seizure control on other AED treatment. Patients were consecutively recruited if they had at least one seizure per month prior to study recruitment. Lacosamide was the first-fifth add-on AED therapy and was started at 100 mg/day and titrated weekly by 100 mg/day to a maximum dosage of 400 mg/day.

Follow up occurred in subjects at a mean of 5.4 months (range < 1–10 months). During this period, nine patients died from tumor progression, no patients' underwent radiation therapy, and nine patients underwent chemotherapy. The mean lacosamide dosage was 332.1 mg/day (range 100–400 mg/day). One subject dropped out due to dizziness and blurred vision. The mean seizure frequency at baseline was 15.4 per month and at follow up decreased to 1.9 per month. Subjects had a median seizure reduction percentage of 79.8%. Five patients (35.7%) had a greater than 50% seizure reduction and six patients (42.9%) were seizure free. One patient (7.1%) had an unmodified seizure frequency. There was a statistically significant difference in the mean monthly seizure frequency from baseline to follow up ($P = 0.022$). The authors concluded that lacosamide is a valid alternative add-on AED in patients with BTRE (18) have had seizures.

ROLE OF LEVETIRACETAM IN ACUTE SEIZURE MANAGEMENT AND SEIZURE PROPHYLAXIS IN BRAIN TUMOR PATIENTS

In this article we chose to focus on the use of levetiracetam. In our experience we have witnessed better efficacy and there seems to be more evidence within the literature supporting levetiracetam in terms of seizure management. An important point to make here is that there are many antiepileptic drug choices, but very little comparative effectiveness data to help physicians decide the best AED to use in the innumerably variable clinical scenarios. This paper attempts to make a contribution and demonstrate what we feel is a good drug choice for managing seizures in the unique population of patients with brain tumors. Levetiracetam is very different from the more commonly used antiepileptic drugs (19, 20). Its action is believed to involve neuronal binding to synaptic vesicle protein 2A (SV2A). Binding to this protein somehow acts as an inhibitor of synaptic vesicle exocytosis (21) decreasing presynaptic neurotransmitter release (21). The mechanisms of action for the more commonly used antiepileptic drugs including benzodiazepines and barbiturates affect gamma-aminobutyric acid potentiation, calcium channels, or sodium channels (22).

Levetiracetam exhibits a relative bioavailability of 100% following both oral (23) and intravenous administration. Levetiracetam, given intravenously, is considered bioequivalent to oral tablets and is well tolerated (12). Being able to give this drug intravenously is an extremely attractive trait which makes treatment in emergency or perioperative situations a possibility. Perhaps more importantly is the fact that levetiracetam is not extensively metabolized by the cytochrome P450 system (23). This is in extreme contrast to the most commonly used antiepileptic drugs. Levetiracetam has not been shown to cause any induction or inhibition of the other important cytochrome P450 enzymes including uridine diphosphate-glucuronyl-transferase or epoxide hydroxylase (23). Levetiracetam has repeatedly been shown to exhibit a low potential for clinically relevant pharmacokinetics both with other antiepileptic drugs or drugs that could possibly be used to treat brain tumors (13, 23). Studies have demonstrated no or very few side-effects with levetiracetam treatment in patients with brain tumors who also received antineoplastic agents (7, 24–26). A few of the larger studies were able to report the occasional occurrence of somnolence with initial doses of levetiracetam (27).

SEIZURE MANAGEMENT WITH LEVETIRACETAM

Over the past 10 years, there have been several studies that looked at both the effectiveness and safety of using levetiracetam in brain tumor patients. Levetiracetam, as both an adjunct therapy and a monotherapy, has shown a complete seizure control rate of between 47.4 and 100% (7, 25–27). Reductions in seizures by more than 50% were recorded in a majority of articles within the literature. These numbers were extremely variable ranging from 29 to 100%. However most of these studies were conducted with limited numbers of patients and were mostly retrospective analyses (6, 11, 24, 25, 28–32). The two largest studies and the ones most likely indicative of what clinicians will see in their patients are from a 2005 *Neurology* supplement by Stevens et al. (31) and a report from Rosati et al. (27) in 2010. The study in *Neurology* reviewed the medical charts of 278 patients with varying brain tumors treated with levetiracetam over a 36-month period. They witnessed a greater than 50% reduction in seizure activity in over 60% of their patients. The second largest study, by Rosati et al., involved 176 patients in a prospective study over a 3-year period (27). In this study, 91% of patients were seizure free with a monotherapy of levetiracetam. Forty-nine of the patients (60%) experienced fast and long-lasting seizure control with initial doses of 1,500–3,000 mg/day. In 23 patients (31.5%) an increase in the dosage up to 3,000–4,000 mg/day was necessary because of subtherapeutic drug levels. The authors experienced no relevant laboratory abnormalities (27). Levetiracetam has demonstrated good potential however; larger cohorts over more extended periods of time would be useful.

While levetiracetam has demonstrated good potential as an adjunct therapy as well as monotherapy, several alternative uses for levetiracetam are currently being explored such as refractory status epilepticus.

Traditional seizure medications have proven woefully ineffective with over half of brain tumor patients continuing to have

seizures despite treatment (32). Status epilepticus is seen in up to 26% of these cases (6) with the overall mortality rate reaching 30–40% (33). First-line treatment for status epilepticus in this patient population is a combination of benzodiazepines and phenytoin (34), which has an efficacy rate of 60–70%. Refractory status epilepticus requires an additional anesthetic drug such as propofol or midazolam to induce an iatrogenic coma (35). Recent data suggest that an alternative combination of phenytoin and levetiracetam has proven to be a very effective cocktail that does not subject the patient to mechanical ventilation or sedation (35).

Swisher et al. conducted a retrospective chart review of electronic medical records for all patients with a diagnosis of primary or metastatic brain tumor who presented with complex partial refractory status epilepticus and received Trifecta (intravenous PHT, LEV, and oral PGB). All patients were >18 years of age and presented between January 2006 and December 2009. Study subjects ages ranged from 25 to 84 with an average age of 56.9. There was a prior history of seizures in 70% of patients. GBM occurred in 52% of patients and was the most common tumor type, yet the tumor locations of all subjects tumors was highly variable. Ninety-one percent of patients had underwent resection for their brain tumor. Thirty-nine percent of patients received radiation, 39% received chemotherapy, and within 1 month of RSE onset 52% underwent a biopsy or brain tumor resection. PHT or LEV was used as first-line therapy in all patients and pregabalin was typically used as second or third-line therapy. The median dosage of LEV was 3,000 mg/day and the median dosage of PGB was 375 mg/day.

Ninety-one percent of patients were already on one AED when RSE was diagnosed, and it was statistically significant ($P = 0.03$) that more patients in the responder group were on an AED at baseline (100 vs. 71%). The average PHT blood level in the Trifecta responder patients was 18.9 at the time of SE cessation. After the administration of Trifecta 30% of patients' (7/23) seizure frequencies were unchanged. After the administration of Trifecta, status epilepticus ceased in 70% of patients (16/23). On average, 24 h after the addition of the third AED status epilepticus was aborted. There was a zero mortality rate in the responder group and only one patient in the responder group required intubation. There were no adverse reactions to Trifecta reported. The authors concluded that in patients with brain tumors presenting in RSE that Trifecta use is highly effective and safe (16).

SURGICAL PROPHYLAXIS

Patients with brain tumors often must undergo neurological surgery for resection or biopsy, which in and of itself can increase risk of seizures. So controlling seizures in the perioperative phase of brain tumor management is an important consideration (3, 36).

Bahr et al. performed an open-label, prospective, single-arm study investigating the use of LEV for perioperative seizure control in patients with suspected primary brain tumors undergoing neurosurgery (e.g., biopsy or resection) (36). Inclusion criteria included age >18 years, neuroradiological imaging suspected primary brain tumor, and planned biopsy or resection neurosurgical procedure. Patients were excluded if in the seven preoperative days any AED other than LEV was used or if there was a known LEV allergy or previous severe side-effects to LEV. Between January 2008 and June 2009, 25 of 30 study patients were enrolled and treated for 4 weeks before and 4 weeks after a neurosurgery procedure with oral and IV LEV. During this time patients had four scheduled visits for monitoring and data collection. Of the 27 patients who underwent surgery, 22 were diagnosed with a primary brain tumor, 3 with meningioma, 2 with brain metastasis, and 1 with abscess. Subjects were started on an oral dose of LEV 500 mg twice daily and titrated to 1,000 mg twice daily after 72 h. The dose was further increased if the patient was at an increased risk of seizures or had seizures in this period. IV LEV was given immediately and for 36 h postoperatively before being transitioned back to oral LEV. Three patients did not have the planned procedure and two patients were lost to follow up.

In the pre-surgery phase (defined as 3 days–4 weeks prior to surgery), 100% of patients were seizure free after the initiation of LEV therapy. In the 48 h post surgery phase and early follow up phase (defined as 48 h–4 weeks post surgery), the seizure free rates were 88 and 84%, respectively. Three patients failed LEV treatment, even after the dose was titrated up to 3,000 mg/day. There were no serious adverse events reported with LEV treatment. The authors concluded that oral and IV LEV for perioperative seizure control was feasible and safe in patients with tumor-related seizures (36). However, to date only four studies have been able to demonstrate the effectiveness of levetiracetam in the perioperative phase (32–35). Zachenhofer et al. (37) retrospectively studied 78 patients with brain tumors who received between 1,000 and 3,000 mg of LEV perioperatively. After a mean follow up time of 10.5 months, 91% of the patients were seizure free. This study demonstrated a very low seizure frequency of 2.5% in the early postoperative period. These studies propose both the feasibility and safety of intravenous levetiracetam in the perioperative treatment, but more long term trials are needed.

INCREASING CHEMOTHERAPY SENSITIVITY

Levetiracetam's role in increasing chemotherapy sensitivity is a fascinating new field of interest. Some antiepileptic drugs have actually shown that they can inhibit histone deacetylase activity within the tumor. Histone deacetylase inhibitors can modulate temozolomide activity by modulating methylguanine-DNA-methyltransferase (MGMT) expression (37) thereby allowing for increased temozolomide efficacy. Levetiracetam has in fact been shown to increase the transcription of histone deacetylase 1 (HDAC1) which ultimately silences MGMT (38). Levetiracetam has also been shown to have a neuroprotective role via free-radical scavenging activity (39), reducing inflammation and neuronal death (40). All of these attributes may lead to the ability of levetiracetam to prevent radiochemotherapy-caused nerve damage. Levetiracetam may in fact increase temozolomide-induced cytotoxicity in patients with GBM who do express the MGMT protein while also experiencing little adverse side-effects (41, 42). This could be a very exciting area of research as GBM tumors are so common.

CONCLUSION

Brain tumor patients require a multidisciplinary approach involving the use of chemotherapy, radiation, possible surgery, and in many cases antiepileptic drugs. The first-line treatments for

these patients have numerous drug interactions to be weary of when using them in addition to anticancer drugs. Increased side-effects have led to renewed interest in antiepileptic drugs that do not induce cytochrome P450 pathways. Levetiracetam, a drug unique in both its mechanism of action and in the way that it does not cause the drug interactions of the first-line drugs, is among the newer antiepileptic drugs. Several studies have demonstrated the effectiveness of levetiracetam in the role of monotherapy as well as adjunct therapy to other antiepileptic drugs. Levetiracetam has also sparked the interest of clinicians for its role in surgical prophylaxis, for refractory status epilepticus, and the ability to increase chemotherapy sensitivity. Some of these attributes have more supporting evidence than others, but levetiracetam does warrant more testing and could very well be a promising drug in the fight to control epilepsy in brain tumor patients.

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Neurobehavioral effects of levetiracetam in brain tumor related epilepsy

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The neurobehavioral profile of anti-epileptic drugs (AEDs) has been a recurrent research topic in the scientific literature. As pharmacological treatments for epilepsy continue to evolve, there is a general consensus that newer AEDs have less detrimental side effects in comparison to their older counterparts. Among newer AEDs and epilepsy patients, potential risk for neurobehavioral changes has been reported with levetiracetam (LEV). Conversely, limited data exists regarding the manifestation of this symptomatology in a subgroup of epilepsy patients with brain tumors. The current paper reviews the literature regarding the neurobehavioral profile of LEV in brain tumor related epilepsy and suggestions for future research will be discussed.

Keywords: levetiracetam, brain tumors, seizures, neurobehavioral

INTRODUCTION

The neurobehavioral profile of anti-epileptic drugs (AEDs) has been a recurrent research topic in the scientific literature. As pharmacological treatments for epilepsy evolve, there is a general consensus that newer AEDs have less cognitive side effects in comparison to their older counterparts. Levetiracetam (LEV) has garnered interest as a treatment for patients with brain tumor related epilepsy. Among patients with epilepsy, potential risk for neurobehavioral changes has been reported with LEV. Conversely, limited data exists regarding the manifestation of this symptomatology in epilepsy patients with brain tumors. The current paper reviews the literature regarding the neurobehavioral profile of LEV in brain tumor related epilepsy and suggestions for future research will be discussed.

ANTI-EPILEPTIC DRUGS

The adverse effects of AEDs have been fairly well documented in the scientific literature and can encompass both physical and neurobehavioral phenomena that can vary in their intensity and their impact on patients' quality of life (1). A meta-analysis conducted by Zaccara et al. (2) revealed somnolence, dizziness, ataxia, diplopia, and fatigue to be the most common adverse physical symptoms of AEDs. Studies have shown that AEDs can negatively impact multiple cognitive domains (3). Further, depending on the choice of medication, AEDs may positively or negatively affect mood. Adverse psychiatric effects often include behavioral changes, depression, and psychosis (4). A benefit of the newer generation AEDs, such LEV, is that they tend to have less severe side effects and a decreased potential to negatively interact with other medications (5). As a result, there is a proclivity to consider newer generation AEDs as the first line of treatment for diverse epileptic syndromes, including brain tumor related epilepsy.

BRAIN TUMOR RELATED EPILEPSY

Seizures are often the initial presenting symptom of intracranial tumors (6, 7). In a meta-analysis of prospective randomized clinical trials involving newly diagnosed brain tumors, Glantz et al. (8) found a 26% incidence of seizures occurring prior to diagnosis and the rates can be even higher depending upon tumor grade (9, 10). The incidence of brain tumor related epilepsy varies based on the type of tumor involved and there is an inverse correlation between tumor grade and likelihood of seizures (7, 11). The mechanisms behind brain tumor related epilepsy are somewhat unclear, although there are a number of proposed etiologic mechanisms including tumor location, histopathological features, the neurochemical profile of the tumor, and changes in neurotransmitter and receptor expression, among others (12).

Brain tumor related epilepsy is characterized by its intractability to anti-epileptic drugs and prior findings suggest that prophylactic AED use is not effective (10). The AAN Quality Standards Subcommittee meta-analysis regarding prophylactic use of AEDs determined that it does not provide substantial benefit and adverse effects are common (8). Another concern with use of AEDs in brain tumor related epilepsy is drug–drug interactions between AEDs and chemotherapeutic agents. Many older AEDs (e.g., phenobarbital, phenytoin, carbamazepine) are hepatic enzyme inducers, which is problematic because several chemotherapeutic agents are metabolized by hepatic chromosome P450 enzymes. AEDs that induce hepatic enzymes increase the metabolism of the chemotherapeutic agent, requiring the chemotherapy dose to

be increased in order to maintain required blood levels (11, 13). Enzyme inducing agents can also interact with steroids that are commonly used to treat edema in patients with brain tumors (14). As a result of these drug interactions, newer generation AEDs that do not have hepatic enzyme inducing properties, such as LEV, have been identified as a pharmacological alternative for treating brain tumor related epilepsy (15).

LEVETIRACETAM

Levetiracetam's mechanism of action is unclear, though some research has supported the idea that it is involved in the expression of synaptic vesicle protein 2A (SV2A) (12) and animal models have shown that SV2A is the binding site for LEV (16). Patsalos (17) noted that the pharmacokinetic profile of LEV meets nearly all the criteria that make an AED "ideal" because of its rapid absorption, limited metabolism, and low risk of drug interactions. It does not inhibit or induce hepatic enzymes like many of the older generation AEDs, which decreases the likelihood of interaction with other drugs. LEV has not been found to induce or inhibit metabolism in other AEDs or non-AEDs, such as chemotherapeutic agents (18). Bobustuc et al. (19) found that LEV sensitized glioblastoma to the chemotherapeutic agent temozolomide in a sample of four newly diagnosed patients, which provides evidence for additional properties of LEV that make it a good alternative for use in brain tumor related epilepsy.

In terms of efficacy, multiple studies have found LEV to reduce seizure frequency by 50% or more in the majority of brain tumor patients when used as monotherapy and/or as adjunctive treatment (6, 20–22). One study compared a group of 105 patients receiving LEV monotherapy with 210 patients receiving phenytoin monotherapy and found both drugs to be effective for early and late post-operative seizures (23). Lim et al. (24) looked at the safety and efficacy of switching patients from phenytoin to LEV following craniotomy in a sample of 29 patients and the two drugs had similar numbers of seizure free patients at 6 months. In addition to its efficacy regarding seizure control, other factors, such as its neurobehavioral profile, need to be considered.

NEUROBEHAVIORAL PROFILE OF LEVETIRACETAM

The literature regarding LEV's neurobehavioral profile in epilepsy has been extensively reviewed (3, 17, 25, 26) and is particularly notable for behavioral changes (27) and increased aggression (28, 29). In particular, patients with premorbid depression or behavioral problems are at greater risk for developing increased aggression (4, 29). In addition, suicidality in some patients prescribed LEV has been reported (30).

Levetiracetam is not generally associated with cognitive side effects (3, 25). Some of the research on LEV and brain tumor related epilepsy has focused primarily on its efficacy related to seizure control and limited information regarding other adverse side effects is reported (22, 31). For the current paper, 14 studies were reviewed that reported on specific physical, behavioral, and cognitive side effects among patients with brain tumor related epilepsy treated with LEV.

Lynam et al. (6) conducted a retrospective analysis of 147 patients with newly diagnosed primary and metastatic brain tumors. Forty one of the patients were on LEV and the most commonly reported side effects included depression, fatigue, and irritability. A limited number of cases had side effects severe enough to discontinue the medication, though the exact number of cases went unreported (6). Newton et al. (32) conducted a retrospective chart review of 41 patients with brain tumors (34 primary brain tumors, 6 metastatic brain tumors) who received LEV as an add on or monotherapy and were followed for 4 weeks. In terms of side effects, 58.5% of the sample experiences side effects, and those on 2,000 mg or more were more likely to experience side effects. Somnolence was the most frequently reported side effect and other symptoms included dizziness, headache, and paresthesias. One patient discontinued LEV after developing panic attacks. This research group conducted a second chart review with a sample of patients with metastatic brain tumors (33) and reviewed the charts of 13 patients receiving LEV as monotherapy (46%) or add-on therapy (54%). At 4 weeks follow-up, 46 percent of the sample had adverse side effects including somnolence, headache, blurry vision, and nausea and vomiting.

Rosati et al. (34) conducted a prospective study with 176 newly diagnosed glioma patients. All patients diagnosed with epilepsy (47% of the sample) were initially treated with LEV. Two patients discontinued LEV during the study because of intolerable diarrhea and visual hallucinations with psychotic thoughts. de Groot et al. (20) also looked at patients with glioma and one patient from their sample of 35 discontinued LEV before follow-up data were gathered due to side effects including leukopenia. Wagner et al. (35) conducted a feasibility study looking at LEV in primary brain tumor patients. Their sample included 26 patients. Thirty-five percent of the sample reported side effects which most frequently consisted of fatigue, somnolence, and dizziness. One patient discontinued LEV after developing psychosis. Maschio et al. (21) also conducted a prospective study exploring the effectiveness of three newer AEDs (LEV, oxcarbazepine, topiramate) in a group of 30 patients with brain metastases. Six of the patients were taking LEV. Only three patients from the sample developed mild side effects. Two of the three were taking LEV and developed mild and reversible restlessness and rash.

Two comparative studies were found that compared LEV to phenytoin (22, 24). One study compared a group of 105 patients receiving LEV monotherapy with 210 patients receiving phenytoin monotherapy and found the patients prescribed LEV had fewer side effects. One patient discontinued LEV due to developing visual hallucinations, and 38 patients discontinued phenytoin due to various side effects (22). Another study that compared LEV to phenytoin included 29 patients post-craniotomy who were randomized into either a phenytoin group or a LEV group. At 3 months follow-up, patients using LEV were reporting fewer side effects in many areas, though they endorsed more difficulty sleeping (33% of LEV subjects) and emotional instability (13%) as compared to the patients in the phenytoin group [(24), p. 353]. One patient reported increased hostility toward others after beginning LEV, though this was not severe enough to discontinue the medication. Zachenhofer et al. (36) retrospectively studied 78 brain tumor patients receiving LEV following surgery. Side effects were reported in 6.4% of the sample, with three patients reporting progressive somnolence and two patients developing reactive psychosis.

Two studies were reviewed that included screens of cognitive functioning in their assessment of side effects. Usery et al. (37)

completed a prospective open-label study looking at the safety and tolerability of intravenous and oral LEV following neurosurgery. The study was hindered by a small sample and only included 17 patients. The patients were followed for 1 month after discharge and six adverse events were reported including three episodes of somnolence, and one episode each of nausea/vomiting, headache, and insomnia. The researchers also used the Telephone Interview for Cognitive Status (TICS) (38) to evaluate weekly for any cognitive changes secondary to LEV. Two patients were deemed cognitively impaired due to their scores on this measure. It should be noted that the TICS is normed for individuals aged 60–98 years, and the current sample's mean age was 56 with a range from 27 to 77. This would suggest that some of the participants were younger than the normative sample of the TICS, and thus their scores may be inaccurate.

Dinapoli et al. (39) conducted a case series to evaluate LEV as a monotherapy for brain tumor related epilepsy in a sample of 18 patients that they followed for 6 months. The Mini Mental Status Examination (MMSE) (40) was used as a measure of global cognitive function and the Karnofsky Performance Scale (KPS) (41) and the Bartel Index (BI) (42) were used to evaluate overall functioning. Sixty one percent of the sample had side effects at the beginning of the trial including rash, somnolence, periarthritis, weight loss, and liver toxicity. At 6 months 22.2% had mild side effects including somnolence and restlessness. At 6 months scores on all three measures were significantly worse than at baseline, though the MMSE mean score was still in the normal range (KPS 85.83, BI 87.78, and MMSE 26.78). This research group has also published two other studies, one of which followed 29 patients for 12 months (43). In terms of side effects, three patients reported restlessness, and one discontinued LEV due to the severity of the restlessness. Two patients reported somnolence. An earlier study by these researchers (44) looked at 19 patients with supratentorial gliomas and found the majority of patients had improved seizure frequency and no adverse side effects were reported.

CONCLUSION

The existing research regarding LEV and brain tumor related epilepsy appears to provide support for the drug's efficacy in reducing seizures. However, its neurobehavioral profile in this population remains somewhat unclear and the existing research has primarily focused on the drug's anti-epileptic properties and tolerability. Given that LEV has a well-established neurobehavioral profile in the general epilepsy population, namely associated behavior change and increased aggression, it is important to examine whether a similar profile appears in patients with co-morbid neurological conditions (e.g., tumors). Several studies were reviewed that reported on side effects seen in brain tumor samples and the results varied widely, ranging from no side effects (44) to 58.5% of a sample reporting side effects (32). In terms of neurobehavioral changes, irritability (6), emotional instability (24), and restlessness (39) were seen in some patients. Interestingly, only once study mentioned a participant developing increased hostility toward others, though it was not severe enough to discontinue LEV (24). Multiple studies had patients who discontinued LEV due to significant psychiatric symptoms including panic attacks and psychotic symptoms (23, 32, 34–36).

The existing research has a number of limitations that make it difficult to draw conclusions across studies. Many of the studies were retrospective chart reviews and sample sizes tended to be quite small. Only one study was found that was prospective and randomized (37) and the study was closed early after only obtaining 17 participants.

Side effect data was typically gathered by self-report and focused primarily on health status (e.g., the Adverse Events Profile). Only two studies were found that included a brief cognitive screening instrument (37, 39) and no research studies included formal neuropsychological testing. In general, the focus of the existing research tended to be on seizure reduction while other areas were not fully explored.

Additional research is necessary to identify the neurobehavioral profile of LEV in brain tumor related epilepsy, as general conclusions cannot be drawn from the existing research. LEV does appear to have some support for its efficacy in terms of reducing seizure frequency and may have fewer side effects in comparison to other AEDs (23). However, the side effects reported across different studies varied widely, and it is unclear if there are particular patient characteristics (e.g., tumor location) that might interact with the drug of choice. Additional research that more fully explores the neurobehavioral effects of LEV in patients with brain tumors would be useful in order to improve patient care and allow providers to make a more fully informed decision when choosing a treatment option.

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Prospects of levetiracetam as a neuroprotective drug against status epilepticus, traumatic brain injury, and stroke

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Levetiracetam (LEV) is an anti-epileptic drug commonly used for the treatment of partial onset and generalized seizures. In addition to its neuromodulatory and neuroinhibitory effects via its binding to the synaptic vesicle protein SV2A, multiple studies have suggested neuroprotective properties for LEV in both epileptic and non-epileptic conditions. The purpose of this review is to discuss the extent of LEV-mediated protection seen in different neurological conditions, the potential of LEV for easing epileptogenesis, and the possible mechanisms that underlie the protective properties of LEV. LEV has been found to be particularly beneficial for restraining seizures in animal models of spontaneous epilepsy, acute seizures, and status epilepticus (SE). However, its ability for easing epileptogenesis and cognitive dysfunction following SE remains controversial with some studies implying favorable outcomes and others reporting no beneficial effects. Efficacy of LEV as a neuroprotective drug against traumatic brain injury (TBI) has received much attention. While animal studies in TBI models have showed significant neuroprotection and improvements in motor and memory performance with LEV treatment, clinical studies suggest that LEV has similar efficacy as phenytoin in terms of its ability to prevent post-traumatic epilepsy. LEV treatment for TBI is also reported to have fewer adverse effects and monitoring considerations but electroencephalographic recordings suggest the presence of increased seizure tendency. Studies on stroke imply that LEV is a useful alternative to carbamazepine for preventing post-stroke seizures in terms of efficacy and safety. Thus, LEV treatment has promise for restraining SE-, TBI-, or stroke-induced chronic epilepsy. Nevertheless, additional studies are needed to ascertain the most apt dose, timing of intervention, and duration of treatment after the initial precipitating injury and the mechanisms underlying LEV-mediated beneficial effects.

Keywords: traumatic brain injury, stroke, acute seizures, chronic epilepsy, post-traumatic seizures, post-traumatic epilepsy, neurodegeneration, epileptogenesis

INTRODUCTION

Levetiracetam [LEV; 2*S*-(oxo-1-pyrrolidinyl) butanamide] is an anti-epileptic drug (AED) often utilized for the treatment of partial onset and generalized seizures (1, 2). LEV has both anti-seizure and anti-epileptogenic properties. It has been also proposed that LEV is an attractive AED for managing post-traumatic seizures (PTSs) owing to its beneficial pharmacokinetic attributes, including excellent bioavailability, linear kinetics, minimal plasma protein binding, and rapid achievement of steady state concentrations (2–4). The underlying mechanisms by which LEV facilitates anti-epileptic and anti-epileptogenic effects are different from classic AEDs. Studies insinuate that LEV bestows its effects mainly through the inhibition of the synaptic vesicle protein 2A (1). Additional investigations have also revealed that LEV can inhibit HVA-Ca^2 channels (N-type), negate the inhibition of negative allosteric modulators such as zinc and β-carbolines of γ-aminobutyric acid (GABA)- and glycine-gated currents, and diminish the calcium release from intraneuronal stores (1, 5).

Moreover, a multitude of studies have proposed that LEV has considerable neuroprotective properties in both epileptic and non-epileptic disorders (2, 6–9). The capability of LEV to augment the manifestation of glial glutamate transporters EAAT1/GLAST and EAAT2/GLT-1 has been proposed as one of the foremost mechanisms through which LEV mediates its neuroprotective properties (2, 10). This hypothesis fits well with one of the conspicuous changes detected following most brain insults, which is increased concentration of glutamate in the extracellular areas causing enhanced activation of *N*-methyl-D-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on neurons and culminating in significant neurodegeneration (2, 11, 12). The efficacy of LEV as a neuroprotective compound has been examined in several brain injury and neurodegenerative disease prototypes. These include brain

damage resulting from status epilepticus (SE) or acute seizures, spontaneous epilepsy, closed head trauma, subarachnoid hemorrhage (SAH), hypoxic-ischemia, and stroke. The goal of this review is to confer the extent of LEV-mediated neuroprotection observed in different brain injury models, the potential of LEV for easing epileptogenesis, and the possible mechanisms that underlie neuroprotective properties of LEV in different neurological conditions.

EFFICACY OF LEV FOR EASING SEIZURES AND SEIZURE-MEDIATED NEURODEGENERATION

Levetiracetam administration appears to be beneficial for restraining seizures, and acute seizure or SE induced neurodegeneration in animal models. A single dose of LEV administered 30 min after the onset of behavioral SE was adequate for transiently attenuating seizure activity in animals treated with LEV at 800 mg/kg or higher (13). Increased doses of LEV (1000 mg/kg or higher) dampened behavioral seizures for prolonged periods. When administered early (i.e., 10 min) after the onset of SE, 400 mg/kg of LEV transiently attenuated behavioral seizures and higher doses dampened seizures for relatively longer periods. Pretreatment with LEV prior to pilocarpine injection delayed the onset of seizures but did not significantly alter ictal discharge measured through electroencephalographic (EEG) recordings (13). Analyses with TUNEL staining however demonstrated reduced neuronal injury in the hippocampus and other limbic brain regions in animals that responded behaviorally to LEV.

Levetiracetam treatment appears to mediate anti-seizure effects through several mechanisms. A study using acute hippocampal slices from spontaneously epileptic rats (SERs) have suggested that LEV modulates Ca^{2+} currents in neurons, as application of 10 μM of LEV decreased the amplitude of the Ca^{2+} current in CA3 pyramidal neurons and application of 100 nM–1 mM of LEV reduced the Ca^{2+} current in a concentration-dependent manner (14). LEV also elevated the threshold potential level for activation of the Ca^{2+} current and reduced the L-type Ca^{2+} current in neurons. Furthermore, LEV can abolish the SE-induced rise in brain-derived neurotrophic factor, a neurotrophic factor believed to contribute to seizures at higher concentrations. Moreover, administration of LEV after SE can enhance levels of Y1- and Y5-like receptors of neuropeptide Y (NPY; an endogenous anticonvulsant) in all subfields of the hippocampus (15). Also, anti-epileptic effect of LEV is apparent from a study in an animal model of hypoxia-induced seizures (16). LEV pretreatment in postnatal day 10 rats significantly decreased the cumulative duration of hypoxia-induced behavioral and electrographic seizures at 25 and 50 mg/kg doses. Additionally, kainate-induced seizures and neuronal loss were significantly diminished in postnatal day 40 rats previously treated with LEV. Thus, LEV treatment can not only suppress acute seizures but also diminish later-life seizure susceptibility and seizure-induced neuronal injury. This suggests that LEV treatment after injury or acute seizures has potential for disease modification.

A recent study has examined the neuroprotective property of LEV against SE in greater detail (17) by administering the drug 2 h after the onset of SE at 50, 100, or 150 mg/kg. Analyses through staining for Fluoro-Jade B (a marker of degenerating neurons) suggested that low dose administration of LEV (50 mg/kg) can reduce SE-induced loss of CA1 pyramidal neurons and dentate hilar neurons but not CA3 pyramidal neurons. However, LEV treatment at a higher dose (100 mg/kg) reduced degenerating neurons in CA1 and CA3 pyramidal cell layers as well as the dentate hilus. Furthermore, a much higher dose of LEV (150 mg/kg) greatly reduced the numbers of degenerating neurons in the CA3 pyramidal cell layer. Interestingly, when different doses of LEV were combined with diazepam (10 mg/kg), neurodegeneration was exacerbated in the hippocampus. Collectively, this study demonstrated that LEV alone is more efficacious for preventing SE-induced neurodegeneration in the hippocampus than other AEDs such as diazepam or valproate (17). Furthermore, the finding that combined administration of LEV and diazepam actually increases neurodegeneration suggested that LEV negatively interacts with diazepam, implying that LEV may be more suitable as a first line drug to minimize SE-induced neurodegeneration rather than as an add-on drug with benzodiazepines (17). Thus, LEV administration after the onset of SE is beneficial for suppressing seizures as well as reducing neurodegeneration. Mechanisms of LEV-mediated neuroprotection likely include its anti-seizure effects as well as its purported ability to decrease the expression of pro-oxidant protein iNOS and increase the expression of the antioxidant protein cystine/glutamate exchanger in the hippocampus (18). A study in pilocarpine model of SE also showed that LEV pretreatment could counteract oxidative stress through maintenance of lipid peroxidation, nitrite-nitrate levels, catalase activity, and glutathione at normal levels in the hippocampus (19).

USEFULNESS OF LEV FOR EASING EPILEPTOGENESIS

Prolonged LEV treatment after SE appears to delay or restrain the development of chronic epilepsy in animal models. A study examined the effects of chronic LEV treatment on hippocampal field responses in rats subjected to pilocarpine induced SE (20). Hippocampal field potentials were recorded *in vivo* in anesthetized animals after 3-day washout period that followed 21-day treatment with different doses of LEV (50, 150, or 300 mg/kg/day) administered via osmotic minipumps. Chronic treatment with LEV yielded clinically relevant plasma concentrations throughout the experiment with complete washout of the drug 3 days after treatment cessation. At this point of time post-SE rats chronically treated with vehicle developed clear signs of hippocampal hyperexcitability typified by increased amplitude of population spike (PS) recorded in the DG and reduced paired-pulse inhibition in the CA1 area. LEV treatment dose-dependently counteracted these long-term effects of SE. Furthermore, at the dose of 300 mg/kg/day, LEV restored these parameters back to control levels (20). Several other studies have also shown beneficial effects of LEV treatment in acute seizure models. For example, the development of kindling (a progressive increase in seizure severity induced by repeated brain stimulation at certain intervals), and kindling-related abnormal gene expression can be considerably modulated through daily application of LEV (15, 21, 22).

Furthermore, LEV administration at 40 mg/kg is efficacious not only for suppressing the development of kindling but also for dampening kindling-induced expression of multiple immediate early genes (IEGs) including many synaptic plasticity-related IEGs, and some late response genes encoding transcription factors,

neurotrophic factors, and proteins that are known to regulate synaptic remodeling (23). An additional potential mechanism by which LEV suppresses the development of kindling is through significant inhibition of kindling-induced synaptic potentiation (24). LEV treatment after kindling can also prevent asymmetric accumulation of hippocampal 7S SNARE complexes [the secretory machinery responsible for neurotransmitter (NT) release] and accumulation of SV2 (25). Thus, LEV treatment can ease multiple abnormalities induced by kindling at cellular and molecular levels.

Moreover, a study in a kainate model of SE examining the long-term effects of LEV treatment, commencing a day after the onset of SE and continuing for 25 days (26) demonstrated that LEV treatment after SE can decrease the mean duration of spontaneous electrographic seizures in the chronic phase after SE. Interestingly, LEV administration also greatly eased SE-induced aberrant migration of newly born neurons into the dentate hilus, an abnormal process that is believed to contribute to the formation of aberrant hippocampal circuitry and epileptogenesis after SE (27, 28). LEV administration has also been found to be effective for easing inflammatory responses in the hippocampus and piriform cortex of epileptic rats (29). These brain regions in epileptic animals typically demonstrate reactive astrocytes and activated microglia displaying strong expression of IL-1β and interleukin-1 receptor subtype 1 (IL-1R1). Interestingly, LEV administration reduced reactive gliosis and expression levels of IL-1β in both of these brain regions. These findings suggested that LEV likely mediates its anti-epileptogenic effects at least partially through modulation of inflammation in epileptic brain regions. Studies in SERs have also suggested anti-epileptogenic effects of LEV (30). Administration of LEV (80 mg/kg/day) to SERs from postnatal weeks 5–8 significantly inhibited seizures at postnatal weeks 5–13. It is of interest to note that inhibition of seizure expression in SERs was still apparent 5 weeks after the termination of LEV treatment, reinforcing that LEV possesses anti-epileptogenic properties.

From the above studies, it is tempting to conclude that chronic treatment with LEV is efficacious for restraining the evolution of initial SE-induced brain insults into a state of hippocampal hyperexcitability and chronic epilepsy. However, currently, there is no clear consensus regarding anti-epileptogenic effects of LEV in SE models. For instance, a study in amygdala kindling model of SE showed that prophylactic treatment with LEV has no effect on epileptogenesis, neuronal damage, or behavioral alterations in rats (31). In one set of studies, LEV treatment was initiated 24 h after onset of electrical amygdala stimulation without termination of SE and continued for 8 weeks using osmotic minipumps. In another set of studies, LEV treatment commenced 4 h after the onset of SE with seizure termination through diazepam and continued for 5 weeks. Interestingly, with either treatment regimen, LEV did not exert anti-epileptogenic or neuroprotective activity. Furthermore, behavioral hyperexcitability and learning deficits were not affected by treatment with LEV after SE. Another study investigating the effects of LEV on visual-spatial memory following SE corroborated these findings (32). Adult rats subjected to SE were treated first with LEV or vehicle for 14 days, tested for visual-spatial memory in the Morris water-maze and then used for unit recording in the CA1 region of the hippocampus. Animals undergoing SE displayed impaired learning and memory function in the water-maze test and abnormalities in firing patterns of pyramidal neurons (place cells) in the CA1 cell layer. LEV treatment had no major effects on water-maze performance or place cell function. Histological analyses however revealed severe neurodegeneration in the CA1 pyramidal cell layer of rats receiving vehicle after SE and relatively reduced neurodegeneration in rats receiving LEV after SE.

Thus, the extent of neuroprotection mediated by LEV treatment was not adequate for preventing SE-induced cognitive dysfunction. However, discrepancy in results between studies may reflect differences in species and strains of animals examined, timing, and dose of LEV treatment after SE, and severity of SE at the time of commencement of LEV treatment. Timing of treatment after SE is particularly important because a study using a rat perforant pathway stimulation model has shown that administration of LEV 5 h after SE does not protect from mitochondrial dysfunction but LEV treatment during established SE prevents mitochondrial dysfunction (7, 33).

PROMISE OF LEV FOR MEDIATING NEUROPROTECTION AGAINST TRAUMATIC BRAIN INJURY

Investigation of the effects of LEV in animal models of closed head injury (CHI) and SAH suggested that LEV is neuroprotective against traumatic brain injury (TBI) (6). In this study, a single intravenous dose of LEV has been shown to improve both functional and histological outcomes after CHI. Moreover, the beneficial effects seemed specific for LEV treatment as fosphenytoin administration did not result in such effects. Administration of LEV also improved functional outcomes and reduced vasospasm following SAH. This was the first study to suggest that LEV could be a therapeutic alternative to phenytoin for TBI in clinical situations where seizure prophylaxis drugs are indicated. Moreover, a recent study has examined the effects of LEV on motor and cognitive function in a rat prototype of TBI (2). Adult male rats were administered LEV (50 mg/kg, i.p) or vehicle daily for 20 days beginning 1 day following a controlled cortical impact (CCI) injury or sham surgery. Animals were assessed for various behavioral tests, which comprised assessment of motor function via beam walking test and spatial learning and memory function through Y-maze and Morris water-maze tests. The results showed that daily LEV treatment for 20 days improved motor function and enhanced novel arm exploration in the Y-maze. Furthermore, LEV treatment promoted greater sparing of hippocampal neurons, decreased contusion volumes, reversed TBI-induced decreases observed in glutamate transporters and markers that promote neuroplasticity, and reduced the expression of pro-inflammatory cytokine IL-1β. However, LEV treatment did not improve spatial learning ability in rats with TBI. Collectively, these animal studies imply that daily LEV treatment has favorable effects on structural, molecular, and some of the behavioral components of neurological improvements after TBI, likely through modulation of excitatory and neuroinflammatory pathways (2).

Additionally, several clinical studies have ascertained the efficacy of LEV for preventing PTS or post-traumatic epilepsy (PTE). Amongst ∼275,000 individuals who are typically hospitalized with TBI every year, ∼7% experience PTS (34). As per guidelines of the Brain Trauma Foundation and the American Academy of

Neurology for the management of severe TBI, administration of AEDs to prevent PTS is recommended only through the initial 7 days after TBI (34). Amid AEDs, the efficiency of phenytoin treatment has been extensively examined for preventing PTS after TBI. However, several clinical studies suggest that LEV treatment after TBI is also effective for decreasing the predilection for developing PTS. Jones and colleagues analyzed EEG recordings from patients receiving phenytoin or LEV for seizure prevention following severe TBI (35). This comparative analysis revealed that LEV is as efficient as phenytoin in averting early PTS but is allied with an increased seizure predisposition based on evaluation of EEG recordings. Another open label, non-randomized phase 2 study assessed the safety, tolerability, and effectiveness of LEV therapy in patients with TBI exhibiting greater susceptibility for PTE (36). LEV treatment was initiated within 8 h after injury and continued for 30 days in this study. Two-year follow-up uncovered that occurrence of PTE in patients receiving LEV (11%) is less than that observed in untreated TBI patients (20%). However, several recent clinical studies in TBI patients report that LEV does not outperform phenytoin as a prophylaxis drug against PTS (37, 38). Another recent clinical study has reported that LEV treatment to children ages 6–17 years with risk factors for the development of PTE decreased the incidence of PTE, as only 1 in 40 patients receiving LEV displayed PTE (39). Collectively, from the above studies, it emerges that LEV has analogous ability as phenytoin for thwarting PTS after TBI. It is also reported that LEV treatment for TBI is linked with fewer adverse effects and monitoring considerations [for details, see the review by Ref. (34)]. Nonetheless, because LEV administration was accompanied by an increased seizure propensity (35), the Brain Trauma Foundation has recommended using phenytoin for early PTS prophylaxis (34).

LEV AS A NEUROPROTECTIVE COMPOUND AGAINST STROKE

Seizures following stroke is one of the causes of epilepsy in adults, particularly in elderly patients (40). Seizures typically occur in ∼10% of stroke patients, depending on risk factors, such as the type of stroke, location of stroke-induced damage in the brain, and severity of the stroke (40). However, stroke accounts for ∼50% of seizures in individuals above the age of 65 years (41). Classically, the use of AEDs to avert recurrent post-stroke seizures is recommended. LEV has been suggested as a first-choice drug against post-stroke seizures, based on safety and efficacy profiles in clinical studies (42). Kutlu and colleagues examined the suitability of LEV monotherapy in individuals aged 60 or older and exhibiting a minimum of two late-onset post-stroke seizures (43). At daily doses of 1000–2000 mg, they reported that 82.4% of the patients were seizure free but seven patients (20.6%) had side effects. These results suggested that LEV monotherapy is efficient and well tolerated in elderly patients with late-onset post-stroke seizures. Consoli and associates compared the efficacy of LEV treatment with carbamazepine (CBZ) in patients with post-stroke seizures in a multicenter randomized open label study (41). Evaluation of results in 106 patients (52 treated with LEV and 54 treated with CBZ) showed no noteworthy variance in the number of seizure free patients between LEV and CBZ. Yet, interval to the first recurrence tended to be longer in patients receiving LEV. The results also suggested that LEV treatment caused considerably less side effects than CBZ, as attention deficit, frontal executive functions, and functional scales (Activities of Daily Living and Instrumental Activities of Daily Living indices) were notably poorer in patients receiving CBZ (41).

Thus, studies conducted so far imply that LEV is a useful alternative to CBZ for post-stroke seizures, predominantly in terms of efficacy and decreased adverse effects. However, another recent study reported that LEV is not effective for the treatment of central post-stroke pain, a severe chronic neuropathic pain state called allodynia resulting from a vascular lesion (44). Considering these, further studies are needed to ascertain the efficacy of LEV as a suitable neuroprotective and seizure-preventing drug after stroke. Additionally, rigorous studies in animal models of stroke are needed to understand the potential anti-seizure, neuroprotective, and anti-epileptogenic effects of LEV following stroke.

OVERALL CONCLUSION

From the analysis of literature pertaining to LEV treatment mediated protection in neurological disorders, it emerges that LEV treatment has potential for restraining SE-, TBI-, and stroke-induced chronic epilepsy development. Particularly, LEV administration has been found to be advantageous for restraining seizures and/or seizure-induced neurodegeneration in animal models of spontaneous epilepsy, acute seizures, and SE. LEV treatment appears to mediate anti-seizure and neuroprotective effects via modulation of Ca^{2+} currents in neurons, inhibition of the up-regulation of brain-derived neurotrophic factor, increases in NPY receptors, and antioxidant proteins, and decreases in pro-oxidant proteins (14, 15, 17). Nonetheless, the capability of LEV for easing epileptogenesis and cognitive dysfunction following SE remains contentious. Several studies report promising outcomes such as delayed development of hippocampal hyperexcitability, restraint of electrographic seizures, diminishment in the abnormal migration of newly born neurons into the dentate hilus, and inhibition of inflammatory responses in SE models (20, 26, 27, 29). There are also reports of mitigation of synaptic potentiation and abnormal expression of IEGs, and prevention of abnormal accumulation of 7S SNARE complexes and SV2 in kindling models (15, 22–24). However, some studies report no beneficial effects of LEV treatment after SE in terms of easing epileptogenesis or cognitive dysfunction (31, 32). Discrepancy in the findings between studies may reflect differences in the timing of intervention with LEV, doses of LEV employed and severity of SE at the time of initial intervention with LEV.

The efficiency of LEV as a neuroprotective drug against TBI has received much consideration. Animal studies in TBI models validate greater sparing of hippocampal neurons, and improved motor and memory function with LEV treatment (2, 6). On the other hand, results of several recent clinical trials convey that LEV has comparable efficacy as phenytoin in terms of its ability for preventing PTE (34, 37–39). The other positive effects of LEV treatment for TBI comprise fewer adverse effects and monitoring issues. However, one caveat of LEV treatment for TBI is the presence of increased seizure propensity in long-term EEG recordings (34, 35). Studies on stroke imply that LEV is a useful alternative to CBZ for preventing post-stroke seizures including elderly patients, in terms of efficacy and safety (40–43) but does

not seem to be useful for easing central post-stroke pain (44). Taken together, studies conducted so far suggest that LEV treatment is useful for easing SE-, TBI-, and stroke-induced chronic epilepsy development. Nevertheless, rigorous additional studies in animal models are needed to ascertain the most beneficial dose, timing of intervention, and duration of treatment after the initial precipitating injury and mechanisms underlying LEV-mediated beneficial effects on epileptogenesis.

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Blast TBI models, neuropathology, and implications for seizure risk

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Traumatic brain injury (TBI) due to explosive blast exposure is a leading combat casualty. It is also implicated as a key contributor to war related mental health diseases. A clinically important consequence of all types of TBI is a high risk for development of seizures and epilepsy. Seizures have been reported in patients who have suffered blast injuries in the Global War on Terror but the exact prevalence is unknown. The occurrence of seizures supports the contention that explosive blast leads to both cellular and structural brain pathology. Unfortunately, the exact mechanism by which explosions cause brain injury is unclear, which complicates development of meaningful therapies and mitigation strategies. To help improve understanding, detailed neuropathological analysis is needed. For this, histopathological techniques are extremely valuable and indispensable. In the following we will review the pathological results, including those from immunohistochemical and special staining approaches, from recent preclinical explosive blast studies.

Keywords: blast, traumatic brain injury, post-traumatic epilepsy, seizures, animal models, neuropathology, histopathology, tissue processing

INTRODUCTION

Blast-related traumatic brain injury (TBI) is a frequent outcome of exposure to explosive device detonation. During the Global War on Terror (GWOT), which includes both Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) in Afghanistan, the use of improvised explosive devices (IED), vehicle borne IED (VBIED), and improvised rocket assisted mortars (IRAM) resulted in a significant number of blast-related TBI (1–4). During the over 10 years of GWOT, almost 290,000 U.S. military personnel suffered TBI of which 68% was due to explosive blast exposure (5, 6).

The use of individual body armor systems (IBAS) reduces the incidence of lethal thoracic and abdominal combat related injuries dramatically when compared to previous wars when this protective equipment was not used. Thus, many soldiers survive who would not have had they not worn IBAS. An untoward consequence of increased survival is that blast-related TBI became more prevalent than in previous conflicts (2). These victims suffer a spectrum of neurological disorders ranging from subtle mild cognitive impairment, affecting the ability of a person to perform under demanding conditions, to severe disruption of brain function as serious as coma. These effects can be temporary or chronic. If the latter, they can have significant negative impact on patients and their families for decades at great emotional and economic costs to themselves and society.

The prevalence of epilepsy among GWOT TBI patients is unknown. From evidence derived from prior wars, it is expected that about 10–25% of patients with closed head TBI and over 50% of patients who have penetrating TBI will develop post-traumatic epilepsy (PTE) (7). The Department of Defense reports that 1.5% all combat related GWOT TBI are from penetrating injury (6). PTE can take any form of epilepsy but temporal lobe epilepsy (TLE) predominates with up to 62% of TBI patients suffering this type (8). It is important for clinicians to be aware that up to 15% of TBI patients from prior wars did not manifest seizures until five or more years after their injury (7). Recognizing this, the Veterans Administration (VA) has established a national network of Centers of Excellence for Epilepsy, which will provide long-term surveillance and care for these patients.

Most seizures occur within the first 2–3 years after the traumatic event, although the risk for developing PTE remains elevated for many years after injury. About 50% of patients, with even mild TBI (mTBI), who suffer early seizures, i.e., within the first 7 days after injury, will progress to PTE (9, 10). The highest risk for developing seizures correlates with TBI severity. Increased risk is associated with structural lesions such as dural penetration and intracranial hematoma. Findings from the Vietnam head injury survey show that cortical involvement, brain tissue loss, and intracranial retained metal fragments are high risk factors (11, 12).

During the last few years, the present and potential long-term impact of blast-related TBI among military personnel has fueled an increasing number of studies aimed to better understand the mechanisms of injury and characterize the pathobiology of blast-related TBI in order to improve its prevention detection and treatment. This effort is particularly relevant to combat related mTBI, where blast accounts for 72% of cases (13).

Confusion is often associated by the use of the words "primary" and "secondary" to define both physical causal mechanisms leading to injury and the neuropathology of the tissue response. In the case of tissue response, "primary" refers to the immediate tissue damage caused by the physical force such as tissue disruption from a blow to the head or a penetrating projectile as it traverses through brain parenchyma. Secondary injury relates to the pathophysiological response to the injury such as inflammation, excitatory amino acid release, or expression of reactive oxygen species. When

referring to the physical explosive blast force causing TBI, primary refers to the direct injurious mechanism of the explosive blast wave. Secondary injury refers to TBI caused by being struck by material (bomb casing fragments, rocks, and dust) propelled by the blast, tertiary to the victim being physically thrown leading to an impact injury and quaternary for all other mechanisms, such as burns from fireball related burns, toxic fumes, radiation, etc. (14).

"Primary blast-induced injury" thus refers to the tissue damage caused by the explosive blast wave alone. A leading hypothesis for a primary mechanism for how explosive blast causes primary brain injury is that shock waves transit across the target tissue causing its acceleration and deformation. The extent of tissue damage depends on the shape of the blast shock wave, its peak overpressure and pulse duration, and the tissues' natural resonant frequencies (3, 15, 16). The ideal blast shock wave can be represented by the Friedlander curve (17). The injurious effect of this primary blast mechanism is most significant in hollow organs including tympanic membranes, lungs, and gastrointestinal tract (18–26).

Another hypothesized primary mechanism is that shock waves impact the torso and are then transmitted to the brain causing TBI (27–31). In particular, it has been proposed that, indirect transmission of kinetic energy from the blast shock wave traveling through the large vessels of the body plays a key role in causing TBI. The blast overpressure compresses large body cavities, which creates oscillating waves inside the fluid contained in large abdominal and thoracic vascular vessels. The oscillating waves are conducted cephalad through these fluid columns into the brain resulting in both morphological and functional damage. Experimental data suggest that both direct (32) and indirect mechanisms (33) have important roles in the pathogenesis of blast TBI.

In order to test these proposed hypotheses and clarify the underlying pathophysiology in blast TBI, different preclinical methods have been developed using either shock or explosive blast tubes or open-field blast experiments (8, 19, 34). From these studies, extensive data has been amassed on blast shock wave–tissue interaction, blast exposure related cognitive, and behavior changes and brain pathology.

In this review, we focus on the pathology of blast-induced TBI from recent animal studies, summarizing gross and microscopic findings, tissue staining methods, and relevant neuropathology.

SEIZURES AND EPILEPSY FOLLOWING TRAUMATIC BRAIN INJURY

Epilepsy is a common disorder for which well-established and widely accepted animal models exist. These methods use either chemical or electrical approaches to induce seizures. For PTE study, injury is recreated using traditional experimental closed head TBI methods, such as fluid percussion injury (FPI) and controlled cortical impact (CCI), and penetrating head TBI, such as balloon inflation penetrating ballistic brain injury (PBBI) (35–39).

Acute TBI causes sudden changes in brain metabolism, blood flow, and homeostasis increasing the risk of immediate and chronic recurrent seizures (40, 41). One leading mechanistic hypothesis of PTE is contact between intracranial blood and the neuropil lowers seizure threshold (42). However, the conditions for closed head PTE are likely more complex and encompass a number of active TBI related processes. The physical forces causing head impact can create a variety of conditions favorable for seizures, such as acceleration, rotation, contusion and shearing of the blood vessels and fiber tracts, leading to hemorrhages, axonal injuries, gliosis, microglia activation, and Wallerian degeneration. Altered cerebral vasomotor regulation leading to blood flow disturbances, intracranial pressure changes, and altered vascular permeability can potentially contribute to by increasing extracellular calcium, glutamate, and reactive oxygen species formation. Iron from hemoglobin and transferrin accumulates in the brain as hemosiderin enhances the formation of toxic free radicals (40, 42, 43). Disrupted fiber tracts results in anterograde transynaptic neuronal degeneration with the loss of inhibitory interneurons thus lower seizure threshold. Release of aspartate, glutamate, and activation of NMDA receptors with reactive gliosis may also be contributing causative events leading to PTE (44). The size of the injury and the underlying pathophysiology can also alter the occurrence and intensity of non-convulsive seizures (NCS).

For penetrating TBI, PBBI generates more delayed and sporadic seizures compared to infarction following permanent middle cerebral artery occlusion (MCAO), a model of a stroke, with more acute and intense NCS soon after the injury (37, 45). There is a correlation between the volume of the infarct and the NCS activity in the MCAO model but the volume of the lesion after PBBI does not correlate with the seizures. However, there is a positive correlation with the ballistic kinetics of the PBBI and the size of the cavity created by different sizes of the inflated balloon (i.e., 5, 10, 12.5% PBBI) (46). It appears that seizure activity is sensitive to both the size of the injury and the ballistic kinetics and there is a significant difference in the timing and intensity of NCS after MCAO and PBBI. The results of these studies suggest that injury-specific treatment strategies need to be considered.

Histopathological findings in experimental models of PTE show similar changes to those of human TLE. Patients with TLE are usually classified in either the mesial TLE group or in the lateral or neocortex TLE group. Mesial structures of the temporal lobe with epileptogenic potential are the hippocampus and occasionally the amygdala and the entorhinal cortex (47, 48). Interestingly, histology analyses of hippocampal tissues from TBI patients with blunt head trauma or acceleration injury show similar cellular and structural changes compared to the pathology from non-trauma patients with TLE. Histology reports from patients operated on for TBI or drug-refractory TLE show direct hippocampal contusion, hippocampal sclerosis, and neuronal cell loss in the CA1–CA4 sectors with relatively mild histological changes in CA2 and the dentate gyrus. In a patient population with prolonged survival following head trauma the neural cell loss was significant in all hippocampal pyramidal cell subfields (49). The hippocampal degeneration appears to be progressive in nature revealing more severe neuropathological alterations in patients surviving more than 6 months than in patients with <1 week survival (50–53). Reactive astrogliosis is also detectable in TLE with increased expression of glial fibrillar acidic protein (GFAP). Blood–brain barrier (BBB) opening can lead to astrocyte activation through albumin-mediated transforming growth factor β (TGFβ)-dependent signaling (54–56). Other neuropathological findings in surgical specimens from patients with TLE described

granule cell dispersion and temporal lobe sclerosis (47). Other, less frequently affected regions of the brain include selective neurons of the thalamus, basal forebrain, cerebellum, and brain stem (57, 58).

In animal models, FPI causes mossy fiber sprouting demonstrated by Timm staining in the ipsilateral hippocampus in rats with the loss of dentate hilar neurons (35). CCI generates common seizure risk factors in the brain, such as epidural hematoma, subdural hematoma (59), cell loss in the cortex and hippocampus, and neurogenesis in the dentate gyrus (60–63). CCI also results in mossy fiber sprouting in the dentate gyrus ipsilaterally in mice with concurrent late spontaneous post-traumatic seizures similar to human TLE (36). Indeed, the hippocampus seems to be one of the primary sites in epileptogenesis as there is increased acetylcholinesterase staining in human temporal lobe seizure specimens, especially in the outer portion of the molecular layer of the dentate gyrus (64).

Interestingly, whereas various experimental blast methods and studies exist to investigate brain injury, none have reported seizures. This raises the issue whether blast causes neuropathology that is distinct from blunt force. Blast waves can create similar neuropathological changes in the brain, most specifically in the hippocampus, as those observed in experimental PTE animal models. There is also evidence of neurodegeneration, axonal injury, and astrocytosis in the molecular layer of the hippocampus and the dentate gyrus at various short and long-term survival times (65–69). One confounding experimental issue is use of anesthetic agents when performing blast experiments. This is to provide humane treatment to subjects but may have the unintentional effect of suppressing spontaneous seizure activity. Furthermore, studies of reduced seizure threshold have not yet been reported. It should be noted that explosive blast study is a relatively new area of neuroscience research. The primary focus of these early blast studies has been to characterize the underlying physical mechanisms and pathophysiology that causes brain injury and not yet the development of PTE. Thus, these studies are limited largely to neuropathological and behavioral evaluations. Moreover, applying well-developed animal models for PTE, such as CCI or LFP, creates more reproducible injury and neuropathological changes in the brain. Injuries caused by CCI, LFP, or PBBI devices could be more circumscribed and focused to the brain area of interest that trigger PTE. Finding an ideal and reliable experimental blast TBI model with equivalent well-established characteristics of brain injury is still in progress. It is clear that more research is needed to study the relationship between blast TBI and PTE (50).

Animal models are useful in elucidating mechanisms underlying and structural alterations associated with PTE. They provide a rational basis by which more effective treatments may be developed. It is also important to be familiar with these experimental methods because the results of these studies, especially the observed histological changes in the central nervous system, provide a deeper understanding of the underlying pathophysiologies of the various types of TBI. It must be noted again that none of these traditional models use explosive blast. Thus, the insights gained from these animal models may be limited as they pertain to combat related explosive blast TBI.

EXPERIMENTAL MODELS OF BLAST-INDUCED NEUROTRAUMA

Appropriate clinical and military-relevant experimental animal methods are essential to characterize injuries and disorders of blast TBI. The injury model should be reproducible with a clearly identified injurious component simulating the features of human blast TBI. Injury severity should be predicted by the different mechanical properties of the injurious agent and the determined end-points of injury should be reflected by the chosen injurious component of the blast (28).

Various test methods are used to model explosive blast injuries suffered by humans. The most frequently used experimental models are open-field blasts, blast tubes, and shock tubes (28). An open-field blast is when an explosive device is detonated in an open area. It may be suspended above or placed directly on the ground. Subjects are located a specific standoff distance away from the device. This is the most accurate representation of the human condition. However, as in actual IED blasts, the shock waves produced are complex as they are subject to reflection off the ground and other surfaces. The fireball and debris cloud may contribute to the injury. Thus, it is difficult to study primary blast effects alone using this approach. For that reason, tubes are used.

In explosive blast tube experiments, a blast wave (shock wave plus blast wind) is created by the detonation of an explosive charge. The advantage of this approach, as compared to open-field blasts, is that equivalent blast intensities at the target can be achieved with significantly smaller explosive charges. Moreover, the experimental setup allows for the exposure of experimental subjects to a "pure" blast event without reflected shock fronts from the ground or other surfaces. Isolation of the primary blast mechanism is facilitated by adequate immobilization (to minimize tertiary mechanism), using uncased explosive (to prevent secondary mechanism), and placement of the subject beyond the detonation fireball (to avoid quaternary mechanism). Examples of blast tubes are the tube developed by Parks used by Bauman et al. (67) and De Lanerolle et al. (70) to study blast-induced TBI in swine, and the Clemedson tube (71) used in Sweden to study the blast-induced TBI in rat (72, 73).

The tube developed by Parks is 70-feet long, open at both ends and has three sections: a 6-feet long heavy walled driver chamber (where the explosive is detonated) with a diameter of 34″, a 10-feet expansion cone, and a 50-feet test section, with a 6-feet diameter. The standoff distance is typically 15–25 feet.

The Clemedson tube is much smaller (about 1.5 m in inner length), closed at the detonation end, represented by a conical shaped chamber about 0.57 m deep. The test section (<1 m long) is cylindrical, with an inner diameter of 0.4 m. The standoff is about 1 m. Two consequence of the difference in size and standoff is that the Clemedson tube can be used only for smaller animals and that blast pulse durations will be shorter.

Obviously, a method using an explosive is the most accurate way to study explosive blast effects. However, there are significant practical considerations when using these blast tubes. Requirements include specialized testing locations (usually, ranges), personnel specifically trained in the safe use of explosives, and expense

associated with these. In addition, explosive blasts, whether in the open-field or in a tube are typically carried out in an outdoor setting and are consequently subject to weather and other environmental conditions (74).

Shock tubes using compressed gas, such as helium, as opposed to explosives are an alternative to blast tubes. They are safer, more cost effective, and can be used indoors. These tubes are smaller than explosive-driven tubes and are closed at one end. They consist of a "driver" section at the closed end, separated from a "driven" section by a frangible or breakable diaphragm composed of mylar or cellulose acetate. The process begins with the generation of high pressure by the pumping of gas within the closed off driven section. When the pressure reaches a critical level, the diaphragm ruptures creating a shock wave. The shock wave characteristics can be controlled or tuned by changing subject standoff from the diaphragm, varying the membrane material or thickness, changing the shape of the closed end of the driver, and using different gases to pressurize the membrane. Similar to blast tubes, most shock tubes are designed to contain the subject animal within their "driven" section. Examples are those used at Walter Reed Army Institute of Research (33), the University of Kentucky in Lexington (75), Wayne State University (76), and Johns Hopkins University (77). In addition, there are smaller models of shock tube that are designed to generate a shock wave to impact a target outside the tube itself so as to study the effect on a specific body region, such as the head or the chest (30, 33, 65, 66, 78–83). Examples of this latter type are those used at the Florida Institute of Technology and Banyan Biomarkers, Inc. (66) and the University of Toronto (83).

Shock tubes have their own important drawbacks. Very importantly, the physics of the gas-driven shock waves may differ from explosive shock waves. If so, the injury pattern produced may not be comparable to the human condition. Gas-driven shock waves are often atypical, showing an apparent pressure plateau following the initial pressure peak. This is likely due by the existence of two successive pressure waves; the first directly coming from the bursting diaphragm and the second reflected back from the tube end. A single and more typical-looking pressure wave is obtained by allowing sufficient standoff, which permits the reflected wave to reach and fuse with the direct one. Another issue is the possible impact of diaphragm fragments on the subject. Even low mass fragments, when accelerating at high rates, will exert significant force on subjects, which means the resultant injury is not primary blast effect alone. Finally, the physical load of multiple small fragments may affect the dynamics of body–head acceleration.

A common issue of both explosive-driven and gas-driven shock tubes is the jet stream effects created near the tube exit. This jet stream creates an unrealistic dynamic pressure effect that can be avoided by placing the target sufficiently far from the tube's exit or, in the case of external exposure, sufficiently off axis to the tube's nozzle (66, 83).

Both explosive and gas-driven shock tubes aim to recreate primary blast conditions with ideal Friedlander waves. Real world exposures are more complicated as reflected shock waves create a complex interaction with primary shock waves. To replicate war related conditions, some investigators have carried out studies using surrogates of military vehicles, buildings, or bunkers (67, 68, 74, 84, 85). Each is appropriate for recreating real world condition but methodological differences interfere with generalization of results (74).

Finally, rodents, pigs, rabbits, and non-human primates (NHPs) used for blast studies widely differ in their neuroanatomy and neurophysiology, which can further contribute to the variations in the observed pathological and physiological changes of experimental blast injuries (28, 74).

PATHOLOGY OF BLAST-RELATED BRAIN INJURY

Recent studies have identified candidate pathophysiological processes that likely play key roles in the genesis of blast TBI. From detailed histopathological analyses, common findings include small and larger intracranial hemorrhages, edema, vasospasm, neuronal damage/degeneration, focal or diffuse axonal injury, glial cell activation, and inflammatory reactions (1, 86). Optimizing identification of tissue injuries is highly dependent on using the most appropriate histological methods and stains as well as on timing after injury ictus and sampled brain region. For general morphological examinations (neuronal injury, cell death, intracranial hemorrhages, edema formation, and inflammation) hematoxylin and eosin (H&E) and cresyl-violet are used. Luxol-fast blue, a special myelin stain, is used routinely for myelin damage. For the detection of more subtle cellular changes, immunohistochemistry (IHC) is the general method. One of the most widely examined features of TBI is diffuse axonal injury. Traditionally, axonal injury is detected by silver staining or β-amyloid precursor protein (β-APP) IHC (87–90). GFAP and various microglia stains are used to label activated astrocytes and microglia cells (91–93). For ultrastructural examinations at the subcellular level electron microscopy is the preferred method.

As part of the research program PREVENT (Preventing Violent Explosive Neurotrauma), Baumann et al. use a swine model and the Parks explosive-driven shock tube to study explosive blast TBI (67). Within the tube the pigs are restrained in a sling that minimizes movement during the blast, and exposes subjects side-on to the blast. In addition, these investigators use both a surrogate military vehicle and 2-room building so as to recreate more typical complex shock waves. Brain specimens are obtained at 2 weeks after blast exposure. For axonal injury, a modified Gallyas silver method, as made available by FD Neurotechnologies, is used (94). This staining technique labels injured/degenerating axons and neurons as early as 24 h after injury. IHC is used to label cells positive for GFAP as well as other markers. Silver staining reveals degenerated axons in the ipsilateral white matter tracts of corona radiata and cerebellum. Astrocyte activation is evident in the ipsilateral white matter of the cortex and in multiple layers of the ipsilateral hippocampus. Elevated GFAP, neuron specific enolase (NSE), and myelin basic protein (MBP) expression are also detected 6, 24, and 72 h after exposure. Additional observations include changes in the electroencephalogram (EEG) patterns, vasospasm in carotid artery branches, and disturbances in the movement of the pigs involving major joints and limbs (knees and metacarpals). Detailed neurological function assessment is made using motion analysis technologies for gait, EEG telemetry, spatial memory testing, and cerebral angiography. However, anatomical differences between swine and human skulls

can generate discrepancies in the interpretation of biological and biomechanical events.

A similar approach to blast exposure is used in a swine study carried out by de Lanerolle et al. (70). Specimens are collected 72 h and 2 weeks after blast. Paraffin-embedded sections are used for standard and immunohistochemical stainings: H&E, Luxol-fast blue, Fluoro-Jade B (neurodegeneration), GFAP, β-APP, and CD68 (macrophage/microglia marker).

Analysis reveals very limited neuronal injury with Fluoro-Jade B failing to reveal positive cells. Intracranial hemorrhages and fiber tract demyelination are not present. Dark, shrunken neurons are noticed but since they are also seen in controls, their presence is attributed to mechanical manipulation of the tissue. Red (eosinophil) neuronal degeneration is occasionally visible throughout the neural tissues both in blast and sham control animals. β-APP IHC is positive in the periventricular white matter close to the lateral ventricle in all groups. The axonal injury, around or close to the ventricles, is explained by a fluid-tissue interface effect generated by local pressure transients at the site, or ventricular volume increase strong enough to cause axonal deformation. GFAP activity is also enhanced in the different layers of the hippocampus and cortical gray and white matters. Their morphology is different from those activated by neuronal injury and the number of activated astrocytes in the hippocampus is significantly higher in the animals exposed in the vehicle or the building. Microglia activation is visible in the central white matter and corpus callosum. One explanation of the glial activation is the transient opening of the BBB triggering the activation of astrocytes by extravasated albumin resulting in excitatory neuronal injury. These findings together support the notion that astrocytosis and periventricular axonal injury may have an important role in the potential for long-term TBI exacerbations, mood, and cognitive disorders.

Lu et al. report their NHP study using open-field blast with either single or double-blast exposure (68). The outcome of exposure to the following conditions is evaluated and compared: single-blast at 80 kPa (equivalent to 11 psi; SBL), single-blast at high intensity at 200 kPa (equivalent to 29 psi; SBH), and double-blast (DBL) at 80 kPa. In the DBL group, exposures are carried out 3 days apart. Specimens are obtained at either 3 days or 1 month post-blast. General morphological analysis uses H&E and TUNEL for apoptosis. IHC is used for the detection of S100B and GFAP (for astrocyte reaction), MBP, neuronal nuclear antigen (NeuN), β-APP, aquaporin-4 (AQP4, for water channel identification), and oligosaccharide-specific agglutinin I anti-lecithin antibody (to reveal microglia cells). Electron microscopy is also performed in order to detect ultrastructural changes.

At gross pathological examination, no visible damage can be detected in the brain, and only minor injuries are noticeable in the lungs. MRI only detects a right anterior lobe cerebellar lesion in a single subject. Microscopically, there are neuronal cell changes in the cortex, the cerebellar Purkinje-cells and the hippocampus such as dark, shrunken neurons with distorted dendrites in all groups with elevated NeuN reaction. Apoptotic cells are rarely co-labeled with GFAP and MBP in the subcortical areas 1 month after injury. The number of apoptotic cells is increased and MBP reaction is reduced in the SBH and DBL groups. Increased β-APP reaction is also observed in the neuronal perykarion and around axons. Besides the neuronal alterations in the cerebellum, the astrocytes show reactive changes in the SBH and DBL groups by S100, GFAP, and AQP4 staining. Electron microscopy on tissues from the cerebellum reveal structural damages in the nucleus, mitochondria, and cytoplasmic filaments of the Purkinje-cells, with the formation of stacks of smooth endoplasmic reticulum, myelin sheath degeneration, astrocyte filamentous and end-feet hypertrophy, microglial activation, and severe oligodendrocyte cell injury. Interestingly, vascular changes are observed in the cerebellum, with obliterated and collapsed capillaries, endothelial cytoplasm vacuolations and accumulation of perithelial cells.

These pathological results correlate with observed behavioral changes in motor coordination and working memory. The lesion detected by MRI shows widespread pathology in the above described area suggesting the vulnerability of the cerebellum. The accumulation of the smooth endoplasmic reticulum in the Purkinje-cells can be a part of a protective mechanism by calcium sequestration. Furthermore, damage to the oligodendrocytes, astrocytes, and capillaries likely contribute to cognitive, motor and other neurological dysfunctions, brain edema, and ischemic-hypoxic damage. Although the study provides a broad pathological overview in blast TBI, the sample size is relatively small and further long-term behavioral studies are required to define neurological deficits.

To determine whether or not torso IBAS mitigates of TBI, Long et al. use a compressed air-driven shock tube to create blast injury in chest-protected and unprotected rats (33). Chest protection is a Kevlar vest that completely covers the rat's thorax but leaves the head exposed. The animals are placed in a transverse prone position in a wire-mesh holder across the mouth of the shock tube. Brain samples are collected 2 weeks after blast exposure. Brains are cresyl-violet, thionine, and silver-stained. The observed pathological alterations are torso protection and intensity dependent. Neural cell loss is observed, along with gliosis, fiber degeneration, hemorrhage, and necrosis, in the brain of unprotected rats exposed to 147 kPa (equivalent to 21 psi), but not 126 kPa (equivalent to 18 psi) blasts. These changes are more severe in the hemisphere facing the blast. Brains from rats exposed to the lower blast show extensive silver-stained fiber degeneration that is bilateral.

Chest protection does not affect the pathological outcome in 147 kPa blast – exposed rats but largely prevents fiber degeneration in the brains of animals exposed to 126 kPa blasts. No evident pathology is observed in the brains at the lowest blast intensity level (114 kPa or 16 psi). These findings suggest that chest protection does contribute to TBI mitigation, particularly at lower blast intensities. Furthermore, these observations lend further support to that of prior studies (31) that the second hypothesized mechanism of how blast injures brain may be valid.

Studying head and torso protection, Koliatsos et al. use a shock tube generating overpressure with compressed helium, with mice placed inside the shock wave tube fixed in a wire-mesh holder disallowing body or head motion (95). The torso and/or head of each mouse are protected by a Plexiglas cover. Animals are exposed to different blast intensities either in a prone or supine position. Social recognition, spatial memory, and motor coordination outcome measures are used. Brains are collected at 1, 3, 5,

7, and 14 days after exposure. Internal organs – lungs, liver, heart, spleen, and kidney – and eyes were also examined. Standard formalin immersion-fixed paraffin-embedded, and perfusion fixed frozen tissues are stained with routine H&E, various special and immunostains (cresyl-violet, Mallory trichrome, elastic fiber stain, Fluoro-Jade, APP, phosphorylated neurofilament, and TUNEL). FD Neurosilver kit is used to detect axonal injury.

Findings are injuries to the internal organs (lung, heart, liver, kidney, and spleen) that are mainly hemorrhages and hemorrhagic infarcts. These correlate with blast wave intensities and body position. Neuropathological results of blast at lower blast intensity include extensive silver-stained axonal injury involving the cerebellum, brainstem, corticospinal tracts, optical, and auditory pathways. Axonal injury is more prominent 14 days after the exposure. No histological reactions are detected in animals at 1, 3, and 5 days after blast. Special stains fail to reveal any brain hemorrhage, neuronal injury, or cell death. Occasional APP and phosphorylated neurofilament positivity is visible in the corpus callosum and anterior vermis. Interestingly, when torso protection is applied, there is no observable white matter tract degeneration and no behavioral deficits.

The finding that torso protection is neuroprotective in blast, especially against diffuse axonal injury, has both important clinical and mechanistic implications. These findings, consistent with those reported by Long et al. (33), point to the likely role of the second mechanism of blast TBI, which is blast chest compression and vascular cephalad conduction of shock waves into the brain. Clinically, this supports the military's use of IBAS as likely helping to protect service members from both blast-related torso and brain injuries.

The importance and usefulness of silver staining is further emphasized and convincing in work by Garman et al. (65). As part of the PREVENT blast program, they conduct an initial neuropathological characterization in body protected rats exposed to blast. Animals are positioned in a helium-driven shock tube within a wedge-shaped holder protecting the torso but leaving the head exposed. Besides protecting the torso, the holder increases the intensity of the shock wave at the target, by creating a mach stem along the side of the wedge. To prevent gross motion, the head is held in place with a leather sling. The shock tube generates a peak pressure of 35 psi, resulting in 25% mortality from apnea. Brains are collected at 1 and 3 days and then 2 weeks. H&E, de Olmos amino cupric silver, and immunostains for GFAP, ionized calcium-binding adapter molecule 1 (Iba1) and CD68 (for microglia activation), APP, and IgG (for brain edema) are performed. Not surprisingly, silver staining is the most sensitive method in identifying TBI, labeling axonal damage as well as neuronal degeneration.

Neuronal degenerations including axons and dendrites are the most prominent histological alterations during the first 2 weeks in blast-exposed rats with body protection. Degenerating neuronal cell bodies are most detectable at 1 and 3 days showing a scattered distribution with some preference in various cortical regions, CA1 pyramidal layer of the hippocampus and the cerebellar cortex, the latter suggesting synaptic or terminal degeneration. The axonal damage marked by silver staining is prominent at all-time points, but most evident after 2 weeks, affecting both sides of the brain except for the entorhinal cortex and hippocampal dentate gyrus, which show stronger contralateral reaction. This is believed to be caused by a diffraction effect or localized shock amplification on the contralateral side of the skull or by the effect of diffraction coupled with skull flexure. The injured fiber tracts include various long tracts such as the optic tract, internal and external capsules, thalamic pathways, cerebral and cerebellar peduncules, trigeminal tracts, and pyramids. APP-based detection of axonal injury is minimal. There is no astroglial reaction and only weak microglia activation is visible adjacent to brain regions with neuronal degeneration. Breach of the BBB using IgG is only seen in the 1 day group mostly on the contralateral side of different brain regions.

This study demonstrate that, in this blast model, silver staining was more effective in revealing axonal injury than APP, a marker which is most prominently detected in axonal injuries related to acceleration/deceleration mechanisms (96, 97). This study also provides evidence of blast-related breach of the BBB. However, its relation with axonal injury, if any, is unclear.

A study by Goldstein et al. (69) examines the connection between blast-induced TBI and chronic traumatic encephalopathy (CTE). Neuropathological examinations of four military veterans who died in blast or concussive injuries show similar brain changes as four athletes who suffered concussive injuries in football, wrestling, etc. The image is correspondent with CTE, a tau protein-related neurodegenerative disease (98–101). These human neuropathological observations are compared with the pathological outcome of mice exposed to blast. In this model, mice are placed prone within a shock tube. Only the heads are exposed, side-on, to the gas-driven shock wave as the rest of the body is protected within the holding fixture. Heads are not secured for some subjects, which allow testing of the hypothesis that blast-induced head acceleration contributes to TBI. The blast is reported to be comparable to detonation of 5.8 kg trinitrotoluene (TNT). Measurements of intracranial pressure at the time of shock wave impact confirm the intracranial transmission of stress waves occurs without significant contribution of torso-transmitted shock waves. Brains are collected at 2 weeks post-blast, saline perfused, prefixed in 10% neutral-buffered formalin, block-sectioned, and post-fixed in 4% paraformaldehyde. Serial sections are cut from paraffin-embedded blocks and stained with various stains including IHC for axonal injury, tau pathology, astrocytosis, and cholinergic motor neurons. Brain tissues are also processed for ultrastructural examinations.

Gross examinations of the brains do not show any visible macroscopic tissue injury. By histological examinations single-blast exposure produces CTE-like changes in the mouse brain such as tau protein immunoreactivity, phosphorylated tau proteinopathy, cortical and hippocampal neurodegeneration, permanent perivascular pathology, myelinated axonopathy, and chronic neuroinflammation with astrocytosis and microgliosis. Blast produces "dark neurons" in close proximity to abnormal capillaries (102, 103). Moreover, axonal conduction velocity is reduced in the hippocampus and synaptic transmission disturbances resulting in learning and memory deficits. Head immobilization prevents blast-induced hippocampus-related behavioral deficits. Electron microscopy verifies persistent microvascular pathology and astrocyte end-feet swelling suggesting BBB compromise, which in

turn possibly plays a role in local hypoxic, inflammatory, and neurodegenerative changes.

The similarities between human CTE cases and the experimental method described above suggest that different scenarios can induce a common pathway leading to similar morphological changes. The results from this mouse blast study are consistent with the morphological, neurophysiological, and cognitive deficits that are reported in military veterans and athletes with blast and/or concussive-related CTE. In addition, this study is also significant because it suggests that head acceleration plays a critical role in TBI.

SUMMARY AND DISCUSSION

In this review, we provide a brief review on experimental models of brain trauma, development of PTE and the pathological/histological features of TBI, including blast. Our intent is to give the reader an overview of the most routinely used and reproducible histopathological methods and neuropathological results published on blast TBI and PTE as these represent the cellular basis of this injury and its clinical consequence, such as seizures. It is at this level that rational comparisons may be made among the different TBI types as well as, very importantly, between preclinical models and the human condition. Increasing demand in the field of blast TBI to understand the physics and pathophysiology of blast-related brain injury has produced a large number of scientific publications reporting, sometimes contradictory, results obtained from animal studies (28, 86). These reports provide information about both morphological alterations in the CNS and also neurophysiological and behavioral aspects of blast injury. While it is extremely important to examine blast TBI and its consequences in every respect, pathological evaluation is probably the ultimate way to prove or disapprove mechanistic theories.

The pathological methods and results reviewed above underpin several technical issues, which need to be taken into consideration when working with tissue specimens, especially brain. The most important is to be able to recognize tissue and cellular changes and responses to a noxious event. It is one of the most crucial rules to learn to recognize common artifacts in CNS tissues, which are of no pathological significance (104). Failure to do so will lead to conclusions that are misleading and erroneous (105–108). Artifacts can be caused by improper tissue handling that, many times, are unavoidable (109) but following current guidelines could help to overcome these potential technical issues. Nevertheless, some of them are worth mentioning (110, 111). Microscopically, underperfused brain tissues demonstrate collapsed microvessels containing blood, with tissue retraction around them and dark, basophilic neurons are readily observable. These artifacts make histological interpretations difficult. Not all parts of the brain will necessarily be evenly well-perfused, but a good perfusion should produce distended vessels throughout the brain with no or minimal artifacts (110, 111).

One of the most frequently noted and long-debated artifacts in surgical human specimens and various experimental studies is the "dark neuron," which is often interpreted as neuronal degeneration or death (112, 113). Neurons are highly susceptible to ischemic/hypoxic injuries that can be detected microscopically after 6–12 h in humans and 30–90 min in experimental animals (104, 114). The cytological hallmark of neuronal injury is the eosinophilic degeneration or "pink neurons." These cells are shrunken with eosinophilic cytoplasm, glassy, basophilic pyknotic nucleus, and absent Nissl substance. After dead neurons and cell debris have been phagocytosed, glial cells appear and proliferate creating a glial scar tissue. Axonal transection, most frequently in lower motor neurons, can produce central chromatolysis when the cell itself is intact, the cell body is rounded and the nucleus and Nissl substance is displaced peripherally (104, 114, 115). Apoptotic, fragmented cells are easily recognizable even for the inexperienced eyes. "Dark neurons" on the other hand, have a shrunken angular cell body with deeply stained cytoplasm, small, irregular, dark basophilic nucleus with loss of details. Dendrites often have a characteristic cork-screw shaped appearance. Such neurons are more frequent in immersion-fixed brains but adequately perfused material can still contain numerous dark neurons in experimental neuropathology (110, 111, 116, 117). Mechanical post-mortem manipulation of the brain can increase the number of these neurons (110, 118). Interestingly, the presence of these contracted neurons has been reported in some acute neuropathological states making the distinction between true neuronal degeneration and artificial dark neurons challenging (119–122). Although neuronal degeneration and cell death can be often detected on routine H&E stained slides, using special stains specific for neurodegeneration can significantly assist to recognize neuronal damage. Fluoro-Jade B and Fluoro-Jade C are both recommended in the identification of neuronal degeneration and the degeneration of fine neuronal processes (111, 123). Silver staining has an important role in experimental neuropathology to detect axonal injury. Even if β-APP fails to label injured axons, silver techniques can help to detect early, and more often, late axonal degeneration (65, 95). Mastering any of the silver staining technique can be challenging but commercially available silver stain kits are easy to use and reliable. In general, for most neuropathological experimental studies, a set of special and immunohistochemical basic stains can provide an initial step toward a close evaluation of the tissue samples. The usage of negative and, ideally, positive tissue controls is of the utmost importance. Finally, it can't be overemphasized that experiments, TBI or others, involving morphological evaluations should be reviewed by experienced morphologists or pathologists to avoid further inconsistencies among researchers (111, 124).

Fortunately, most researchers working with neural tissues are using appropriate current pathological methods but future investigators in the field, especially those without a background in pathology, should take into consideration the above discussed technical details. Moreover, the validity of some of the methods used to reproduce blast phenomenon may be lacking. The heterogeneity of results may be partly the result of this inadequacy and partly reflect differences in experimental designs. When considered in balance, the collective work still reveals important insights on mechanism of blast-related injury.

Some key findings are that explosive blast, when of sufficient severity, leads to brain pathology. The most consistent neuropathological findings are multifocal axonal and neuronal injuries detected by silver staining, astroglial alterations, inflammation with elevated cytokine and reactive oxygen species activity, BBB anomalies, and intracranial hemorrhages. This pathology

correlates with behavior changes such as spatial and cognitive performance and coordination. Very important clinically is the evidence supporting the benefits of body armor in mitigating blast TBI as well as torso protection. This also provides supporting evidence to the notion that caudal transmission of shock waves through the thoracic and intracranial blood vessels plays a role in TBI genesis. Also very important is the demonstration that torso protection also mitigates diffuse axonal injury. The role of primary blast in causing TBI is still unclear. However, it does appear that head acceleration is an important contributor to TBI as well.

Seizures are an important clinical consequence of all TBI. Although the precise impact of this clinical condition on explosive blast TBI recovery is still being elucidated, the finding that explosive blast leads to consistent neuropathological brain changes raises significant concern that seizures and epilepsy may be more prevalent than previously suspected. Fortunately, the VA is taking a comprehensive prospective longitudinal approach to study PTE in blast TBI victims.

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Role of intravenous levetiracetam in seizure prophylaxis of severe traumatic brain injury patients

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Traumatic brain injury (TBI) can cause seizures and the development of epilepsy. The incidence of seizures varies from 21% in patients with severe brain injuries to 50% in patients with war-related penetrating TBI. In the acute and sub-acute periods following injury, seizures can lead to increased intracranial pressure and cerebral edema, further complicating TBI management. Anticonvulsants can be used for seizure prophylaxis according to the current Parameters of Practice and Guidelines in a subset of severe TBI patients, and for a limited time window. Phenytoin is the most widely prescribed anticonvulsant in these patients. Intravenous levetiracetam, made available in 2006, is now being considered as a viable option in acute care settings if phenytoin is unavailable or not feasible due to side-effects. We discuss current data regarding the role of intravenous levetiracetam in seizure prophylaxis of severe TBI patients and the need for future studies.

Keywords: levetiracetam, seizure prophylaxis, traumatic brain injury, phenytoin, epilepsy

INTRODUCTION

POST TRAUMATIC SEIZURES

The annual incidence of traumatic brain injury (TBI) is 1.7 million in the United States (1). One well-recognized complication of moderate to severe TBI is post traumatic seizure (PTS). A study by Temkin et al. shows a 2-year seizure rate of 21% after severe TBI (2). In another study by Salazar et al. the incidence of seizures is almost 50% after penetrating war-related TBI (3). It is generally accepted that mild TBI or concussion can increase the risk of developing epilepsy but the incidence is uncertain. This uncertainty is largely due to incomplete knowledge of the incidence of mild TBI itself.

Post traumatic seizure are divided into two subgroups. The seizures occurring within the first 7 days after brain injury are classified as early PTS. Those occurring after 7 days of injury are classified as late PTS. Non-convulsive electrographic seizures can also occur. These can lead to cerebral metabolic crisis, delayed increase in intracranial pressure, and worse clinical outcome (4).

The deleterious effects of PTS exacerbate the existing brain injury and contributes to greatly worse TBI outcome. Consequently, an important clinical goal in TBI care is preventing seizures. To this end, Temkin et al. conducted a series of randomized placebo controlled trials (RCT) that demonstrated efficacy of phenytoin, valproate, and carbamazepine in reducing the incidence of early PTS (2, 5, 6). These same studies showed that there was no benefit for late PTS.

Phenytoin is the preferred agent of choice for early PTS prophylaxis. It is generally well tolerated, can be administered once per day, can be given IV and most medical practitioners are familiar with its use. Valproate is less desirable as it is shown to be associated with increased mortality. Carbamazepine is not yet clinically available in an IV formulation. This issue is important because most moderate to severe TBI patients cannot take medications orally due to inability to protect their airways. Thus, valproate and carbamazepine should be considered as alternatives to phenytoin for early PTS prophylaxis (7, 8).

Phenytoin has significant adverse effects. The most common are hypersensitivity reactions, irritation of the skin, phlebitis, arrhythmias, and hypotension during parenteral administration (9). One particularly severe toxicity is Stevens Johnson Syndrome. Phenytoin has a narrow therapeutic index and non-linear kinetics so a small increase in dose may result in much greater increase in levels resulting in toxicity. Additionally, it is more prone to drug–drug interactions because of the induction of the hepatic cytochrome P450 system which further limits its use in critically ill patients (10). Phenytoin has also been shown to exacerbate acute adrenal hyporesponsiveness, a phenomenon seen in patients with severe brain injury by decreasing the cortisol concentration (11–14). A limitation frequently encountered with phenytoin is the use of weight based dosing, which often leads to subtherapeutic levels of the drug (15).

Because of these complications and the limitations of valproate and carbamazepine, levetiracetam is more often being considered as a viable option.

Intravenous levetiracetam was approved by the FDA in 2006 and has a number of advantages over phenytoin. Levetiracetam has linear pharmacokinetics (PKs) and is thus easier to titrate. It has lower potential of drug–drug interaction than phenytoin. It has not yet been shown to have enzyme inducing properties. Finally, there is no need to monitor of serum drug levels (16). In this review, we will discuss the studies which provide evidence of efficacy of intravenous levetiracetam in the prevention of PTS.

Preclinical studies using animal models of TBI have shown efficacy of levetiracetam as a neuroprotectant. Zou et al. report

a study of rats treated with levetiracetam after receiving a controlled cortical impact (CCI) TBI. In this study, 50 mg/kg of either intraperitoneal levetiracetam or saline control were administered daily for 20 days starting 1 day after CCI or sham injury. To determine neurobehavior outcome, rats were tested on balance beam, Y-maze, and the Morris Water Maze. Levetiracetam treatment was shown to be beneficial to neurobehavior functional recovery and hippocampal cell sparing. It also decreased contusion volumes by almost 33%. Finally, TBI-induced decreases in regional glutamate transporter expression and neuroplastic markers were reversed by levetiracetam. The investigators concluded that levetiracetam treatment post-TBI, lead to improved histological, molecular, and neurobehavioral outcomes (17).

In spite of these promising preclinical studies, levetiracetam has not yet been conclusively shown to have neuroprotective or neuro rescue effects in humans.

However, there is human clinical evidence to show that levetiracetam is a reasonable alternative to phenytoin for seizure prophylaxis. In a prospective multicenter comparison of levetiracetam vs. phenytoin for early PTS prophylaxis by Inaba and colleagues, patients with closed head TBI are treated with either medication. A total of 813 patients are analyzed of which 406 received levetiracetam and 407 received phenytoin. The two groups are balanced for age, gender, Injury Severity Score (ISS), Marshall score of $\geq$3 or craniectomy. There results reveal no statistically significant difference in terms seizure rate, adverse drug reactions, or mortality. The authors conclude that levetiracetam did as well but not better than phenytoin as an early PTS prophylaxis. The cost of Levetiracetam is higher than phenytoin and availability is also an issue. However, the need and cost of monitoring favors Levetiracetam (18).

For other than TBI brain pathological states, Zafar et al. report a meta-analysis of studies that compare levetiracetam to phenytoin for seizure prophylaxis for patients who suffer from TBI, intracranial hemorrhage, intracranial neoplasms, and/or craniotomy. A comprehensive electronic data search is performed on studies with a primary outcome of seizures and have balanced the baseline population characteristics, type of intervention, and study design. Of 2489 studies, 8 meet inclusion criteria. Of these, two are RCT and six observational studies. The results show odds ratio equal to 1.12 so there is no superiority of either agent in the prevention of early seizures. The conclusion of the meta-analysis is that levetiracetam and phenytoin have equal efficacy in seizure prevention after TBI as well as intracranial hemorrhage, intracranial neoplasm, and craniotomy. The limitation of this study is that the conclusions are based on a few RCT. Thus, additional trials are recommended (19).

Szaflarski et al. describe the first prospective randomized comparative trial of intravenous levetiracetam vs. phenytoin for seizure prophylaxis in the neuro intensive care unit setting. Fifty-two neuro intensive care unit patients are enrolled in this study. Many (89%) have severe TBI. Thirty-four patients receive levetiracetam and 18 phenytoin. Standard intravenous doses are used with doses of phenytoin adjusted to maintain therapeutic serum levels. Continuous EEG monitoring is performed during first 72 h of treatment. After controlling the baseline severity, there are better outcomes with levetiracetam therapy as evidenced by lower disability rating scales at 3 months and higher Glasgow outcomes scales at 6 months. No differences in occurrence of seizures are seen in either group during or at 6 months. No differences are seen in adverse effects in either group except for lower incidence of worsened neurological status and fewer gastrointestinal problems in the levetiracetam treated group. The authors conclude that levetiracetam is a reasonable alternative to phenytoin for seizure prophylaxis in the neuroscience ICU setting. The limitations of this study include a small sample size, and a lack of reported data on the use of sedating agents in these patients. Propofol is the drug of choice in patients with severe TBI, which also has anticonvulsant properties. The concomitant use of both agents may have confounded the results of the trial (20).

A retrospective, observational study is conducted by Kruer et al. to evaluate patients treated with phenytoin vs. levetiracetam for seizure development within 7 days after TBI. Of 1,552 TBI patients identified through the adult trauma center registry, 354 met inclusion criteria of $\geq$3 on the Abbreviated Injury Scale (AIS), and $<$8 on the Glasgow coma scale. A total of 245 patients are excluded, mostly because no prophylactic antiepileptic drug was given (34%) or there was no history of acute TBI (30%). The remaining 109 adults who received phenytoin ($N = 89$) or levetiracetam ($N = 20$) have additional information gathered from the electronic medical record and paper chart. One patient in each group had documented post-traumatic seizures. Sixty-five percent of patients received prophylactic AEDs for $>$7 days, and 68/109 patients survived to hospital discharge. Between 2000 and 2007, nearly all of the patients (98%) received phenytoin. This was reversed during 2008–2012 such that the majority (64%) patients instead received levetiracetam. Thus, after FDA approval of its IV formulation, a clinical trend favoring levetiracetam use over phenytoin is observed but the lack of difference between these drugs is due to the restricted results on PTS (21).

To determine efficacy for early PTS prophylaxis following severe TBI, Jones et al. compare levetiracetam vs. phenytoin. The study is conducted in 32 patients, all of whom receive IV levetiracetam for the first 7 days after TBI. These data are compared to results from a historical cohort of 41 patients who received phenytoin for seizure prophylaxis. Patients are evaluated for 1 h by EEG if they have persistent coma, decreased mental status, or clinical signs of seizures. The results show that 15/32 patients in the levetiracetam group and 12/41 in the phenytoin group require EEG monitoring. Of the levetiracetam group, the EEG from seven patients were normal and eight were abnormal. Of the EEG abnormal subgroup, one patient had EEG evident seizure activity and the rest had signs of seizure tendency but no overt evidence of seizure. The EEG results were all normal in phenytoin treated patients. For seizure activity, there was no statistical difference between these two treatment groups. The authors conclude that levetiracetam is as effective as phenytoin in preventing early clinical PTS, but a higher incidence of epileptogenicity with levetiracetam. The limitations of this study included lack of randomization, small sample size, and duration of the EEG recording. The rationale was that only 1-h recordings would not be able to capture actual seizures for which more prolonged monitoring is required (22).

Studies have shown that the PK profile of intravenous levetiracetam, which includes linear kinetics and lack of drug–drug

interactions, favors this new agent over phenytoin. However, the PK properties of levetiracetam are not etiology specific and more studies are required in this subset of patients.

In a prospective, open-label, steady-state PK study by Spencer and colleagues, steady-state PKs of IV LEV is assessed in neurocritical care patients. The sample size of 12 adults, comprised of five men and seven women aged 54 ± 14 years, all required anticonvulsant prophylaxis in the neurocritical care unit after TBI ($N = 1$), subarachnoid hemorrhage ($N = 10$), or subdural hematoma ($N = 1$). Patients were eligible if they were >18 years old, presence of arterial or central venous access for blood sampling, and required IV LEV for seizure prophylaxis. Patients were excluded if they had multisystem trauma, end-stage renal disease, or hemoglobin concentration <7.0 g/dl. An IV infusion of 500 mg LEV given over 15 min every 12 h was administered to patients. After a minimum of four doses of LEV, serial blood samples were collected from all patients. Ultraperformance liquid chromatography with tandem mass spectrometry detection was used to determine the serum levetiracetam concentration. LEV maximum serum concentration was found to be a mean ± SD of 28.0 ± 8.0 μg/ml, minimum serum concentration 3.1 ± 1.8 μg/ml, and half-life 5.2 ± 1.2 h. The systemic clearance was 5.6 ± 1.8 l/h and the volume of distribution at steady state 36.8 ± 6.3l. The probability of achieving a target trough concentration of 6 μg/ml or greater was increased by greater doses of LEV, but it also increased the probability of reaching trough concentration greater than 20 μg/ml. The highest probability of achieving a target trough concentration of 6–20 μg/ml was when 1,000 mg of LEV every 8 h and 1,500–2,000 mg every 12 h. The authors concluded that the LEV systemic clearance was faster and the terminal elimination half-life was shorter in neurocritical care patients than in previously reported results in adults in status epilepticus or healthy volunteers. The study limitation is the sample size (23).

Klein et al. conducts a fixed dose, open-label, non-randomized, phase II safety, and PK study of patients, including children ≥6 years old, treated with levetiracetam after TBI with a high risk of post-traumatic epilepsy. A total of 26 children and 15 adults are enrolled, whose ages range from 6 to 87 years. TBI inclusion criteria are any intracranial hemorrhage, except for isolated subarachnoid hemorrhage or with penetrating wound injury, depressed skull fracture, or early PTS. Beginning ≤8 h after injury and lasting for a duration of 30 days, all subjects receive levetiracetam 55 mg/kg/day orally, nasogastrically, or intravenously. The initial dose is followed by two divided doses every 12 h for the study duration. On treatment days 3 and 30, all 41 subjects undergo PKs analysis. Thirty-six of 41 subjects are randomized to undergo PK study on treatment day 3, and 24/41 subjects are randomized to undergo PK study on day 30. Mean T_{max} on day 3 is 2.2 h, C_{max} was 60.2 μg/ml and area under the curve (AUC) is 403.7 μg/h/ml. T_{max} is shorter in children than in adults and elderly subjects (respectively, 1.5 and 1.8 vs. 5.96 h; $p = 0.0001$). Compared with adults and the elderly, the AUC is non-significantly lower in children (461.4 and 450.2 vs. 317.4 μg/h/ml). C_{max} is non-significantly higher after administration IV (0.4 μg/ml) vs. tablet (59 μg/ml) or NG (48.2 μg/ml). AUC of IV and NG administration is 88 and 79% of the AUC of oral administrations. Between days 3 and 30, the PKs are not significantly different. The authors conclude that TBI study subjects with a high PTS risk are treated with the same dose with antiepileptogenic effect in animals (55 mg/kg/day) achieve plasma LEV levels comparable to those in animal studies (24).

FUTURE DIRECTIONS

The available data have shown limitations and one cannot clearly establish the efficacy and tolerability of intravenous levetiracetam over phenytoin in early seizure prevention in severe TBI. Phenytoin still remains the drug of choice based on the existing evidence and wide availability of PHT titration makes it easy to be managed safely. However, based on the existing data, levetiracetam seems to be a favorable choice for early seizure prophylaxis in patients with severe TBI. Levetiracetam can be used in situations where there is risk of drug–drug interactions and drug toxicity because of the narrow therapeutic index of phenytoin. However, further larger, prospective, randomized double blind multicenter trials are needed to further define the role of this anticonvulsant in short and long term seizure prophylaxis in patients with severe TBI.

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Neurobehavioral effects of levetiracetam in patients with traumatic brain injury

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Moderate to severe traumatic brain injury (TBI) is one of the leading causes of acquired epilepsy. Prophylaxis for seizures is the standard of care for individuals with moderate to severe injuries at risk for developing seizures, though relatively limited comparative data is available to guide clinicians in their choice of agents. There have however been experimental studies which demonstrate potential neuroprotective qualities of levetiracetam after TBI, and in turn there is hope that eventually such agents may improve neurobehavioral outcomes post-TBI. This mini-review summarizes the available studies and suggests areas for future studies.

Keywords: neurobehavioral effects, levetiracetam, traumatic brain injury, neurological functioning, neurobehavioral outcomes

INTRODUCTION

Traumatic brain injury (TBI) refers to a spectrum of neurological injury resulting from external forces applied to the brain that cause changes in neurological functioning ranging from a brief alteration of consciousness (known as a mild TBI or concussion) to severe TBI which is marked by extended periods of coma or altered consciousness (1). The severity of the initial injury is a fairly reliable predictor of neurobehavioral outcomes, with more severe injuries likely leading to permanent neurobehavioral impairments (2). For individuals who survive the acute phase of a moderate to severe TBI, a period of functional recovery occurs for up to 2 years post-injury (3). Despite the potential for recovery, those who survive moderate to severe TBI frequently experience a host of persistent neurobehavioral symptoms, such as cognitive deficits, difficulties with social judgment, fatigue, and mood changes (4).

In addition to the permanent neurobehavioral problems that can result from moderate to severe TBI, 5–20% of patients who sustain a severe TBI will develop seizures, a phenomenon known as post-traumatic epilepsy (PTE) (5, 6). Injury characteristics impact risk for PTE, with 50% of patients sustaining penetrating head injuries developing seizures, while closed head injuries appear less likely to develop such complications (7). The presence of visible contusions, hemorrhages, longer coma duration, and older age all also increase the risk for PTE (8).

Post-traumatic epilepsy may account for up to 5% of all cases of epilepsy in general (6). It is unclear which if any neurophysiological markers are most strongly associated with outcomes, as well as the development of PTE (9, 10), but there is increasing evidence that PTE is associated with worse functional outcomes in general (11). Experimental models have revealed that the post-injury time period is marked by a host of excitatory neurochemical changes as well as structural brain changes such as cellular loss and changes in organization which may foster the development of PTE (12). Therefore, the acute stage presents an opportunity to intervene prophylactically with anti-epileptic medications in hopes of preventing or limiting seizure activity. Although there remains debate about whether prevention of seizures in the acute post-TBI time period actually prevents the development of epilepsy over longer time periods (13), current guidelines recommend anti-epileptic drug (AED) use in at risk patients during the first 7 days post-injury to prevent acute seizures (14).

The choice of AED agent utilized in severe TBI cases has begun to shift over the years. Because of its availability in intravenous format and clinical utility, phenytoin (PHT) was historically utilized for PTE prophylaxis, despite its need for ongoing clinical monitoring and potential for serious adverse side effects (15). However, since levetiracetam (LEV) became available in an intravenous formulation, it has been increasingly utilized because it requires no loading dose or ongoing monitoring (16). Recent meta-analysis (17) and clinical data (18) suggest that both agents are equally effective in preventing post-traumatic seizures during the first 7 days post-injury, though to date no data is available to indicate the agents ability to prevent PTE.

Although there are encouraging findings for LEV's use to prevent acute seizures following TBI, the agents impact on neurobehavioral outcomes has been relatively unexplored. As noted above, moderate to severe TBI by itself is associated with a host of neurobehavioral symptoms which vary in severity from patient to patient and evolve over the course of recovery. Memory impairments, difficulties with executive functioning and social regulation, fatigue, depression, and irritability/aggression are common post-TBI sequelae (4). Given that some of these symptoms have been associated with AED use in general (19, 20), it is important to fully understand any potential interactive or additive effects in the TBI population in an attempt to avoid or mitigate any untoward clinical outcomes.

NEUROBEHAVIORAL IMPACTS OF LEV IN POPULATIONS OTHER THAN TBI

Levetiracetam has proven to be a popular agent in many neurological populations, in part because it has been relatively well-tolerated from a neurobehavioral standpoint. However, a review of the evidence from epilepsy samples suggests that LEV treatment is associated with changes in emotional functioning. Specifically, studies are suggestive of increased aggression and possibly suicidality, especially in individuals with premorbid depression or behavior problems (21–23). In children, there is some evidence that LEV use may be particularly associated with untoward behavioral outcomes. Schiemann-Delgado studied LEV in children with partial onset seizures and found that LEV was associated with stable cognitive performance versus placebo, but also mild neurobehavioral adverse effects, including increased aggression and irritability (24).

Summarizing the available data, Mbizvo and colleagues suggested that in patients with epilepsy, LEV add on treatment was associated with increased somnolence, changes in behavior in 23% of children studied (but few adults), and no significant impact on cognition (25).

From a neuropsychological standpoint, the medication seems well-tolerated. In a small (16 subject) but well-designed experiment involving healthy controls, LEV had cognitive and electrophysiological effects comparable to that of placebo, suggesting that at least over the short run in healthy subjects, it had little adverse neurobehavioral impact (26). LEV may even provide some cognitive benefit in select populations. For example, LEV has been associated with improved memory in patients with high grade gliomas (27, 28). Similarly, in a retrospective review of patients with a history of intracranial hemorrhage, patients treated with LEV were discharged home more often, had higher Glasgow coma scale (GCS) scores, and demonstrated a trend toward better global cognitive status (defined as oriented and cooperative versus not) (29).

THE INTERACTION OF LEV AND TBI ON NEUROBEHAVIORAL OUTCOMES

In contrast to other populations such as epilepsy or general neurosurgery patients, the study of the neurobehavioral profile of LEV in TBI is still in its infancy, with most data culled from recent efficacy studies. These studies tend to utilize relatively broad self or caregiver reports of neurobehavioral changes with little formal cognitive testing or more granular assessments of neurobehavioral outcomes.

What information is available, based largely on a series of papers from the same study suggests an increase in fatigue with LEV use in TBI during the acute phase. Klein et al published data on the pharmacokinetics of a PHT + LEV treatment arm and noted that around 3% of subjects discontinued the LEV secondary to somnolence (30). In a follow-up safety study, Klein and colleagues (31) found approximately 15% of their sample reported fatigue, somnolence, and headache, with most of these symptoms reported as mild in nature.

Pearl and colleagues (32) recently published data specifically evaluating the pediatric subjects from the aforementioned study, followed over 2 months and later 2 years. This study included measures of problematic behavior and depression, and interestingly there was no difference between LEV treated patients and controls on these measures. However, during active treatment, LEV patients showed higher rates of headache, fatigue, drowsiness, and irritability. Eighty-five percent of patients complained of fatigue, but only 5% rated it as severe. One patient had a psychosis which resolved with LEV discontinuation. While fatigue may be considered a minor side effect, it may interfere with participation in brain injury rehabilitation, which in turn could lead to other untoward outcomes in a TBI population, and this warrants further investigation. For example, Nair and Kadies (33) published a case study of an older individual participating in rehabilitation for a TBI who was having persistent sleep wake cycle disorder and agitation. While these symptoms had been attributed to his TBI, after removing LEV he gradually resumed a normal sleep wake cycle and had less agitation which in turn led to better participation in rehabilitation.

At a more global level, there is some evidence for better neurobehavioral outcomes in both the short and long term with LEV versus PHT. Szarflarski et al (34) studied a group of 52 patients the majority of which suffered a severe TBI in a randomized single-blinded study comparing PHT and LEV. They included global outcome measures including the disability rating scale (DRS) and Glasgow outcome scale (GOS), which assess in a broad way neurobehavioral status. In this study, there was no difference in seizure outcomes over both short term and long term outcome, and similar rates of mortality in each group. Side effect profiles were similar between groups, with LEV patients having fewer instances of a decrease in neurological status and fewer gastrointestinal problems. Most notably, the LEV patients demonstrated a statistically significant lower (better) score on the DRS and a higher (better) GOS score than their PHT matched controls. In contrast to these findings though, Jones and colleagues (35) found similar 3 and 6 months GOS outcomes when comparing PHT and LEV, and noted that their LEV patients had stronger tendencies to seizure activity on EEG (but no greater increase in seizures). Thus the potential for an actual neurobehavioral benefit to LEV use in post-TBI care remains to be definitively established.

Unfortunately at the time of this writing no studies were found which specifically evaluated the neurobehavioral impact of LEV in the chronic phase of the recovery or in individuals who had developed PTE.

POTENTIAL FOR NOVEL THERAPEUTIC USES

While current work has focused on LEV as a prophylactic agent for PTE, there is a history of laboratory work as well as clinical observations suggesting LEV is a neuroprotective agent which may improve behavioral outcomes even in the absence of seizure activity. As noted above, LEV use was associated with better cognitive outcomes in brain tumor patients (27), and one study revealed LEV to be associated with improved global outcome (including neurobehavioral functioning) in severe TBI cases (34). Similarly, in the suspected prodromal phase of Alzheimer's disease (amnestic mild cognitive impairment) LEV use was associated with reduction in hippocampal activity and paradoxically, improved cognition (36). The authors suggest that increased hippocampal activation

may be a sign of potentially damaging overactivation of the brain, and that LEV may reduce this and thus preserve neurons.

Consistent with this finding, in a rat model of TBI involving controlled cortical impacts, animals treated early with LEV versus a saline control had improved motor function, increased exploratory behavior, better preserved hippocampal cells, and reduced total volume of contusions (37). The authors propose that despite LEV still not having a fully elucidated mechanism of action for the prevention of seizures, its ability to upregulate glutamate transporters may lead to increased neuroprotection as well as improved anti-epileptic impact. A similar study conducted by Wang and colleagues (38) demonstrated a similar pattern of neuroprotective effects that were not present in animals treated with fosphenytoin. If replicated and extended to humans, such a finding would support the use of LEV not just to prevent seizures but also to prevent the secondary damage of excitotoxicity in the peri-injury period.

DIRECTIONS FOR FUTURE RESEARCH

Studying neurobehavioral phenomena in TBI is a complex endeavor, given the heterogeneity of initial injury, different recovery courses, and the difficulty of measuring complex phenomena such as mood, cognition, and behavior. Partialing out the impact of a medication such as LEV from the disorder itself which can result in many of the same symptoms will require careful study design. A well-designed study to evaluate the neurobehavioral impacts of LEV would have to include control and treatment groups which are carefully randomized or matched to control for the impact of variability in initial injury severity, time since injury, and relevant demographic and other medical factors (for example controlling for the presence of other neurobehaviorally active drugs such as anti-depressants and pain medications). Given the difficulty of relying on self-report in patients with potential impairments in cognition and self-awareness, multi-modal assessment end points, including neuropsychological testing and informant ratings will be necessary to adequately capture the phenomena of interest. Electrophysiological markers may be helpful to quantify the nature and extent of physiological impact of LEV in this population, and imaging techniques to quantify the interaction of specific structural abnormalities and medication effects would also be intriguing.

While much research remains to be done on establishing the efficacy or superiority of LEV for seizure prophylaxis post severe TBI, future studies may also want to move toward studying LEV as an adjunctive neuroprotective agent. Adding a longitudinal neurobehavioral component to an acute LEV vs. placebo or active control study with more granular neurobehavioral ratings for each stage of recovery (i.e., time to follow commands in the acute phase, ranging to neuropsychological evaluations later in the recovery course) would allow for evaluating LEV as a potential neuroprotective agent. Naturalistic studies which look at the subset of a sample who continue LEV treatment beyond the current 7 days window may also yield insights into a potential benefit from this medication. Finally, functional neuroimaging may provide insight into how LEV alters the functional activation and connectivity of the recovering brain, unlocking the mechanisms into its neurobehavioral impact.

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Levetiracetam differentially alters CD95 expression of neuronal cells and the mitochondrial membrane potential of immune and neuronal cells *in vitro*

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Epilepsy is a neurological seizure disorder that affects over 100 million people worldwide. Levetiracetam, either alone, as monotherapy, or as adjunctive treatment, is widely used to control certain types of seizures. Despite its increasing popularity as a relatively safe and effective anti-convulsive treatment option, its mechanism(s) of action are poorly understood. Studies have suggested neuronal, glial, and immune mechanisms of action. Understanding the precise mechanisms of action of levetiracetam would be extremely beneficial in helping to understand the processes involved in seizure generation and epilepsy. Moreover, a full understanding of these mechanisms would help to create more efficacious treatments while minimizing side-effects. The current study examined the effects of levetiracetam on the mitochondrial membrane potential of neuronal and non-neuronal cells, *in vitro*, in order to determine if levetiracetam influences metabolic processes in these cell types. In addition, this study sought to address possible immune-mediated mechanisms by determining if levetiracetam alters the expression of immune receptor–ligand pairs. The results show that levetiracetam induces expression of CD95 and CD178 on NGF-treated C17.2 neuronal cells. The results also show that levetiracetam increases mitochondrial membrane potential on C17.2 neuronal cells in the presence of nerve growth factor. In contrast, levetiracetam decreases the mitochondrial membrane potential of splenocytes and this effect was dependent on intact invariant chain, thus implicating immune cell interactions. These results suggest that both neuronal and non-neuronal anti-epileptic activities of levetiracetam involve control over energy metabolism, more specifically, m$\Delta\Psi$. Future studies are needed to further investigate this potential mechanism of action.

Keywords: epilepsy, Keppra, splenocytes, C17.2, *in vitro*, Fas, FasL

INTRODUCTION

Epilepsy is a neurological disorder that affects approximately 65 million people worldwide. While numerous treatment options are available to control seizures associated with epilepsy, approximately 20–30% of epileptic patients are resistant to treatment. Pharmaceutical and biomedical device companies continue to develop new treatments that are more efficacious, while minimizing undesirable side-effects. One such drug, levetiracetam, alone as a monotherapy, or combined with another treatment as an adjunct, is widely used to control partial onset and generalized seizures (1). Despite its use as a relatively safe and effective anti-convulsant treatment option, the precise mechanism(s) of action are not fully understood.

The anti-epileptic effects of levetiracetam may occur, at least in part, by acting directly on neurons. For example, levetiracetam is known to bind to the synaptic vesicle protein SV2A (2) and to inhibit presynaptic calcium channels (3). Studies have shown that levetiracetam may also exert its anti-epileptic effects by reducing calcium currents in CA3 pyramidal neurons of the hippocampus (4), or by rescuing neurons in the hippocampus and dentate gyrus from death (5). Another possibility is that levetiracetam alters mitochondrial membrane potential (m$\Delta\Psi$), although the reports using the *in vivo* perforant pathway stimulation paradigm are conflicting. In one study, levetiracetam effectively mitigated mitochondrial dysfunction in the hippocampus following established *status epilepticus* (6), but not at acute time points after the onset of *status epilepticus* (7). Thus, it is unclear if, or to what extent, the therapeutic effects of levetiracetam can be attributed to its neuronal interactions.

While the purpose of the current study is to determine if there are direct effects of levetiracetam on neuronal and immune cells, other studies have suggested glial cell mechanisms of action. For example, Ueda et al. (8) suggested that levetiracetam exerts its neuroprotective effects through its actions on glial cells. Similarly, Haghikia et al. (9) showed that levetiracetam has anti-inflammatory effects on astrocytes. Consistent with this finding,

Kim et al. (10) showed that levetiracetam reduced gliosis in epileptic brains and inhibited IL-1B, and Stienen et al. (11) showed that the anti-inflammatory effects of levetiracetam on astrocytes may be mediated by TGFβ1.

In addition to effects in the CNS, levetiracetam could also be exerting its effects in the periphery. Supporting this notion are the results of a previous study demonstrating that levetiracetam inhibits the function of some $CD8^+$ T Lymphocytes (12). Such interactions with the peripheral immune system might explain the increased incidence of pharyngitis and rhinitis in levetiracetam-treated patients (13–18). However, studies are lacking that provide a thorough analysis of the effects of levetiracetam on peripheral immune cells.

Understanding the mechanism(s) of action of levetiracetam is important because this knowledge could lead to more efficacious treatments and better understanding of the epileptic condition. Due to the lack of a unified theory for the mechanism(s) of action, the current study was designed to determine if levetiracetam affects the $m\Delta\Psi$ of peripheral immune cells and neuronal cells. Moreover, this study sought to address possible immune-mediated mechanisms by determining if levetiracetam alters the expression of immune receptor–ligand pairs.

MATERIALS AND METHODS

CELL LINES

C17.2

The C17.2 cell line is an immortalized mouse neural progenitor cell line capable of differentiation *in vitro*. The cell line was established by retroviral-mediated transduction of the avian *myc* oncogene into mitotic progenitor cells of neonatal mouse cerebellum from a CD1 × C57BL/6 mouse. The C17.2 line of neural stem cells responds to NGF by differentiating into more mature neuronal phenotypes and has been used extensively to monitor developmental regulation of mouse neurons (19). We employed this cell line as a model of mouse neuronal cells.

In vitro stimulations

C17.2 cells were either untreated, or treated with nerve growth factor (NGF) at 0.4 nM final concentration. All cells were treated with levetiracetam or vehicle for 48 h, at the following concentrations: 0.5 μm, 15 μm, 0.15 mM, or 1.5 mM.

Mice

Eight- to ten-week-old C57BL/6J male mice were purchased from Jackson Labs. Invariant chain (CD74)-deficient mice (Ii^{Def}) (C57BL/6 background) were purchased from Jackson Labs and bred at the Scott and White Healthcare animal facility to maintain homozygosity. Mice were housed in the Scott and White Healthcare animal facility according to IACUC regulations.

Spleen cell isolation

Mice were sacrificed and spleens were removed. Splenocytes were dissociated by passing spleens through 40 μm cell strainers. Red blood cells were lysed using GEY'S buffer (20). Cells were then cultured at 1.010^6 cells/mL in 6 well plates. Cells were grown in RPMI 1640 (Invitrogen) supplemented with 5% fetal bovine serum (Invitrogen) in a humidified 5% CO_2 incubator at 37°C for the designated time period. Splenocytes were then treated with levetiracetam or vehicle for 48 h, at the following concentrations: 0.5 μm, 15 μm, 0.15 mM, or 1.5 mM.

Flow cytometry

For cell surface markers the cells were first blocked with FC Block (BD Bioscience) and then stained with the following antibodies; MHCII, CD3ε, CD80, CD86, Fas (CD95), and CD178 (BD Bioscience). Cells were analyzed using a BD FACS Canto II flow cytometer and the data was analyzed using FlowJo software (TreeStar Inc.).

Mitochondrial membrane potential ($M\Delta\Psi$)

To assess the possibility that levetiracetam has direct effects on mitochondrial function, mitochondrial activity was assessed using MitoTracker Red CM-H2XRos (Life Technologies), a mitochondrial dye that fluoresces as a function of $m\Delta\Psi$. Tightly regulated $m\Delta\Psi$ is essential for maintaining physiological function(s), including appropriate mitochondrial substrate selection for generating ATP and for maintaining cell viability. Cells were treated with MitoTracker Red and allowed to incubate in the dye for 20 min prior to analysis using a BD FACS Canto II flow cytometer. The flow cytometer measures mean fluorescent intensity per cell. Cells were untreated, treated with NGF, treated with levetiracetam or NGF + levetiracetam, as described above in the *in vitro* stimulation. For each treatment group, a minimum of four separate assays were performed in triplicate.

Lysosomal acidity

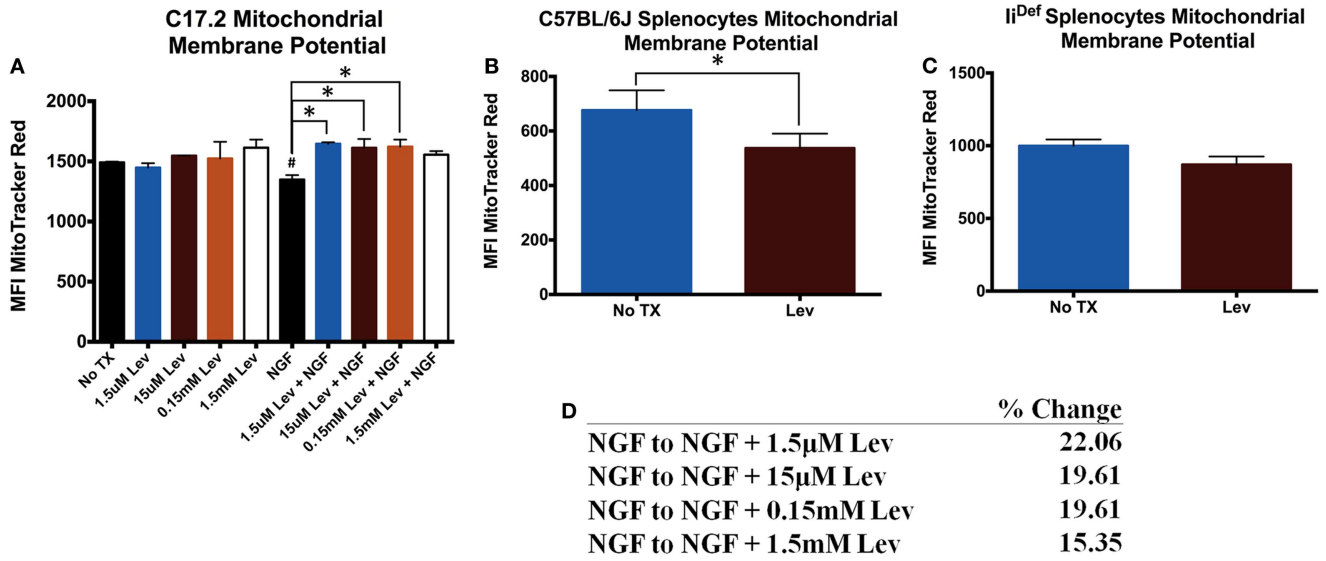
To assess the effects of levetiracetam on lysosomal pH, we used the fluorescent dye Lysosensor Green (Life Technologies). Lysosensor Green produces increased fluorescence intensity at lower pH. Cells were analyzed using a BD FACS Canto II flow cytometer.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software Inc.). For comparisons between splenocytes from C57BL/6J and Ii^{Def}, a paired *t*-test was used with a significance cut-off of $P < 0.05$. For all other analysis, repeated measures ANOVA was used with *post hoc* planned comparisons using Dunnett's correction factor.

RESULTS

Previous studies have indicated mitochondrial differences in the presence of levetiracetam (6, 7). Therefore, we determined if these differences were specific for neuronal or immune cells. Analysis of $m\Delta\Psi$ in C17.2 cells revealed no significant differences in the absence of NGF (**Figure 1A**). In the presence of NGF, levetiracetam resulted in a significant increase (**Figure 1A**) in $m\Delta\Psi$ at all concentrations tested (1.5 μm. $p < 0.03$; 15 μm, $p < 0.05$; 0.15 mM, $p < 0.04$; 1.5 mM, NS). It is pertinent to note that treatment with levetiracetam did not cause any observable alterations to the morphology of the C17.2 cells, either with or without NGF (data not shown). In contrast to the increased $m\Delta\Psi$ in the presence of NGF and levetiracetam, the impact of levetiracetam on spleen cells (**Figure 1B**) was a significant reduction in $m\Delta\Psi$ ($p < 0.007$). This reduction appeared to be invariant



D

	% Change
NGF to NGF + 1.5μM Lev	22.06
NGF to NGF + 15μM Lev	19.61
NGF to NGF + 0.15mM Lev	19.61
NGF to NGF + 1.5mM Lev	15.35

FIGURE 1 | Changes in mitochondrial membrane potential following treatment with levetiracetam. **(A)** Mean fluorescence intensity (MFI) of Mitotracker Red as a measure of relative mitochondrial membrane potential in C17.2 cells at 48 h post treatment with NGF with or without levetiracetam (Lev). **(B)** MFI Mitotracker Red in C57BL/6 splenocytes 48 h after treatment with or without 0.15 mM Lev. **(C)** MFI Mitotracker Red in in Ii^{Def} splenocytes 48 h after treatment with or without 0.15 mM Lev. **(D)** Table depicting percent change from NGF treatment alone, compared to NGF treatment in the presence of doses of levetiracetam. *Denotes a p-value < 0.05.

chain dependent, as splenocytes from mice deficient in invariant chain showed no significant changes in $m\Delta\Psi$ in response to levetiracetam (**Figures 1C,D**).

Elevated $m\Delta\Psi$ can be associated with elevated CD95 (21, 22). Therefore, to address the possibility that levetiracetam alters receptor–ligand pairs on neurons, we used the mouse neuronal stem cell line, C17.2, which can be differentiated in the presence of NGF. We assessed CD95, a member of the BGF superfamily and its ligand, FasL (CD178) to determine if levetiracetam can influence cell proliferation, differentiation, and survival. We also examined alterations in the co-stimulatory molecules B7.1 (CD80), or B7.2 (CD86) to assess the potential of levetiracetam to alter co-stimulation of T cell activation. The results from analysis of C17.2 cells revealed that levetiracetam treatment alone had no significant effects on CD95 (**Figure 2A**), CD178 (**Figure 2B**), CD80 (**Figure 2C**), or CD86 (**Figure 2D**). In the presence of NGF, no significant differences were observed for CD95, CD178, CD80, or CD86 at the 1.5 or 15 μm concentrations. However, at 0.15 and 1.5 mM, a significant increase in CD95 ($p < 0.02$ and $p < 0.001$, respectively) was observed. At these latter two concentrations, no significant differences were observed for CD80 or CD86. For CD178, no significant differences were observed for the three lowest concentrations of levetiracetam, but at the 1.5 mM concentration, a significant increase was observed for CD178 ($p < 0.05$).

In addition to examining neuronal cells, we also examined peripheral immune cells from the spleen. We examined numbers of T cells and numbers of $MHCII^+$ cells (which includes macrophages and B cells), as well as CD95 expression on these cells. The results showed that levetiracetam treatment resulted in no significant effect on the number of $CD3^+$ T cells (**Figure 3A**), $MHCII^+$ (**Figure 3B**) cells, nor on the levels of CD95 expression by T cells (**Figure 3C**), and non-T cells (**Figure 3D**). In addition, we examined overall levels of MHCII and CLIP on non-T cells (**Figures 4A,B**) to address the possibility that levetiracetam alters immunogenicity of peripheral immune cells. No changes were observed for either of these variables (**Figures 4A,B**). To further detect levetiracetam-induced changes in processing or presentation by immune cells, we assessed lysosomal acidity and found no significant changes (**Figure 4C**).

DISCUSSION

Levetiracetam is well established as a beneficial anti-seizure medication and as an adjunct to other anti-seizure medications. The molecular mechanisms accounting for the efficacy of levetiracetam for seizure activity are largely unknown. The results from the present study suggest that levetiracetam induces expression of CD95 and CD178 on NGF-treated C17.2 neuronal cells. The results also demonstrate that the increased $m\Delta\Psi$ in response to levetiracetam on C17.2 neuronal stem cells requires the presence of NGF. This is likely due to the differentiating effect of NGF on neural stem cells. In contrast, the study shows that levetiracetam lowers the $m\Delta\Psi$ of splenocytes and this effect is dependent on intact invariant chain. These results suggest that both neuronal and non-neuronal anti-epileptic activities of levetiracetam involve control over energy metabolism, more specifically, $m\Delta\Psi$.

Epilepsy has traditionally been considered primarily a neuronal disease. Growing evidence also implicates astrocytes, microglia, peripheral leukocytes, and blood–brain barrier breakdown in the pathogenesis of epilepsy. Here, we show two novel observations

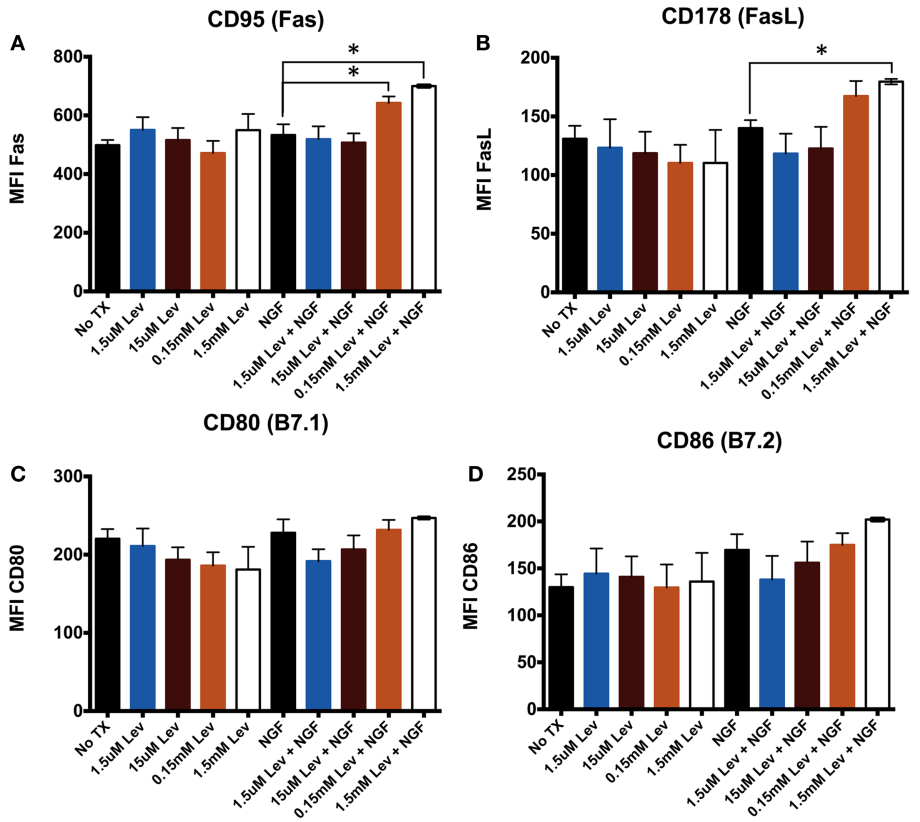


FIGURE 2 | Levetiracetam alters cell surface Fas expression on C17.2 cells. Mean flouresecence intensity (MFI) as measure of relative expression level of **(A)** CD95, **(B)** CD178, **(C)** CD80, and **(D)** CD86 48 h after treatment with or without Lev. *Denotes a p-value < 0.05.

that potentially link peripheral leukocytes to neurons. Our results suggest that levetiracetam affects mitochondrial energy metabolism as reflected by changes in $m\Delta\Psi$. Interestingly, these changes are inversely related when comparing splenocytes to NGF-treated neuronal stem cells. That is, levetiracetam causes a statistically significant increase in the membrane potential of NGF-treated C17.2 cells and a significant decrease in $m\Delta\Psi$ of levetiracetam-treated spleen cells. It is pertinent to note that in mice deficient for CD74, the levetiracetam-induced change to splenocyte $m\Delta\Psi$ was ameliorated. Thus, it is possible that levetiracetam-induced changes in mitochondrial activity result from cell–cell contact because CD74 can be expressed on the cell surface and mediate interactions with other cells through its cognate ligand, CD44. Alternatively, the requirement for CD74 to see the effects.

Previous studies have suggested that mitochondrial dysfunction contributes to the epileptic condition (23). The putative functional significance of levetiracetam-induced alterations to $m\Delta\Psi$ in the epileptic brain is its known effects on proton transport. Previous studies have demonstrated that alterations to the $m\Delta\Psi$ in neurons (24) and non-neuronal cells (25), directly influences ion concentrations in the cytosol, thereby influencing plasma membrane potential. Alterations to $m\Delta\Psi$ have also been shown to influence oxidative stress (26), which may be another anti-epileptic mechanism. A third potential mechanism through which altered $m\Delta\Psi$ could influence seizures is by altering the cytosolic pH. An acidification of cytosol as a result of protonation is known to hyperpolarize the plasma membrane (27), which may raise the seizure threshold. Support for this latter suggestion is observed in epileptic hippocampal slices where the pattern of epileptic activity corresponds to $m\Delta\Psi$ and ion concentration (23).

Previous work from our lab demonstrated that Fas/FasL interactions can facilitate neurite outgrowth subsequent to nerve crush injury (28). CD95 and its ligand CD178, a member of the NGF/NGF receptor superfamily of death-inducing receptor–ligand pairs. Many members of this superfamily are involved in cell fate decisions including cell death, cell proliferation, and differentiation. We addressed the possibility that if levetiracetam altered mitochondrial activity, it might also affect CD95 expression because elevated $m\Delta\Psi$ can be associated with elevated CD95. The results from the current study are consistent with this idea because we found that in neuronal cells, in the presence of NGF and >0.15 mM levetiracetam, $m\Delta\Psi$ is increased as is Fas expression. Therefore, levetiracetam may be involved in stabilizing $m\Delta\Psi$ in the presence of elevated levels of NGF.

Another potential effect of levetiracetam on leukocytes could be related to some of the side-effects associated with levetiracetam. In particular, an increased incidence of pharyngitis and rhinitis has been observed in levetiracetam-treated patients (13–18). These

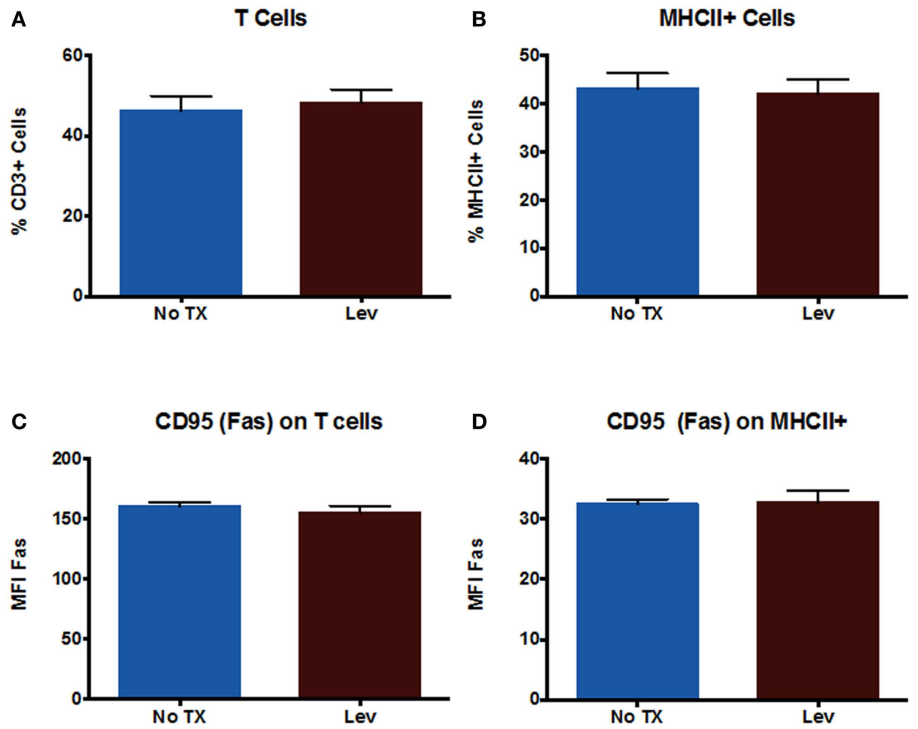


FIGURE 3 | Levetiracetam does not Alter Immune Cells *in vitro*. The percentage of **(A)** T cells and **(B)** MHCII$^+$ cells in splenocytes 48 h after treatment with or without 0.15 mM Lev. Mean fluorescence intensity (MFI) as measure of relative expression level of CD95 on **(C)** T cells and **(D)** MHCII$^+$ cells in splenocytes 48 h after treatment with or without 0.15 mM Lev. *Denotes a p-value < 0.05.

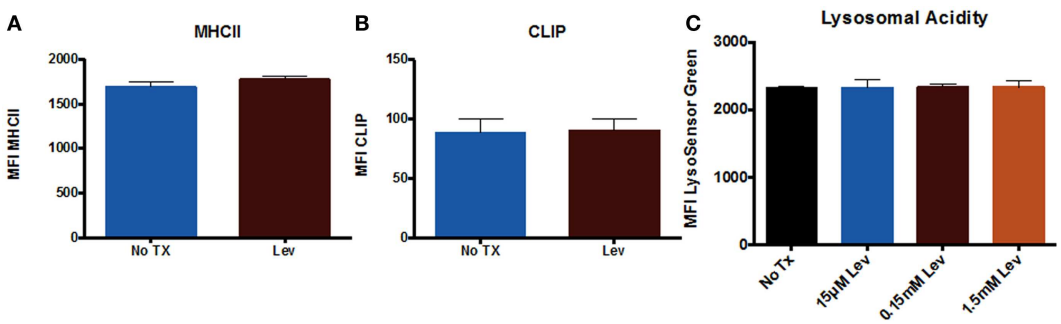


FIGURE 4 | Levetiracetam does not alter antigen processing and presentation machinery *in vitro*. Mean fluorescence intensity (MFI) as measure of relative expression level of **(A)** MHCII$^+$ and **(B)** CLIP on splenocytes 48 h after treatment with or without Lev. **(C)** MFI Lysosensor Green as a relative measure of lysosomal acidity of splenocytes 48 h after treatment with Lev. *Denotes a p-value < 0.05.

findings are consistent with a role for alterations to an effective immune response that may involve alterations in CD74 expression and function. A second possibility is related to the finding of reduced m$\Delta\Psi$ in splenocytes, which may reflect altered levels of immune function, including increased inflammation accounting for pharyngitis and rhinitis.

Overall, the data from the current study indicate that levetiracetam differentially affects the m$\Delta\Psi$ of neuronal C17.2 and non-neuronal splenocytes. The results also show that in the presence of elevated NGF, neuronal C17.2 cells express CD95 and CD178. The results from this study could help to explain some of the mechanisms of action of levetiracetam, including some of its side-effects. More studies are needed to better understand the implications of these findings so that more efficacious treatments with minimal side-effects can be developed.

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