Ultrasonic enhancement of drug penetration in solid tumors

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Increasing the penetration of drugs within solid tumors can be accomplished through multiple ultrasound-mediated mechanisms. The application of ultrasound can directly change the structure or physiology of tissues or can induce changes in a drug or vehicle in order to enhance delivery and efficacy. With each ultrasonic pulse, a fraction of the energy in the propagating wave is absorbed by tissue and results in local heating. When ultrasound is applied to achieve mild hyperthermia, the thermal effects are associated with an increase in perfusion or the release of a drug from a temperature-sensitive vehicle. Higher ultrasound intensities locally ablate tissue and result in increased drug accumulation surrounding the ablated region of interest. Further, the mechanical displacement induced by the ultrasound pulse can result in the nucleation, growth and collapse of gas bubbles. As a result of such cavitation, the permeability of a vessel wall or cell membrane can be increased. Finally, the radiation pressure of the propagating pulse can translate particles or tissues. In this perspective, we will review recent progress in ultrasound-mediated tumor delivery and the opportunities for clinical translation.

Keywords: ultrasound, sonoporation, vascular permeability, tumor penetration, enhanced drug delivery

THE PROBLEM

The goal of this Frontiers issue is to explore methods to enhance the penetration of drugs within solid tumors. Combining ultrasound with a drug does indeed have the potential to enhance delivery; however, due to the requirement to guide the beam to the tumor such treatment will be a possibility only for localized primary tumors and well-characterized metastases that are accessible to sound waves. Ultrasound is easily directed to superficial organs such as the breast and prostate, as well as most abdominal organs, and has also been applied in the treatment of brain tumors. The effects of high intensity ultrasound on biological tissue, and particularly on the central nervous system, have been recognized for more than 70 years; the ability to heat and ablate tissue was described initially (1–5). Within studies in the 1940s and 1950s, ultrasound was also determined to have non-thermal effects on tissue, typically characterized as mechanical effects (6). The mechanical effects of ultrasound can act directly upon the tumor tissue or on injected microbubbles whose oscillations enhance vascular or cell membrane permeability. Although early studies were not geared toward drug delivery, these same mechanisms of high temperature ablation or mild hyperthermia can increase drug accumulation within a lesion and lesion boundary. In recent strategies, the increased temperature is applied to influence both the tissue and the drug capsule.

ENHANCED EXTRAVASATION OF NANOTHERAPEUTICS THROUGH MECHANICAL AND THERMAL EFFECTS ON TISSUE

The direct effects of ultrasound on tissue and vasculature have been reported to enhance the extravasation of antibodies and nanotherapeutics (7–9). In some cases, the mechanical effects of ultrasound have been shown to enhance therapeutic penetration.

With a center frequency of 1 MHz ultrasound at a peak negative pressure (PNP) of 8.95 MPa, antibody penetration has been shown to be enhanced at the tumor periphery, presumably through mechanical effects (8). The compression and rarefaction resulting from the ultrasound wave can produce the nucleation, growth, and collapse of gas bubbles within tissues. Such cavitation is assumed to facilitate transport within tumor tissue.

The thermal dose delivered by ultrasound is typically measured in cumulative equivalent minutes at 43°C (CEM 43) which is defined as $tR^{(43-T)}$, with t being the time of treatment, T the average temperature during treatment, and R a constant that equals 0.25 for temperatures between 37 and 43°C and 0.5 above 43°C (10, 11). Hyperthermia has been demonstrated to increase tumor blood flow and microvascular permeability (12). While it has long been recognized that heat increases the accumulation of small particles in the heated region of interest, the typical protocol has involved 1 h or more of heating. However, by combining the mechanical and thermal effects of ultrasound, enhanced delivery has been achieved with a shorter treatment (13). In such studies, the temperature goal is ∼41–42°C and insonation continued for ∼5–20 min. As a result of hyperthermia and the mechanical effects of ultrasound, we have observed that the accumulation of liposomes in an insonified tumor can be increased up to threefold to as much as 22%ID/g. While ultrasound was shown to enhance accumulation in syngeneic murine tumors, the ultrasound parameters that were required to enhance nanoparticle accumulation were shown to differ between epithelial and epithelial-mesenchymal transition (EMT) tumor phenotypes (7). While mild hyperthermia enhanced accumulation in the epithelial tumors, likely through decreased intratumoral pressure and enhanced apparent permeability, higher ultrasound pressure was

required to enhance delivery in the poorly vascularized EMT phenotype. Further, excessive temperature or thermal dose can result in vascular stasis, particularly in highly vascular epithelial tumors. The requirement to personalize the ultrasound parameters to the tumor biology will likely require image guidance to insure clinical success.

In part due to the differing effects of mild hyperthermia with tumor biology, the use of high temperature ablation to enhance delivery has been explored as a methodology that is likely to be generally effective in increasing delivery. While it seems counterintuitive that tissue ablation can greatly enhance accumulation, edema, enhanced blood flow, and increased transport in the region surrounding the ablated site can successfully improve delivery. In our experience, the peak delivery in regions surrounding ablation can exceed 30%ID/g. Also of clinical interest, the hyperthermia surrounding radiofrequency (rf) ablation lesions has been used to enhance local delivery; however, the temperature obtained with such devices ranges from 50 to 90°C (14). Rf ablation has been applied in previous studies to achieve a similar enhanced delivery, and such techniques are now in clinical trials (15, 16). High intensity focused ultrasound similarly enhances delivery surrounding the site of ablation, although combinations of ablation and drug delivery remain primarily under pre-clinical investigation.

RELEASE OF DRUG FROM NANOPARTICLES WITHIN THE VASCULATURE

Nanoparticles that can be triggered to release a small molecule cargo within a tumor have shown the potential to increase both the local concentration of the drug and tumor penetration. Yet, the challenge of developing particles that are stable in circulation and release their cargo upon activation has long been recognized as a major challenge in pharmaceutical development. While many activatable particles are under development (11, 17–23), thermally sensitive liposomes have been frequently combined with ultrasound in recent pre-clinical and clinical studies and will be considered here. In studies of thermally sensitive liposomes, imaging has been used to verify that amphipathic cargo released within the tumor vasculature remains concentrated within the tumor in the region of release (18). We have found that release of drug from such temperature-sensitive vehicles can be highly effective, resulting in a complete response in aggressive murine tumors (unpublished data).

Temperature-sensitive liposomes were initially proposed containing 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) with a phase transition of $T_m = \sim 41°C$ and multiple formulations containing DPPC have been proposed (24, 25). The incorporation of lyso-phospholipids in DPPC-based liposomes decreases the phase transition temperature and speeds the release of the cargo, likely due to the creation of local defects within the lipid bilayer (26). The Thermodox™ formulation, with the incorporation of a lyso-phospholipid, releases at a clinically desirable temperature of ~39°C. The incorporation of the lyso-phospholipids also enhances the ion permeability and drug release rates at the membrane phase transition (27). Unfortunately, using the conventional ammonium sulfate loading, liposomes containing lyso-phospholipids also rapidly release their cargo within the blood pool. As a result, while local delivery can be achieved within tens

of minutes after injection, the dose limiting toxicities of such formulations have typically limited their application to single dose administration. Although the pre-clinical data using Thermodox has been very exciting, this activatable doxorubicin formulation reportedly failed to meet its primary endpoint in the Phase III HEAT Study in patients with hepatocellular carcinoma (HCC). Yet, in spite of this setback, the potential for temperature-sensitive vehicles to have a significant impact on the concentration and penetration of drugs within solid tumors is substantial. Although early clinical studies have typically been limited to one-time treatment, with new formulations repeated treatment should be feasible and the resulting clinical impact enhanced. Multiple alternative formulations have been proposed and compared and have been shown to enhance circulation time (25, 28–30). Alternative strategies using metal-drug complexes, a Brij surfactant and phosphatidylglycerol have been reported to enhance the stability of temperature-sensitive liposomes and are promising alternatives for future investigation. The ultrasound parameters used to enhance delivery with temperature-sensitive liposomes have also varied widely with the center frequency typically ranging from 1 to 3 MHz and duty cycle ranging from ~10 to 100% (20, 31, 32).

MICROBUBBLES

Micron-scale gas bubbles with a stabilizing shell are used in ultrasound imaging to improve imaging of the blood pool and have been widely applied in pre-clinical studies of enhanced drug delivery. The microbubble shell can be coupled to nanotherapeutics, such as liposomes, or coated with a drug (33, 34). The gas core can transport oxygen or other useful gas cargo, although for imaging the gas core is selected to reduce diffusion through the shell material (35). Alternative formulations in which liquid perfluorocarbon particles are injected and change to a gaseous phase *in vivo* have also been shown to have efficacy in the delivery of drugs to solid tumors (36).

Reflections of ultrasound waves from tissue increase in proportion to variations in density and compressibility of the medium and therefore highly compressible gas bubbles produce strong ultrasound echoes. These small bubbles expand and contract in response to ultrasound waves. When driven at a frequency near the resonance frequency that is determined by the size and physical composition of the microbubble, a multi-fold expansion can result. During the subsequent collapse, the velocity of the microbubble wall can reach hundreds of meters per second (37, 38), and the gas core can fragment into a set of small gas particles (39). Also, during microbubble collapse, small jets can impact nearby cell membranes and result in enhanced transport of materials into the cell. *In vitro* studies in phantom materials and *ex vivo* studies within tissues have confirmed that the oscillating microbubble can travel through the vessel wall or can affect the mechanical integrity of the vessel (40–42). Still, such jets affect cells only within a distance on the order of tens of microns. Therefore, the application of microbubbles to alter vascular, rather than tumor cell, permeability is attractive since the vascular concentration is initially high and large numbers of microbubbles are required to effectively change the membrane permeability of a large fraction of cells within a tissue. Within the vasculature, catheters have also been applied to direct streams of bubbles to a

region of interest (43). Also, microbubble-enhanced gene delivery has been widely studied since the biological amplification resulting from transfection is expected to increase the impact of treatment although the protocols have varied (44–48). Within such studies, microbubbles and DNA have been co-injected or combined into a single vehicle and the administration has been intratumoral or intravascular. Typical ultrasound parameters for enhanced tumor gene delivery have included a center frequency on the order of 1 MHz and a low pulse repetition frequency; however, the peak negative ultrasound pressure has varied significantly and successful transfection at higher ultrasound frequencies has also been reported.

The ultrasound parameters used to increase vascular permeability must be chosen carefully as insonation of microbubbles with low ultrasound frequencies has been shown to reduce blood flow (49). A parameter space for safe and effective use of microbubbles for enhancing vascular permeability has been established (41, 50–52). Many parameters, including microbubble dosage and size, the ultrasound center frequency, pulse duration, pulse repetition frequency, and the PNP determine the effect of the oscillating microbubble on the surrounding tissue.

In addition to the formation of jets, physical mechanisms exploited in microbubble-enhanced delivery include radiation forces and microstreaming of fluid (53, 54). Radiation force refers to a mechanism by which oscillating microbubbles or other particles are displaced, most typically in the direction of wave propagation (55–57). This displacement can be used to enhance vascular targeting of a microbubble or microbubble-drug conjugate. Further, local motion of fluid surrounding the oscillating bubble is known as microstreaming and has been shown to increase cellular uptake of therapeutics (53, 54).

One of the most important applications for the use of microbubbles to enhance delivery has been the enhancement of blood brain barrier (BBB) permeability (58–65). In general, the technique consists of systemically injecting microbubbles and insonifying the region of the brain where enhanced permeability is desired. Here again, a parameter space has been established within which enhanced BBB transport is achieved with minimal hemorrhage and cell death. Still, the extension of these techniques into the clinic is expected to require the use of real-time cavitation detection, as individual variations in the skull penetration of the ultrasound wave are significant and the therapeutic window for effective and safe therapy is relatively small (66). As noted above, pre-clinical-application of microbubble-enhanced delivery is widespread. In addition, the authors are aware of yet unreported small clinical studies of microbubble-enhanced delivery to solid tumors.

APPLICATION OF IMAGING TECHNOLOGY

A major reason for the expansion of the application of therapeutic ultrasound is the development of methods to monitor the treated location and the temperature using MRI (67) or ultrasound (68). While mild hyperthermia (CEM 43 < 0.5) is associated with increased metabolism, blood flow, and tissue repair, higher thermal doses are associated with enhanced cell death and therefore the methods to carefully control and monitor the delivered temperature are critically important (69). Image guidance using nuclear

medicine techniques is also attractive due to their high sensitivity and the opportunity for quantitation of delivery (70, 71). By radiolabeling nanoparticles, the rate and magnitude of extravasation can be directly estimated from PET data (71). Even with the relatively low spatial resolution of PET ($\sim$1 mm), the penetration of nanoparticle-based therapeutics has been assessed and shown to differ from small molecular weight agents (72).

In order to fully evaluate the enhanced penetration of a drug resulting from ultrasound, multiple imaging labels can be incorporated with drug accumulation and penetration assessed at the whole body, organ, and cellular scales (8, 9, 70, 73, 74). Multiple MRI protocols can be proposed for the guidance of ultrasound therapies including diffusion-weighted (75, 76), T2-weighted (77, 78), and contrast enhanced T1-weighted imaging (79–81), fluid attenuated inversion recovery (FLAIR) (82), heteronuclear (^{23}Na) (83), spectroscopy (84), and displacement sensitive sequences via MR elastography (85). Following HIFU ablation of the prostate, gadolinium enhanced MRI is often used to evaluate the extent of tissue damage. Although contrast enhanced T1-weighted MRI can detect tissue damage following HIFU ablation (86, 87), it does not correlate to histological results (intensity of necrosis, presence of foci of viable cancer) immediately following HIFU (87). However, for follow-up examinations, DCE MRI has demonstrated good sensitivity and diffusion MRI has shown specificity in identifying tumor progression after HIFU ablation (75, 88).

In addition to endogenous contrast mechanisms that can be used to guide and assess ultrasound therapies, exogenous agents can be used to report on specific changes. For example, co-administration of two paramagnetic contrast agents (gadolinium and thulium) within liposomal drug carriers has been previously utilized to follow internalization and cellular trafficking of the vehicle (89). Similarly, multi modal liposomal agents spanning CT and MRI have been used to assess the penetration of liposomes within tumors and have been proposed for cross modality registration and as a means to guide imaging-based interventions (73, 74). Many physiological parameters can also be assessed by MRI and coupled with the soft tissue anatomical information motivate MRI as an excellent tool for guiding thermal therapies (90, 91).

In addition to the role of MRI in the assessment of drug penetration and distribution, MR thermometry can be applied to monitor the temperature of a region during an intervention (92–100). The proton resonance frequency (PRF) of water is frequently used to detect changes in temperature (101) both because it has a thermal coefficient that is linear over a wide temperature range and, excepting adipose tissue, the PRF shift has little dependence on tissue type even following coagulation (99, 102). The PRF shift can be measured rapidly with gradient echo sequences, which is advantageous during thermal therapies where high temporal resolution is desirable, especially during ablative processes, to avoid damage to surrounding tissue. Further increases in temporal resolution can be gained via partial parallel imaging techniques using phased arrays (103–105) utilizing various algorithms (105).

Neither clinical focused ultrasound (FUS) systems, which typically operate around 1 MHz (106, 107), nor clinical MR scanners (e.g., 1.5, or 3 T) are ideal for small animal imaging. In the former,

the focal spot depth may encompass an appreciable portion of the animal, while the latter may have insufficient signal-to-noise ratio (SNR) to easily obtain detailed images of murine tumors. The smaller focal depth at higher FUS frequencies makes them more suitable for pre-clinical imaging of small animals (108). High field scanners are especially useful for small animal thermal imaging because in addition to providing higher SNR, which can be used for higher spatial resolutions, they also improve the sensitivity of thermal measurements made with the PRF shift method, which itself has a first order dependence on magnetic field strength.

FUTURE APPLICATIONS

The use of the thermal and mechanical effects of ultrasound to enhance delivery to solid tumors is expanding. With the increasing availability of MRI-guided high intensity focused ultrasound, well-controlled and calibrated clinical studies are feasible. Both the use of ultrasound to alter tissue properties and to release a drug from a carrier are in widespread pre-clinical evaluation. With the addition of microbubbles, drug penetration through the endothelium can also be increased, although the protocols are currently more complex due to the need to co-inject the therapeutic and microbubbles.

In the future, the effects of ultrasound may transcend the local effect through enhanced immune response. The addition of immunotherapy to standard-of-care cancer therapies has shown evidence of efficacy in the pre-clinical and clinical settings (109–115). The immune system is often tolerant to antigens presented by the tumor and therefore strategies to induce tumor-specific immunity must overcome obstacles including: insufficient and dysfunctional populations of antigen-presenting cells and lymphocytes, the difficulty of inducing potent immunity without inducing unacceptable autoimmune toxicities, the low immunogenicity of antigens expressed by tumor cells, and immunoregulatory pathways that dampen the tumor-specific immune response (116). The use of ultrasound ablation to generate an immune response has been shown to be a promising technique for immune activation (110–112, 114, 115, 117–121). Ultrasound ablation is thought to act through dendritic cell maturation and T-cell immunity (122), and is particularly advantageous because it is completely non-invasive, can be controlled with high spatial precision and uses no harmful ionizing radiation (123, 124).

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