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Driving delivery vehicles with ultrasound ★

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Abstract

Therapeutic applications of ultrasound have been considered for over 40 years, with the mild hyperthermia and associated increases in perfusion produced by ultrasound harnessed in many of the earliest treatments. More recently, new mechanisms for ultrasound-based or ultrasound-enhanced therapies have been described, and there is now great momentum and enthusiasm for the clinical translation of these techniques. This dedicated issue of *Advanced Drug Delivery Reviews*, entitled “Ultrasound for Drug and Gene Delivery,” addresses the mechanisms by which ultrasound can enhance local drug and gene delivery and the applications that have been demonstrated at this time. In this commentary, the identified mechanisms, delivery vehicles, applications and current bottlenecks for translation of these techniques are summarized.

Keywords

Ultrasound; Therapy; Drug delivery; Gene delivery; Sonoporation; Cavitation

As an imaging modality, ultrasound is widely employed and valued for its real time applications, low cost, simplicity, and safety. Waves of alternating increased and decreased pressure propagate from the transducer, typically resulting in a maximum intensity in a focal region chosen by the operator. The dimensions of the focal region can vary from tens of microns for high frequency ultrasound to centimeters for an unfocused therapeutic beam. Most clinical instruments are engineered to effectively image from the body surface to 24 or more centimeters in depth. The ability to locally control and maximize energy deposition deep within the body facilitates a broad range of clinical opportunities for ultrasound imaging and therapy. Developing over four decades, clinical and commercial application of ultrasonic therapies spans hyperthermia, drug delivery, lipoplasty, and thrombolysis [1,2]. A complete and precise summary of the potential applications for ultrasonic enhancement of drug and gene delivery remains challenging as the mechanisms are multiple and not fully characterized. From an engineering viewpoint, the methods by which ultrasound facilitates drug and gene delivery can be summarized as direct changes in the biological or physiological properties of tissues facilitating transport, direct changes in the drug or vehicle increasing bioavailability or enhancing efficacy, and indirect effects by which ultrasound acts on the vehicle to produce changes in the surrounding tissue. While there are common mechanisms between these methods, the engineering challenges associated with the optimization of ultrasound systems and drug and vehicle design are unique for each method. Promising examples of each of these methods can be identified at this time and are addressed below.

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1. Mechanisms

Historically, the scattered waves resulting from regions of varied tissue density and compressibility have provided the basis for ultrasound images. More recently, the tiny displacements of tissues by the propagating wave have also been utilized to image local tissue properties [3,4]. These same displacements result in secondary effects that can be harnessed for therapeutic benefit. With each ultrasonic cycle, a fraction of the energy in the propagating wave is absorbed by tissue, resulting in local heating. The rate of absorption is tissue dependent and increases with increasing ultrasound frequency. With low ultrasound intensities, these thermal effects have been associated with increases in perfusion and changes in drug-carrying vehicles. At higher ultrasound intensities, these thermal effects can locally ablate tissue. While local ablation by high intensity heating has a substantial set of clinical applications, the resulting changes in tissue properties impact future imaging studies, e.g. potentially obfuscating the boundaries of a tumor. The secondary ultrasound effects used to release drugs from within activatable vehicles may ultimately have advantages in locally treating a region without resulting in local fibrotic changes.

Further, in limited circumstances these mechanical displacements can result in the nucleation, growth, and collapse of gas bubbles, commonly referred to as acoustic cavitation. Inertial cavitation involves the rapid growth and collapse of bubbles in which the surrounding tissue or fluid inertia drives rapid collapse; alternatively, stable cavitation involves sustained oscillation of gas bubbles. Acoustic cavitation induces high fluid velocities, shear forces, and local temperature increases, thus producing biological effects and altered transport kinetics near the site. Mechanical oscillations are locally amplified as a result of the injection of ultrasound contrast agents, which are small stabilized gas bubbles whose walls are displaced with each ultrasonic cycle by substantially larger distances than an equivalent tissue volume. Thus, therapeutic effects associated with ultrasound are reported at far lower acoustic pressures when combined with intravenously-injected microbubbles, as compared with the mechanical effects of ultrasound alone.

1.1. Enhanced membrane transport

Changes in cell membrane permeability, resulting from cavitation of endogenous gas bubbles or *in vitro* and *in vivo* insonation of exogenous microbubbles [5–8], are now well-established. Small holes are produced within cell membranes by the collapse of a microbubble and production of a jet, and this has been confirmed by optical and electron microscopy [5,6]. Pores produced within the cell membrane may be transient (facilitating successful therapeutic delivery) or permanent (resulting in cell death) [7,8]. Early analyses demonstrated that microbubbles must be very close to the cell membrane in order to produce such changes, and therefore the local density of microbubbles must be high in order to transfect a substantial fraction of the local cell population.

1.2. Changes in tissue/vascular transport properties

Insonation of microbubbles at a sufficiently high concentration or with a sufficiently high mechanical stress (low frequency, high acoustic pressure) results in changes in the permeability of capillary wall, with resulting extravasation of particles into the interstitial space [9,10]. In such cases, convective transport can result in transport of drugs or particles over distances on the order of tens of microns from the vessel wall and therefore far from the location of the microbubble oscillation. The change in capillary permeability is strongly dependent on bubble size and concentration, and acoustic pressure and frequency. With a sufficiently high microbubble concentration, ultrasonic pressure on the order of 0.75 MPa at a center frequency of 1 MHz can produce capillary rupture in the intact rat muscle microcirculation; and the pressure required increases with increasing ultrasound frequency [11,12]. Direct visualization

of microbubble–vessel interaction has been reported [13], where the expansion of the microbubble within small vessels was decreased, the lifetime of the microbubble increased, and larger microbubbles were shown to directly contact the wall.

1.3. Radiation force and streaming

Momentum transfer from the propagating wave to delivery vehicles can result in the translation of vehicles across a blood vessel and facilitate vascular targeting [14–16]. Combining radiation force (to bring a microbubble in contact with the vessel wall) with destructive ultrasound pulses (to localize the drug on the wall) has resulted in a ten-fold increase in deposition of oil on cell membranes *in vitro* in comparison to ultrasound alone [17]. Radiation force and streaming effects can also be combined with other therapeutic mechanisms (e.g. heating, cavitation) to further enhance drug delivery.

1.4. Ultrasonic drug enhancement

Reviews within this issue are complemented by an excellent recent summary of mechanisms for ultrasonic drug enhancement, which is often referred to as sonodynamic therapy [18]. The insonation of sonosensitizers is thought to generate free radicals, destabilize cell membranes, and enhance trans-membrane transport. A subset of anticancer drugs act as sonosensitizers, where the combination of ultrasonic therapy with the drug results in a greater effect than the additive effect of the two mechanisms [18].

1.5. Mechanical or thermal changes in delivery vehicles

Lipids and polymers can be engineered to self-assemble into vehicles that protect or concentrate drugs, particularly those with systemic toxicity [19–22]. The mechanical or thermal properties of ultrasound can release the drug from the vehicle in the selected region of interest. Engineering such vehicles to stably incorporate the drug or gene during circulation and yet effectively release the contents at the desired site remains challenging; however, the impact of such strategies could be very high. Particularly as we learn to identify early indications of disease and seek to preempt disease processes, an effective local delivery of drugs and genes that minimizes systemic toxicity could be transformational. While other energy sources (e.g. magnetic field, light) can also produce such changes, ultrasound can effectively focus energy deep within the body facilitating local treatment of a broader range of conditions than alternative approaches.

2. Vehicles

The use of vehicles that are activated by the thermal or mechanical properties of ultrasound and the co-administration of drugs that passively accumulate in regions affected by ultrasound are discussed below. Major classes of vehicles activated by ultrasound include microbubbles, micelles and liposomes, and perfluorocarbon nanoparticles.

Early studies involved the systemic co-injection of a particle or imaging probe together with a microbubble, followed by local insonation to activate the microbubble and change local vascular permeability, allowing for extravasation of the agent [9,23]. Recent studies have incorporated a drug within the shell of an activatable nano- or micron-sized agent, locally delivering the drug after insonation [24,25]. Without a targeting ligand, our initial studies have indicated that the mass of lipophilic radioactive material delivered from a monolayer microbubble shell to a vessel wall is small (unpublished data). However, with high concentrations of microbubbles and genes loaded on the shell, therapeutic levels of transfection have been achieved [26], and the incorporation of targeting ligands seems likely to further increase the mass of the drug or gene delivered.

Lipid-shelled microbubbles have been employed in many studies of drug and gene delivery. The lipid shell expands, buckles and compresses with each acoustic cycle, recoating as needed to cover bubble fragments [27–32]. A thick layer of DNA can be applied to the surface or, alternatively, liposomes or other nanoparticles can be attached to the membrane, each enhancing the payload [16,33,34]. Protein and polymer-shelled microbubbles have also been functionalized to carry targeting ligands [35] and genetic payloads [36,37]. The major advantages of microbubbles as carriers include the enhanced transport of drugs or genes across cell membranes and vessel walls resulting from nearby oscillation of the microbubble. Disadvantages of microbubbles as carriers involve the relatively low payload and short circulation half-life (on the order of minutes for lipid-shelled vehicles but extended with polymer-shelled vehicles).

An array of ultrasonically-activated vehicles with a diameter on the order of tens to hundreds of nanometers has also been described. The smaller size of these particles facilitates extravasation from leaky tumor vasculature and molecular targeting to vascular receptors. Perfluorocarbon-containing nanoparticles have been widely applied as agents for ultrasound and magnetic resonance imaging [38,39], and more recently for therapeutic delivery [14]. In a particularly exciting development, polymer-coated perfluorocarbon nanoparticles were recently reported to produce an effective cancer therapeutic when combined with drug-carrying particles and ultrasound [40]. With this coating, liquid perfluorocarbons with low phase transition temperatures can be stably incorporated within vehicles.

Activatable liposomes can release a drug by the mechanical effects of ultrasound, enhanced by the inclusion of small air pockets within the membrane [19,20] or by heating beyond the phase transition temperature of the lipids within the membrane [21]. Advantages of liposomes as carriers include, the fusogenic nature of the membrane, and a reasonably high drug loading efficiency (up to 0.25 mg drug/mg lipid). Polymeric coatings of liposomes can enhance the circulation half-life and stability. Alternatively, small polymeric carriers can have substantial advantages in extended *in vivo* stability and controlled release, and can also respond to mechanical and thermal activation [22]. Doxorubicin has been shown to release from Pluronic® P105 micelles with insonation at 20 kHz [41], where such micelles are expected to be stable *in vivo* for extended periods.

3. Applications

The following reviews describe many of the important applications of ultrasound-enhanced drug delivery, including transdermal delivery, delivery to solid tumors, delivery across the blood brain barrier, and enhanced thrombolysis. Transdermal delivery is perhaps the most widely recognized application of ultrasonically-enhanced drug delivery based on the approval of the Sontra Medical (Echo Therapeutics, Inc) system for transdermal delivery of topical therapeutics in 2004 [42]. Other technologies, including transdermal glucose monitors and systems to enhance the delivery of macromolecules, are advancing [2].

Enhancement of delivery across tight endothelial junctions, such as those present within the blood–brain barrier, is one of the most important applications of ultrasound-enhanced delivery, as it represents one of the few possible approaches for enhancing the transport of many molecules. In the presence of microbubbles, transport of a magnetic resonance contrast agent was shown to be enhanced with an ultrasound center frequency of 260 kHz and peak rarefactional pressure of 0.3 MPa without tissue necrosis [43]. Enhanced transport of doxorubicin has been demonstrated with the insonation of microbubbles and systemically injected drug using a center frequency of 1.6 MHz and acoustic intensity of 0.6 W [44]. While the generation of liquid jets (resulting in holes within cell membranes has been clearly demonstrated with high microbubble concentrations and higher ultrasound intensities,

mechanisms responsible for enhanced transport with lower ultrasound intensities are still under investigation.

Enhanced delivery to tumors is a similarly important application of ultrasound-enhanced drug and gene delivery, since transport of drugs within solid tumors can be very limited. Many of the vehicles described here (liposomes, polymeric micelles, microbubbles, etc) have been harnessed to enhance delivery to solid tumors, with reported increases in localized delivery of up to 5–10 fold [40].

Vascular lesions, such as thrombus, are also important targets for ultrasound-enhanced delivery, due to their accessibility to a wide range of therapeutic particles. Ultrasound enhances thrombolysis, particularly when applied in conjunction with intravenous administration of thrombolytic agents [45–47]. Microbubble oscillation has also been shown to enhance the breakup of a clot, with maximal efficacy achieved with the combined effect of insonation, thrombolytic agents and microbubbles [46,47]. Challenges for clinical translation include the development of systems for reliable control of the applied ultrasound dose and the great challenges of achieving the required timely intervention (3–6h after the thrombus formation) in the critical applications of stroke and myocardial infarction.

4. Other issues

While limited applications of ultrasonic drug and gene delivery have been approved for human use, other applications may be hastened by auxiliary developments. Many endothelial cell surface receptors have recently been identified — and these are accessible to the nano- and micron-sized particles frequently employed in ultrasound delivery systems [48–50]. These accessible receptors greatly expand the opportunities and methodologies to be considered in ultrasound-enhanced delivery. Combining the complex pharmacokinetics of individual drugs, their vehicles, and molecular targeting approaches with the range of physiological effects induced by ultrasound, the need for validation of each step in the delivery cascade seems clear. Creation of a clinically-optimized ultrasound-enhanced drug or gene delivery vehicle is challenging due to the vast parameter space of potential materials, targeting ligands, cargos, and ultrasound parameters. We and others are developing cross-modality imaging methods in which nuclear medicine, magnetic resonance imaging, and optical imaging probes are used to visualize the delivery vehicle or the model drug [51–54]. The great strength of nuclear imaging techniques, particularly positron emission tomography, is the real-time and highly sensitive full-body pharmacokinetics currently possible in pre-clinical studies [54,55]. Magnetic resonance imaging (MRI) techniques can be used to visualize the biodistribution of drugs or vehicles [52] and the activation of delivery vehicles in limited cases [51], and MRI can also be used to monitor local temperature in pre-clinical and clinical studies [56]. Optical imaging can be broadly applied to monitor the activation of a delivery vehicle or release of a drug, although *in vivo* studies of ultrasound-based drug delivery do not yet incorporate real-time optical imaging. With correlative imaging techniques, the effect of each component (materials, molecular targeting, cargo, ultrasound parameters) on the biodistribution can then be assessed with and without ultrasound, quantifying these changes in an absolute manner. Such tools seem to be essential for the optimization of these complex processes and the translation of these techniques into the clinic. Similarly, the development of transducers and systems to combine therapeutic ultrasound arrays with confocal high-quality imaging arrays is in process at several sites. In our UC Davis Center for Molecular and Genomic Imaging, we invite collaboration with investigators wishing to quantify the efficacy of new ultrasound-based drug and gene delivery approaches using correlative imaging and confocal imaging and therapy.

The safety of the relatively large (100 nm and larger) particles used for ultrasound-enhanced drug and gene delivery is also receiving considerable attention. While the interactions of

ultrasound with microbubbles and the resulting biological effects have been under investigation for a number of years, complement activation and related anaphylaxis are recently receiving attention. Complement attachment to lipid-shelled microbubbles with a net negative charge [57] and those exposing biotin or RGD [58] has been shown. Albumin-encapsulated microbubbles also bind complement C3 and as a result bind to the vascular endothelium [59]. Similarly, the complement activation resulting from the administration of liposomes has been examined recently, with a report that the removal of the negative charge from the lipo-polymer used to sterically stabilize microbubbles prevented complement activation [60]. Although few reports of adverse reactions to microbubbles have been published [61], the United States Food and Drug Administration has recently limited the use of microbubble contrast agents in patients with acute coronary syndrome and now requires monitoring of cardiac function after contrast imaging. These limitations have been assumed to result from anaphylaxis associated with complement activation. Fortunately, the engineering of delivery particles to eliminate unnecessary charged lipids and lipopolymers is straightforward.

5. Conclusion

In summary, the many mechanisms by which ultrasound can enhance the efficacy of drugs and genes and alter drug delivery vehicles are currently areas of intense study, with several promising clinical applications. The design of ultrasound systems, correlative imaging systems, and drug delivery vehicles each engineered to capitalize on the known mechanisms is well underway. Many challenges remain to be solved in optimizing these techniques, but there is great excitement and enthusiasm generated by the prospective utility, practicality, and wide applicability of ultrasound-based drug delivery.

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