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Ultrasound for Drug and Gene Delivery to the Brain

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Abstract

Noninvasive, transient, and local image-guided blood-brain barrier disruption (BBBD) has been demonstrated with focused ultrasound exposure in animal models. Most studies have combined low pressure amplitude and low time average acoustic power burst sonications with intra-vascular injection of pre-formed micro-bubbles to produce BBBD without damage to the neurons. The BBB has been shown to be healed within a few hours after the exposure. The combination of focused ultrasound beams with MR image guidance allows precise anatomical targeting as demonstrated by the delivery of several marker molecules in different animal models. This method may in the future have a significant impact on the diagnosis and treatment of central nervous system (CNS) disorders. Most notably, the delivery of the chemotherapy agents liposomal Doxorubicin and Herceptin has been shown in a rat model.

Keywords

Blood-brain barrier; brain; targeted drug delivery; image-guided therapy; drug delivery; HIFU; focused ultrasound; ultrasound

Keywords

Ultrasonics; Magnetic Resonance Imaging; Drug Delivery Systems; Ultrasound Contrast Agents; Blood-Brain Barrier

1. Introduction

The endothelial cells of the central nervous system (CNS) are unique cells that are tightly fused to each other by intercellular attachments known as “tight junctions” [1,2]. In addition to the endothelium the blood -brain barrier (BBB) is formed by astrocytes, neurons, microglia and pericytes. The endothelial cells have low pinocytic activity and thus form a barrier between the blood and the cells in the CNS [3]. The factors that determine penetration of substances from the blood to the CNS are lipid solubility, molecular size and charge, and whether or not they can utilize any carriers in the endothelial cells. Thus the BBB limits the passage of most molecules (over 95% of potential therapeutically useful molecules) from circulation into the brain parenchyma, precluding their use in the treatment or diagnosis in the CNS [1,2,4,5]. There

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are two ways to enhance propagation through the barrier: Chemical modification of the drugs, or the use of other carriers such as amino acid and peptide carriers can increase transport through the BBB. The tight junctions (TJs) can also be opened temporarily by an intra-arterial injection of certain chemicals such as mannitol or other hyper-osmotic solutions. It is assumed that these agents cause the endothelial cells to shrink, resulting in a disruption of the TJs that lasts for a few hours [6]. Both the above methods produce diffuse BBBD within the entire tissue volume supplied by the injected artery branch [1],[2] and they cannot be used to selectively target a small CNS volume. This type of localized BBB disruption currently can be accomplished clinically only by direct invasive injection of agents into the targeted region of the brain [2].

Local BBBD was observed after experimental exposure of animal brains to ultrasound [7], [8], [9] indicating that ultrasound may offer a local method for drug delivery. In this paper we will review the literature and our experience in using focused ultrasound for image-guided focal and transient BBBD.

2. Ultrasound exposure through the skull

A major limitation in the utilization of ultrasound for the disruption of the BBB has been the poor penetration of ultrasound through the skull, and for several decades it was believed that the skull bone had to be removed to perform ultrasound treatments in the brain [10–12]. Although focusing was attempted for overcoming the attenuation losses [13], the high speed of sound in the bone coupled with its dependence on the density of the bone further complicates ultrasound transmission through the skull. The distortion of the wave front by the heterogeneous bone makes focusing in the brain difficult with traditional single focus transducers [13]. Phased arrays with CT-derived phase and amplitude corrections have been proposed to overcome this problem [14,15]. Such methods have been able to focus ultrasound through human skulls [14] and create thermal lesions in rabbit [16,17] and sheep brains [18] *in vivo*, and a magnetic resonance imaging (MRI) guided system is currently in a clinical phase I trial for thermal ablation of malignant brain tumors [19]. Since the time averaged ultrasound power is at least two orders of magnitude larger for thermal ablation than for the BBBD, it is clear that such systems can be used in humans for BBBD. It has also been proposed that skull induced distortions could be limited by reducing the frequency of the sound beam, thus increasing the wavelength. Both experiments [13] and simulations [20] have shown that the use of low frequency (around 250–300 kHz) will allow focusing through human skull without patient-specific distortion corrections. There is also another route for using higher frequencies that allows more precise focusing without the need of CT-derived corrections. This method uses the transmission of shear waves that distort less than longitudinal waves [21].

3. Ultrasound methods for BBBD

3.1. Temperature effects

Although local BBBD was observed after exposure of animal brains to ultrasound [7], [8], [9], there were no experiments that deliberately tried to exploit this phenomenon for molecular delivery into the brain until Patrick et al. [22] proposed it for the delivery of chemotherapy agents into brain tumors. They demonstrated that the BBB is leaky around ultrasound-induced thermal lesions, but not if the exposures did not result in tissue coagulation. This thermally-induced BBBD has been further investigated, but it has always been associated with tissue damage [23]. However, in tissue cultures, elevated temperatures have been shown to induce reversible BBBD [24].

3.2. Cavitation effects

Although short, high-intensity ultrasound exposure above the cavitation threshold was found to produce BBBB, sometimes without brain tissue damage [25], this was not able to be reproduced consistently. Similar results were observed with pulsed, higher frequency (2 MHz) sonications [26] where the association with cavitation was not clear. There is also clinical evidence that sonications can disrupt the BBB [27]. In a clinical study, low frequency continuous wave ultrasound (approximately 300kHz) was used for blood clot lysis in combination with intravenous injection of tissue plasminogen activator (TPA). After some treatments, contrast-enhanced CT demonstrated that the BBB was disrupted in a remote location. It is not possible to determine exactly why the disruption occurred, but it is possible that the low frequency exposures in combination with the injected fluid caused cavitation. It is not clear if the BBBB was associated with tissue damage.

3.3 Micro-bubble aided BBBB

A few years ago, noninvasive and reversible disruption of the BBB was demonstrated using focused ultrasound bursts in conjunction with an ultrasound contrast agent [28]. Contrast agents that contain preformed micro-bubbles are now extensively investigated for many therapeutic applications (see review [29]). Since this method has been the only one to produce reversible BBBB without brain tissue damage, it has been extensively investigated by several research groups. These studies will be reviewed in the following pages.

3.3.1. Description of the method—This method for BBBB with ultrasound utilizes pre-formed micro-bubbles injected into the blood stream before the ultrasound burst exposures. The working hypothesis for this research was that since the micro-bubbles act as energy concentrators and are contained in the blood, the induced biological effects would be confined to the blood vessel walls, for the most part (figure 1)[28]. So far all of the animal experiments support this hypothesis.

The ultrasound exposures used in most experiments have been 10-ms bursts at low pressure amplitude (<1 MPa) repeated at the frequency of 1 Hz for the duration of 20–30 s. The micro-bubbles, developed for ultrasound imaging, contain gas (air or perfluorocarbon) encased in a shell, most often made of albumin or lipids, and have diameters between approximately 1 and 5 micrometers such that they pass through the capillary network. When the bubbles pass through the tissue volume exposed to ultrasound they expand and contract at the frequency of the propagating acoustic wave due to the cyclic pressure reductions and increases associated with the wave propagation. The bubble oscillations also cause the surrounding fluid to flow (microstreaming) thus creating large shear forces around the bubbles [30]. In addition, the bubbles are pushed by a radiation force [30,31] in the direction of wave propagation. Above a threshold, the bubble oscillations become so large that the inertia of the surrounding fluid causes the bubble to collapse, inducing high temperatures and pressures and resulting in a shock wave that propagates at supersonic speed radially from the collapse site [32,33]. If the bubbles collapse close to a vessel wall, they can create fluid jets that can puncture the wall [32–34]. As a result the bubbles absorb and concentrate energy from the ultrasound wave into a microscopic tissue volume reducing the ultrasound power levels by at least two orders of magnitude from that required to induce bio-effects without the bubbles [28]. The acoustic signals emitted by the micro-bubbles and their relationship to the BBBB and vascular damage has been investigated [35]. It was discovered that the bubble collapse demonstrated by wideband emission was associated with extravasations of red blood cells (RBCs). The results also showed that bubble oscillations, detectable from the emission spectrum, were present when BBBB without vascular damage was induced. Therefore, the acoustic signals may provide a method for exposure control.

The micro-bubbles used so far are standard, clinically proven, diagnostic ultrasound contrast agents. Optison™ (GE Healthcare, Milwaukee, WI), containing bubbles of perfluorocarbon gas (Perflutren) with a human albumin shell, has been the most commonly used agent. According to the manufacturer, the bubbles have a mean diameter of 2–4.5 micrometers with a maximum diameter of 32 micrometers. The bubble concentration in the agent is between 5 and 8×10^8 bubbles/mL. Most of the animal experiments have been performed at the clinically recommended diagnostic dose of 0.2 ml of Optison/kg. As expected, experiments have shown that other ultrasound contrast agents containing pre-formed micro-bubbles can be used for BBBD [36,37] and thus the method is not specific to one agent.

Ultrasound contrast agents contain some large bubbles that are too large to pass through the capillary network. Since the contrast agent is injected IV, the large bubbles are filtered away by the lungs. However, if intra-arterial injection is needed, then large bubbles should be removed to avoid development of micro-embolizations. These large bubbles were most likely the cause for the BBB disruptions that were seen in experiments with rats with intra-arterial injection of Optison without sonications [38].

3.3.2. The mechanism of ultrasound induced BBBD—In order to determine the time course between the sonications and the disruption of the BBB, a series of mouse experiments was performed using *in vivo* multi-photon microscopy [39]. In these experiments two dyes of different molecular weights were injected IV into a mouse positioned in a microscopy system that allowed simultaneous ultrasound exposures. The results showed that the dye leakage occurred without extravasation of RBCs (which was also seen on one occasion) via two observed routes. First, micro-disruptions were observed where the dye rapidly leaked from a point on the vessel wall. This could be caused by bubble collapse with associated jet formation that punctured the vessel wall. These micro-disruptions happened more in arteries than veins and were associated with points of bifurcation. Second, the dye leaked slowly through apparently intact endothelium. The results demonstrated that the sonications were associated with an almost instantaneous constriction of at least some of the arteries and arterioles followed by the slow leakage of dye molecules through the vessels walls. The smaller (10kD MW)(Alexa Fluor 488, Molecular Probes, Eugene, OR, USA), molecules leaked at a higher rate than the larger (70 kD MW)(dextran-conjugated Texas Red, Molecular Probes, Eugene, OR, USA) molecules which showed a much lower intensity. The vessel diameter relaxed slowly after reaching a minimum during or just after the sonication. It is not known whether or not the constriction was the cause of the BBBD or if it was just an unrelated byproduct of the sonications. The most likely reason for the vessel constriction is mechanical stimulation induced by the radiation force caused by the sonication or by the micro-streaming associated with the bubble oscillations. One potential mechanism for the BBBD could be that the vasoconstriction may reduce the oxygen transfer temporarily but long enough to trigger ischemia related receptors. It is known that ischemia can compromise the BBB [40].

The ultrastructural changes that may be responsible for the BBBD were investigated using electron-microscopy [41,42]. The marker molecules used were immunoglobulin and horseradish peroxidase. These studies identified three main mechanisms: First, rupture of the blood vessel was associated with extravasations of the RBCs. Second, there was an occasional widening of the TJs. This was similar to what was shown by Mesiwala et al. [26] with high frequency ultrasound exposures without micro-bubbles. Third, it was observed that vacuoles were transporting marker molecules through the endothelial cells.

Another study evaluated the impact of the sonications on the molecular integrity of TJs after focused ultrasound disruption of the BBB. Using immunoelectron microscopy, the distribution of TJs-specific trans-membrane proteins occludin, claudin-1, claudin-5, and of sub-membranous ZO-1 was studied at 1, 2, 4, 6 and 24 h after sonications. The protein expression

was quantified by counting immunosignals per micrometer of length of junctional clefts. The BBB disruption was demonstrated by the leakage of intravenously injected horseradish peroxidase (m.w. 40,000 Da) and lanthanum chloride ($\text{La}^{3+} \sim 139 \text{ Da}$) at 1 h, 2 h and in a few vessels at 4 h after ultrasound application. These changes were paralleled by disintegration of the TJs complexes manifested by redistribution of molecular components and loss of immunosignals for occludin, claudin-5 and ZO-1, while claudin-1 seemed less involved. At 6 and 24 h after the sonication there was no leakage through paracellular clefts and the barrier function of the TJs was completely restored [43] (figure 3).

Further experiments were performed to investigate the trans-endothelial vesicular traffic (figure 4) after ultrasound exposure in the rabbit brain, using ultrastructural morphometry and horseradish peroxidase (HRP) as a tracer. The mean endothelial pinocytotic density (the number of HRP-containing vesicles per μm^2 of the cell cytoplasm) was over an order of magnitude higher in the arterioles after sonication than in similar vessels in the unexposed control locations. Conversely, the sonications did not increase the pinocytotic density significantly in the capillaries and only a small number of HRP-positive vesicles were observed in the venules (Table 1.). Therefore the ultrasound exposure-induced trans-cytoplasmic traffic is important only in the pre-capillary microvessels [44].

3.3.3. The influence of exposure parameters on the BBB disruption—The disruption of the BBB with focused ultrasound and micro-bubbles has been investigated extensively using standard commercial MRI agents such as gadopentetate dimeglumine (Magnevist, Berlex Laboratories Inc, Wayne, NJ) (928 MW) that does not penetrate through the BBB. These studies showed that focal disruption of the BBB is feasible at pressure amplitudes that do not induce necrosis, ischemia or apoptosis of the brain tissue (burst length = 10 ms, repetition frequency = 1 Hz, sonication duration = 20 s, rarefactional pressure amplitude = 0.4–0.5 MPa, and frequency = 0.69 MHz) [42]. Figure 2 shows the focal enhancement induced in rabbit brain. The amount of contrast enhancement was found to be dependent on the pressure amplitude. However, at higher pressures, the exposures induced tissue damage (figure 5). The frequency of 1.7 MHz with the same burst sonications was associated with a few locations of extravasated RBCs that appeared not to have any long term effect on the brain tissue [45]. At lower frequencies the pressure threshold of the BBB disruption decreased and the number of extravasated RBCs decreased such that at the frequency of 250 kHz the BBB disruption was possible without any extravasated RBC [35]. The disruption of the BBB healed approximately in 6 h after the sonications and only a small signal enhancement was observed with contrast enhanced MRI (figure 6). The imaging studies performed at 2–5 days, and 4 weeks after the sonications revealed that the BBB was completely healed [42,45]. These studies were done with only one small molecule contrast agent and the BBBD may be different for different molecules. Similarly a light microscopy study demonstrated intact brain tissue and vasculature at these follow up time points. In order to determine the potential impact of the BBBD on the brain, they were examined with light microscopy and several histology stains. As stated earlier, the only tissue effect that was seen at the pressure amplitude levels close to the threshold of BBBD was occasional extravasations of RBCs. The number of extravasations decreased with decreasing ultrasound frequency and increased with increasing pressure amplitude. For example, at the 1.7 MHz approximately 5 % of the detected vessels had extravasated RBCs. These extravasations appeared to be absorbed by the tissue over the course of four weeks with no detectable adverse reaction. No apoptotic or ischemic tissue regions were observed at these exposure levels. However, when the pressure amplitude was increased, both ischemic and apoptotic cells were detected with the number increasing with the pressure amplitude. A further elevation in the pressure amplitude resulted in tissue necrosis and hemorrhage [46].

Both the 50% threshold of the BBB disruption detected by MRI contrast agent injection and the red blood cell extravasations were found to increase almost linearly as a function of frequency [47,48]. The threshold stayed constant at 10 and 100 ms bursts but increased when the burst length was reduced. An increase in the duration of the exposure has been shown to increase the amount of the contrast agent leaked across the BBB. The burst repetition frequency did not have a large impact on the BBB, however, the higher repetition frequency of 10 Hz used by Choi et al [49] appeared to require significantly higher pressure amplitudes (approximately 2.5 MPa at the frequency of 1.5 MHz) perhaps indicating dependency on the burst repetition frequency. When exposures similar to Doppler (10 us bursts repeated at the frequency of 1 kHz) were used, the threshold for the BBB was also higher and always associated with tissue damage [50]. This agrees with a clinical study demonstrating that transcranial Doppler studies (lower pressure amplitude) are not associated with the BBB disruption [51].

The studies investigating the impact of the concentration of the micro-bubbles on the BBB are inconclusive. These studies were performed with the same burst sonications as stated above. Yang et al [37] demonstrated a linear increase in the BBB with increased bubble quantity agreeing with Treat et al. [52] who demonstrated higher drug delivery with higher bubble concentration. Conversely, McDannold et al. [48] did not see a significant increase in the BBB with increasing bubble quantity. It is logical that the BBB should increase with the number of bubbles, and it may be that the large variation from experiment to experiment seen in the study of McDannold et al masked the trend in their study.

Many of the potential CNS therapies require large molecules and particles to be delivered through the BBB. In order to test the feasibility of such treatments, an intravascular MRI contrast agent MION (20 nm nanoparticulate contrast agent, MION-47, Center for Molecular Imaging Research, MGH, Boston, MA, USA) was used to study the feasibility of these treatments. The experiments showed that the leakage of MION was the largest when the agent was present in the blood during the sonications (burst length = 10 ms, PRF = 1 Hz, sonication duration = 20 s and frequency = 250 kHz) (figure 7). The MION delivery was much diminished when it was injected IV post sonication [30,42]. These experiments, together with the dual-photon and electron microscopy observations, demonstrated that large molecules and particles can be delivered through the BBB when the agent is in the blood during the sonication.

3.3.4. Delivery of antibodies through BBB—Many potential brain treatments could use antibody based approaches. For example, Herceptin, which is an anti-HER2 monoclonal antibody, could be used for some brain metastasis of breast cancer [53] and the anti-CD20 monoclonal antibody, Rituximab, for malignant lymphoma [54]. There is also evidence suggesting that antibodies against the A β may reverse cognitive deficits in early Alzheimer's disease [55]. There are, however, major problems in using these promising agents *in vivo* in the CNS because the antibodies have a large molecular size, and thus, they are blocked by the BBB if administered into the circulation.

In order to study the feasibility of using ultrasound (burst length = 10 ms, PRF = 1 Hz, sonication duration = 20 s and frequency = 680 kHz) induced BBB disruption for antibody delivery, the first study [56] used a dopamine-4-receptor-targeting antibody to test the functionality of the antibody after its delivery through the BBB. Post-mortem antibody staining of brain sections of the exposed volume showed positive signals in the brain. When the sections were evaluated under the microscope, the signals detected were characteristic for location of the Dopamin D₄ receptors in the hippocampus and small cells in the basal ganglia within the sonicated tissue volume. The staining was limited to the sonicated location with no obvious signal in the contra lateral site. The staining intensity correlated with the MRI contrast

enhancement seen after the sonications, indicating that the MRI signal may be useful in quantifying and controlling the antibody delivery in the brain.

The second study investigated a delivery of the antibody-based therapy agent Herceptin with the same sonication parameters and demonstrated that it can be delivered through the BBB [57]. Although much more work needs to be done to show that adequate quantities of the antibody based agents can be delivered into the brain, the initial studies show promise.

3.3.5. Delivery of Chemotherapy agents through the BBB—Targeted BBBD could potentially aid in the delivery of chemotherapy agents in brain tumors [58]. The first study investigated the delivery of liposome-encapsulated doxorubicin which does not penetrate the BBB and demonstrated that increased concentrations were detected in the sonicated (burst length = 10 ms, PRF = 1 Hz, sonication duration = 120s and frequency = 1.5 or 1.7 MHz) locations of rat brains [52]. The sonicated locations showed significantly higher concentrations of doxorubicin than the contra-lateral side. The concentration of the drug in the brain tissue was observed to increase linearly with increasing micro-bubble concentration with approximately 1000 ng/g of tissue at clinical diagnostic micro-bubble concentration of 0.1 ml/kg. The measured doxorubicin concentrations should be adequate to produce a clinically significant response [59] if they can be produced in human tumors. Although the treatment looks promising, the effectiveness and side-effects of the treatment in an animal brain tumor model needs to be investigated before the clinical potential can be explored.

3.3.6. Gene delivery to the brain—Gene therapy could have significant potential in the treatment of various CNS diseases [60] but the delivery into the brain is limited by the large size of the agents. It is likely that the focused ultrasound method could be used to deliver gene vectors through the BBB. So far there is only one preliminary study that demonstrated feasibility [44]. That study used genetically engineered Herpes Simplex Virus (HSV) that was radiolabeled using ^{111}In -oxine, and injected intravenously into the animals after focused ultrasound BBB disruption on one side of the brain. The brains were harvested 24 hours after injection of the virus, and subjected to serial sectioning. Autoradiography and histology were performed on the sections and there was a clear Right-Left difference in the brains at the sites of the sonications, indicating focal viral delivery. Serial autoradiographs demonstrated the viral distribution in each of the locations. ROI analysis showed a maximal R/L ratio of 3.56 for a 2.3 MPa location, and 1.74 for a 1 MPa location at the frequency of 1.63 MHz with 100ms bursts repeated at the rate of 1 Hz for 20s.

4. Ultrasound aided delivery of molecules to other neural tissues

Other neural tissues such as spinal cords are also protected by endothelial cells with TJs similar to the brain. Although the spinal cord is protected by a more variable bony structure than the brain it is likely that the same methods could be used to disrupt the BBB in this structure. We are currently performing a preliminary study investigating large molecular agent delivery into the spinal cord. The initial experiments demonstrate feasibility, but much more work needs to be done for exposure optimization.

5. Future potential of ultrasound in agent delivery to the brain

The development of a method that can induce focal and transient disruption of the BBB without permanent tissue damage may make image-guided targeted molecular therapeutic and imaging agent delivery feasible in the brain. For example, advances in tumor cell biology have led to the availability of new types of anti-cancer chemotherapeutic agents that are superior to the conventional agents in that they can precisely target the signal-transduction system unique to malignant tumor cells, thereby lowering their toxic effects on normal cells. Herceptin®

(Trastuzumab) is a humanized monoclonal antibody (mAb) that targets human epidermal growth-factor receptor 2 (HER2/c-erbB2) expressed in the breast cancer cells of some patients. Herceptin® has been used to treat breast cancer patients and succeeded remarkably in controlling local and distal breast cancer lesions [53]. Although breast cancer often metastasizes to the brain [61], Herceptin® could only be used to treat extracranial lesions because there is currently no efficient method to deliver it to the CNS. The increased use of Herceptin® to treat breast cancer patients has resulted in a higher incidence of brain metastasis from primary lesions [62,63]. When Herceptin® was used as a first-line therapy in breast cancer patients, metastatic extracranial lesions responded to the agent in 71% of the patients who continued to develop metastatic lesions in the brain [62]. If this method can be implemented clinically it could have significant application in treating patients with metastatic brain disease. There are also other effective anti-cancer agents that do not penetrate the BBB. For example, Rituxan® (Rituximab), an anti-CD20 mAb, has also been shown to be effective in patients with lymphoma [54]. The ability to aid the delivery of these new agents through the BBB could have a large impact in health care due to the large number of patients that may benefit from these new treatments but who eventually develop brain metastases. This is just one example of the potential of image-guided, focused, ultrasound-induced BBB disruption, and we believe that there are many other potential uses for this technology after it has been clinically tested for the tumor treatments.

6. Conclusions

A number of animal studies have demonstrated that local BBBD is possible with burst focused ultrasound exposures and intravascular micro-bubbles. The required acoustic power values are over 100 times smaller than those required to produce thermal damage in tissue and are thus safe to deliver through intact skull. Two major mechanisms have been identified for the disruption including opening of the TJs and active transport by vacuoles. BBBD is associated with minimal or no damage to the vasculature or the surrounding brain tissue. Survival studies have verified the lack of adverse events in test animals. Focal delivery of antibodies and chemotherapy agents has been shown. However, the quantity of brain uptake and the therapeutic effectiveness of drug delivery for a specific intervention has to be demonstrated in appropriate models before the clinical potential can be determined.

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Figure 1.
A diagram of the ultrasound induced BBBD.

Figure 2.

Top: An example of T1-weighted MR image of a rabbit brain obtained after five sonications (arrows) through a craniotomy and an injection of a bolus of gadolinium contrast agent. The image was acquired across the focus of the ultrasound beam and show local contrast enhancement at the sonicated locations. Bottom: The post mortem slice of the same brain showing trypan blue leakage at the sonicated location. The dye was injected a few minutes after the sonications.

Figure 3. The number of TJ proteins Occludin and Claudin 5 as a function of time before and after sonications of rat brain through intact skull [43].

Figure 4.
An electron microscopy image of horseradish peroxidase transport through the endothelium showing a vacuole emptying its content after transporting it through the endothelial cell [44]. These rat brain sonications were done through the intact skull.

Figure 5.

A. The percentage of sonicated locations in rabbit brain that showed contrast enhancement in T1-weighted MR images after sonication as a function the pressure amplitude during the 10 ms ultrasound bursts at the frequency of 0.68MHz. The sonications for this graph were done through a craniotomy window but the study demonstrated that equivalent response can be induced also through intact skull. The percentage of the locations that showed light microscopy evidence of neural damage is also plotted in the graph [42].

Figure 6.

The normalized T1-signal intensity change after injection of gadopentetate dimeglumine contrast agent in rabbit brain as function of time after sonication when the contrast was injected demonstrating the healing of the BBB [30]. The 10 ms burst sonications at the burst repetition frequency of 1 Hz and at a frequency of 0.26 MHz were done through intact skull.

Figure 7.

The signal intensity change in the sonicated and the unsonicated control brain locations as a function of time after sonication. The was sonication before (A) and after (B) the contrast agent (MION) injection.

Table 1

Mean density (vesicles per μm^2) of HRP-containing vesicles in the endothelial cells' cytoplasm for control and sonicated brain vessels 1h after sonication (mean $\pm$ SD) [44]

	Capillaries	Arterioles	Venules
Controls	0.7 ± 0.23	0.14 ± 0.03	0.17 ± 0.12
Sonicated at 0.69 MHz	0.92 ± 0.25	$1.63 \pm 0.23^*, \dagger$	0.36 ± 0.10
Sonicated at 0.26 MHz	1.05 ± 0.20	$2.43 \pm 0.27, **\dagger$	0.55 ± 0.17

* $P < 0.05$ compared with capillaries at same sonication

** $P < 0.001$ compared with capillaries at same sonication

$\dagger$ $P < 0.001$ compared with arterioles controls and to venules at same sonication