Advances in neurosonology — Brain perfusion, sonothrombolysis and CNS drug delivery

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Summary Advances in microbubble pharmacology together with novel ultrasound technologies are leading to new emerging applications for both assessment and treatment of stroke patients. Ultrasound perfusion imaging, for example, has added new perspectives for diagnosis and monitoring of both ischemic and hemorrhagic stroke. Real-time brain perfusion imaging of cerebral infarctions is now possible and quantitative algorithms for evaluation of regional cerebral blood flow are being applied for the first time in humans. There is growing interest in therapeutic applications of ultrasound, particularly in the field of sonothrombolysis. Recent results indicate that ultrasound and microbubbles may be effective in clot lysis of ischemic stroke even without additional thrombolytic drugs. Moreover, this combination may be effective in treating the microcirculation, which will give new dimensions to the application of sonothrombolysis in stroke patients. Further therapeutic avenues include opening of the blood–brain barrier (BBB) with ultrasound and microbubbles to enable novel drug delivery to the brain.

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Brain perfusion imaging — towards new clinical applications

The most important advance in brain perfusion imaging during the last several years has been low-mechanical index (MI) real time perfusion scanning. This technique allows the detection of ultrasound contrast agent (UCA) in the cerebral microcirculation with little or no bubble destruction as compared to the high MI-imaging. Because of minimal contrast agent bubble destruction, a high frame rate can be applied, which leads to a better time resolution of bolus kinetics (Fig. 1). Low-MI imaging of contrast agent also avoids the shadowing effect, a significant problem associated with high mechanical index imaging. Because of the high acoustic intensities that are emitted by bursting bubbles, bubbles that are “behind” the emitting bubbles (farther away from the ultrasound transducer) are “shadowed” by this effect and thus obscured from data analysis. Thus, areas of tissue that are shadowed may not be available for analysis of tissue perfusion. The problem of shadowing is basically eliminated with low mechanical index imaging, since bubbles are not destroyed with such low acoustic pressures. Moreover, the technique can obtain multi-planar real-time images of brain perfusion [1]. This is a significant breakthrough for ultrasound perfusion imaging, since previous approaches were confined to a single image plane and therefore limited in their assessment of the extents of brain infarction and low perfusion states. The disadvantage of this new low-MI technique, however, is the limited investigation depth due to the low MI used. Recent technological advances suggest, however, that perfusion imaging of the contralateral hemisphere through the temporal bone window will be possible.

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Figure 1  Real-time acoustic intensity curve of the contrast agent SonoVue™ after bolus injection on a Philips IU22 platform using a 1–5 MHz dynamic pulsed array transducer. A very low mechanical index of 0.017 is used, which is then attenuated about 90% by the skull bone before entering the brain. A region of interest has been drawn in the upper image to determine where mean acoustic intensity will be measured. The smaller images in the middle of the figure are the individual image frames at 14 Hz. The red curve displays the mean acoustic intensity of contrast bolus arrival from the region of interest for a total of 264 frames during 18.81 s.

If a constant concentration of contrast agent is delivered to brain tissue using a constant UCA infusion rate, then after destruction with high MI ultrasound, new microbubbles will enter this field with a certain velocity, will travel a determined distance and fill a certain tissue volume depending on blood velocity. The intensity of the echo response signal is directly related to the contrast agent concentration in the tissue; therefore, the blood flow assessment is based on monitoring the intensity of the echo response signal of the insonated volume after bubbles destruction. Low-MI ultrasound imaging can be used to monitor microbubble replenishment in real time (Fig. 2) following the application of destruction pulses at high MI. The behavior of the refill kinetics can be assessed with an exponential curve fit [2]. The parameters of this exponential curve are related to cerebral blood flow: blood flow velocity is directly related to the rate constant $\beta$, the fractional vascular volume is related to the plateau echo enhancement ($A$), and the product of both ($A \cdot \beta$) is associated with blood flow [3]. More sophisticated algorithms for characterization of microbubble refill have been recently introduced [4,5], which should increase reproducibility and improve the quantification of cerebral blood flow with ultrasound perfusion imaging.

Since individual microbubbles can be depicted flowing through small vessels in the brain with low MI imaging, it is possible to track these bubbles and map perfusion over time. Dynamic microvascular microbubble maps provide excellent demarcation of MCA infarctions (Fig. 3) and provide impressive displays of low velocity tissue microbubble refill following destruction with high mechanical index imaging. In brain regions showing delayed contrast bolus arrival on perfusion-weighted MRI, ultrasound shows decreased or absent microbubble refill kinetics. This new technique has been applied for diagnosis of acute ischemic stroke. Recent results demonstrate that real-time ultrasound perfusion imaging with analysis of microbubble replenishment correctly identifies ischemic brain tissue in acute MCA stroke [6]. Pulse compression methods are being combined with the nonlinear bubble imaging techniques discussed above for highly sensitive contrast imaging with very low noise. These advances will lead to further improvements of microbubble imaging of the brain microcirculation, making ultrasound perfusion imaging a viable application for bedside assessment of acute stroke patients.

Therapeutic applications of ultrasound — sonothrombolysis

Ultrasound applied as an adjunct to thrombolytic therapy improves recanalization of occluded intracerebral vessels and microbubbles can amplify this effect. New data suggest that ultrasound and microbubbles alone may be as
Figure 2  Real-time acoustic intensity curve of the contrast agent SonoVue\textsuperscript{TM} demonstrating refill following bubble destruction with transient high mechanical index imaging. Mean acoustic intensity is measured in the region of interest in the upper image. The smaller images in the middle of the figure are the individual image frames at 14Hz. The yellow arrow shows the first of a series of high mechanical index images for microbubble destruction.

Figure 3  Microvascular map of left MCA infarction (arrows) characterized by absent or diminished contrast agent. The excellent infarction demarcation compares well with MRI diffusion-weighted imaging (bottom images).
Ultrasound-sensitive thrombolytic drug delivery combined with specific targeting is highly attractive. Targeting of clot-dissolving therapeutics can potentially decrease the frequency of complications while simultaneously increasing treatment effectiveness by concentrating the available drug at the desired site and permitting a lower systemic dose [9].

Clinical studies support the use of ultrasound for therapy of ischemic stroke, and first trials of enhancing sonothrombolysis with microbubbles have been encouraging. A recent meta-analysis of all published clinical sonothrombolysis studies confirmed that ultrasound and tPA (with or without microbubbles) increases recanalization compared to tPA alone [10]. These observations have led to design of CLOTBUSTER, a phase III controlled clinical trial of sonothrombolysis.

Sonothrombolysis of spontaneous intracranial hemorrhages

One emerging clinical application is sonothrombolysis of intracranial hemorrhages for clot evacuation using catheter-mounted transducers. As compared with MISTIE (Minimally Invasive Surgery plus T-PA for Intracerebral Hemorrhage Evacuation) and CLEAR (Clot Lysis Evaluating Accelerated Resolution of Intraventricular Hemorrhage II) studies data, the rate of lysis during treatment for IVH and ICH was faster in patients treated with sonothrombolysis plus rt-PA versus rt-PA alone [11]. Thus, lysis and drainage of spontaneous ICH and IVH with a reduction in mass effect can be accomplished rapidly and safely through sonothrombolysis using stereotactically delivered drainage and ultrasound catheters via a bur hole.

MRI-guided focused ultrasound for clot lysis

Histotripsy is a process which fractionates soft tissue through controlled cavitation using focused, short, high-intensity ultrasound pulses. Histotripsy can be used to achieve effective thrombolysis with ultrasound energy alone at peak negative acoustic pressures >6 MPa, breaking down blood clots in about 1.5—5 min into small fragments less than 5 μm diameter [12]. Recent developments in using MR-guided focused ultrasound therapy through the intact skull suggest that this technology could be useful for clot lysis in humans. Experimental studies are currently being undertaken to test this possibility, both in ischemic and hemorrhagic stroke.

Ultrasound and microbubbles for treatment of the microcirculation

Ultrasound and microbubbles may improve flow to the microcirculation irrespective of recanalization, thus opening new opportunities for application of sonothrombolysis in acute ischemic stroke. This was suggested by results of a study on possible adverse bioeffects [13] of 2 MHz ultrasound and microbubbles (Sonovue™) in a middle cerebral artery permanent occlusion model in rats at different steps in the cascade of tissue destruction after ischemic stroke [14]. While deleterious effects were not observed, infarctions were unexpectedly smaller in the treatment group, despite the fact that in all animals recanalization of the MCA did not occur. This suggested a beneficial effect of ultrasound and microbubbles in the microcirculation. A similar tissue protective effect has been found in an in vivo animal study using intravenous microbubbles and transthoracic ultrasound to treat acute coronary thromboses. Pigs treated with ultrasound and intravenous perfluorocarbon microbubbles (PESDA) had significantly greater improvements in ST segments over a 30-minute treatment period when compared with pigs treated with ultrasound alone or with control animals. Moreover, there was a significantly smaller myocardial contrast defect size after treatment with ultrasound and PESDA [15]. Recently, nano-CT was used to demonstrate complete reversal of microcirculatory impairment in a rodent reperfusion model following treatment with rt-PA, ultrasound and microbubbles [16]. The mechanism of the microcirculatory effect of ultrasound and microbubbles may involve improvement of blood flow to risk tissue via collaterals and changes in the microenvironment of damaged tissue, like decreased cell damaging factors, e.g. glutamate or enhanced enzyme activity of endothelial nitric oxide [17]. Further work is necessary to elucidate the exact mechanisms of salvaging of tissue-at-risk by ultrasound-mediated microbubble thrombolysis.

Opening the blood—brain barrier with focused ultrasound and microbubbles

The blood—brain barrier is a significant obstacle for delivery of both small molecules and macromolecular agents. Indeed, potential therapeutic substances, which cannot be applied in the presence of an intact BBB are neuropeptides, proteins and chemotherapeutic agents. Likewise, large-molecules such as monoclonal antibodies, recombinant proteins, antisense, or gene therapeutics do not cross the BBB.

There is a good deal of evidence showing that ultrasound can be used to permeate blood-tissue barriers. Large molecules and genes can cross the plasma membrane of cultured cells after application of acoustic energy [18]. Indeed, electron microscopy has revealed ultrasound-induced membrane porosity in both in vitro and in vivo experiments [19]. High-intensity focused ultrasound has been shown to allow selective and non-destructive disruption of the BBB in rats [20]. If microbubbles are introduced to the blood stream prior to focused US exposure, the BBB can be transiently opened at the ultrasound focus without acute neuronal damage [21]. Thus, the introduction of cavitation nuclei into the blood stream can confine the ultrasound effects to the vasculature and reduce the intensity needed to produce BBB opening (Fig. 4). This can diminish the risk of tissue damage and make the technique more easily applied through the intact skull. In most studies, the confirmation of BBB disruption has been obtained with MR contrast imaging at targeted locations [21—23] or with post mortem histology [20,24].

Targeted delivery of antibodies to the brain has been accomplished with focused ultrasound. Dopamine D(4) receptor-targeting antibody was injected intravenously and shown to recognize antigen in the murine brain following disruption of the BBB with ultrasound [22]. Likewise,
doxorubicin, a chemotherapeutic drug that does not cross the BBB, has been administered to the brain using ultrasound and microbubbles [25]. Different levels of doxorubicin in the brain were accomplished through alteration of the microbubble concentration. These results are encouraging and provide an important framework for future studies aimed at local disruption of the BBB for delivery of macromolecular agents to the brain.

Several avenues of transcapillary passage after ultrasound sonication have been identified. These include transcytosis, passage through endothelial cell cytoplasmic openings, opening of tight junctions and free passage through injured endothelium [26]. One study investigated the integrity of the tight junctions (TJs) in rat brain microvessels after BBB disruption by ultrasound bursts (1.5-MHz) in combination with Optison [27]. BBB disruption, as evidenced by leakage of i.v. administered horseradish peroxidase (HRP) and lanthanum chloride, was paralleled by the apparent disintegration of the TJ complexes, the redistribution and loss of the immunosignals for occludin, claudin-5 and ZO-1. At 6 and 24 h after sonication, no HRP or lanthanum leakage was observed and the barrier function of the TJs, as indicated by the localization and density of immunosignals, appeared to be completely restored. The results of these studies demonstrate that the effect of ultrasound upon TJs is very transient, lasting less than 4 h.

Although much effort has been undertaken to demonstrate the safety of BBB opening with ultrasound and microbubbles, further work is needed to elucidate the molecular effects of this application. Recent data demonstrate that at the upper thresholds of acoustic pressure for safe BBB opening a reorganization of gap-junctional plaques in both neurons and astrocytes may occur [28]. This is important because gap junctions allow transfer of information between adjacent cells and are responsible for tissue homeostasis. Likewise, there is evidence that focused ultrasound-induced opening of the BBB in the presence of ultrasound contrast agents can lead to increased ubiquitinylation of proteins in neuronal cells [29], indicating that brain molecular stress pathways are affected by this treatment. Nevertheless, this new technology for delivering drugs across the BBB will offer exciting opportunities for treatment of a variety of brain diseases in the future.

References


