Local and reversible blood-brain-barrier disruption by noninvasive focused ultrasound at frequencies suitable for trans-skull sonications

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Introduction: Advances in neuroscience have resulted in the development of new therapeutic agents and genes that may be used to study and treat many central nervous system (CNS) diseases [1]. However, the use of these agents is often limited by their access to the CNS via the blood supply because the blood brain barrier (BBB) protects the brain from foreign molecules [2]. There is experimental evidence that focused ultrasound can selectively disrupt the BBB locally in the presence of a circulating agent consisting of preformed gas bubbles [3]. Since the bubbles are restricted to the vasculature, the injury to the surrounding brain tissue has proven minimal. Since ultrasound is strongly attenuated by bone, for brain applications, (according to conventional practice) an acoustic window must be constructed by a craniotomy. Recently it was shown that a sharp focus can be created with exposures through the intact skull, and that the optimal frequency for this is below 1 MHz [4,5]. The first tests showing BBB disruption were at an ultrasound frequency of 1.63 MHz [1]. Since the behavior of gas bubbles in an ultrasound field is highly dependant on the frequency, it is not known whether the BBB disruption can be safely induced at such low frequencies. The purpose of this study was to test MRI guided focal BBB disruption by focused ultrasound at frequencies that penetrate skull and to use electron microscopy (EM) establish the cellular route for the substance delivery.

Methods: The transducer (diameter/radius of curvature: 10/8 cm, frequency: 0.69 MHz) was moved within the MRI (1.5 T, GE Medical Systems) with a positioning device. Pulsed sonications (burst length: 10 ms, repetition frequency: 1 Hz) were delivered to 1-4 locations at a focal depth of 10 mm in the brains of 22 rabbits at peak acoustic pressure amplitude levels ranging from 0.4 to 3.1 MPa. Approximately 10 s prior to the start of the sonication, a bolus of ultrasound contrast agent (Optison, Mallinckrodt Inc. St. Louis, MO) containing micro bubbles was injected in the ear vein. Contrast-enhanced T1-weighted fast spin echo images (TR/TE = 500/17 ms, echo train length = 4, three NEX, FOV = 10 cm, matrix size = 256×256, slice thickness = 1.5 mm, BW=16 kHz, contrast agent: gadopentetate dimeglumine (Magnevist)) were used evaluate the BBB opening. EM was performed in tissue samples from the focal spot locations of four animals after injection of Horse Radish Peroxidase (HRP).

Results: The BBB opening increased as a function of pressure amplitude (Fig.1). Pressure amplitudes of 2.3 MPa and higher demonstrated consistent tissue necrosis. The EM findings confirmed effective BBB disruption, and the cellular mechanisms of HRP passage through the vessel walls included both transendothelial (via caveolae and cytoplasmic vacuolar structures) and paraendothelial (via intercellular clefts) routes (Fig. 2).

Discussion: This study demonstrated for the first time that BBB disruption can be induced at a frequency that can be focused through human skull bone while sustaining little damage to the brain tissue. This finding may have considerable impact on future brain research, diagnostics, and therapy. The mechanisms seen in EM are similar to those from other means of BBB disruption (hypertension, injection of bradykinin, hyperosmotic agents, etc.) [6]. The BBB disruption took place in capillaries, arterioles, and venules, demonstrating similar mechanisms. The data obtained show that blood-circulating macromolecules (at least with molecular weight 40,000) invade the brain interstitial space and thus demonstrate that many therapeutic and diagnostic agents may be used in the brain.

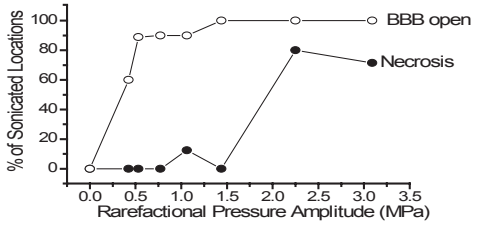

Figure 1: The percentage of sonicated locations that demonstrated BBB disruption and tissue necrosis as a function of the applied pressure amplitude.

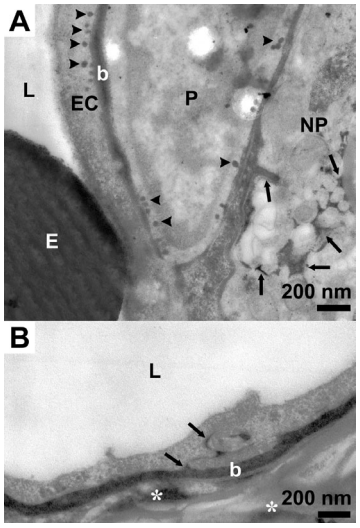

Figure 2: Vessel and perivascular neuropil 19 min after sonication in the presence of HRP (perfusion fixation of the brain). A. Numerous caveolae containing peroxidase (arrowheads) have moved to abluminal front of the endothelial cell (EC) and into the pericyte (P). The tracer has infiltrated the basement membrane (b) and the interstitium of the neuropil (arrows). B. The passage of HRP through interendothelial clefts with evidently opened tight junctions is shown with arrows. Peroxidase has reached the middle and abluminal part of the cleft and has penetrated the basement membrane (b). Staining for HRP also is seen outside the vessel's wall in the neuropil (asterisks). L-lumen; E – red blood cell in the lumen; NP – neuropil.

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Acknowledgments: This research was supported by NIH grants CA76550 and CA089017 and a grant from CIMIT