BBB Disruption With Low-Intensity Ultrasound

Low-Intensity Pulsed Ultrasound Reversibly Opens The Blood-Brain Barrier For Drug Delivery

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ABSTRACT

Treatment of many neurological diseases is impeded by the blood-brain barrier (BBB) that prevents most drugs from entering into the brain. Accidental occurrence of MRI contrast agent extravasation in a patient after treatment with low-intensity ultrasound prompted us to investigate whether low-intensity pulsed ultrasound (LIPUS) could be used to safely and reversibly open the blood-brain barrier, allowing large molecules to pass through. In three pre-clinical rodent studies and an ongoing primate study, evidence has been collected that a localized, safe, and reversible opening of the BBB can be enabled by LIPUS exposure.

LIPUS increases Evans Blue concentration in rat brains

Huang Z[1] , Herken U[2] , Moskowitz M[1]

In this study male Sprague-Dawley rats were used to investigate whether low-intensity unfocussed ultrasound applied over an extended time period would increase extravasation of Evans Blue (EB) dye into the brain tissue.

Methods

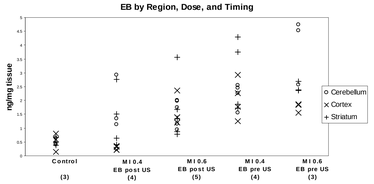
Animals were anesthetized using 2% Isoflurane in an oxygen/nitrogen mixture. 0.1g/kg EB was injected into the right femoral vein of each animal. EB injection in groups #1 and #2 was performed ~15 minutes after the termination of the ultrasound exposure. In groups #3 and #4, EB was injected 30-45 minutes before starting the ultrasound exposure. For ultrasound exposure, animals were placed in a stereotaxic frame and kept under inhalation anesthesia throughout the procedure.

Ultrasound parameters: frequency 300 +/- 30 kHz, pulse repetition frequency 300 Hz, duration of exposure two (2) hours. Each pulse comprised either two (for 10% duty cycle) or four (for 20% duty cycle) bursts of 50 cycles each. Frequency was swept from 270 kHz to 330 kHz during each burst. Ultrasound intensity was either MI 0.4 and 20% duty cycle, or MI 0.6 with 10% duty cycle over a period of 2 hours

Animals were sacrificed after 24 hours and immediately perfused with 500ml 0.9% saline solution. Brains were removed and separated into hemispheres and further into corpus, striatum, and cerebellum for photo spectrometric measurements of EB concentration.

Results

All groups exposed to ultrasound showed increased EB concentration in brain tissue (see graph). When EB was injected after ultrasound exposure, concentration increased by about 100% (MI 0.4) and 200% (MI 0.6). EB injection prior to ultrasound exposure resulted in an increase of tissue concentration by about 380% (MI 0.4) and 450% (MI 0.6). These results appear to indicate that ultrasound at these intensities will reversibly increase BBB permeability, and that the effect will last only for a short time after ultrasound exposure is terminated.



Evans Blue concentration after LIPUS exposure depends on ultrasound intensity and application time

BACKGROUND

The BBB represents both a safeguard against the penetration of physiologically harmful substances into the central nervous system (CNS), and a considerable hurdle to the delivery of therapeutic agents. Blood vessels in the brain differ from those in the rest of the body in that they prevent most drugs from passing into the surrounding tissue. While this is often beneficial, it can inhibit the range of therapies that can be employed to successfully treat diseases such as Alzheimer's and brain cancer.

A technology allowing safe, targeted, reversible opening of the BBB would potentially revolutionize both the study and treatment of CNS disorders, including neurodegenerative conditions and brain malignancies that have proven resistant to conventional approaches.

BBB disruption with hypertonic solutions (typically mannitol) enhances CNS penetration of macromolecules [1], but at the cost of pronounced fluid shifts and lack of regional specificity. Ultrasound-mediated BBB disruption has been another approach explored. High intensity focused ultrasound (HIFU) has been demonstrated to open the BBB at energy levels that do not result in cellular injury[2], but a drawback is the long duration of opening induced.

LIPUS has been used since the early 1990's to accelerate bone healing, but only recently some insight into potential mechanisms of action has been gained [3-6]. The upregulating effect of LIPUS on cytokines and chemokines that has been observed here might also occur in other tissues.

LIPUS enables transfection of Adeno Virus in rat brain

Gianni D[3] , Abbed K[3], Herken U[2], Chiocca EA[3]

In this study male Sprague-Dawley rats were used to investigate whether low intensity ultrasound could enhance delivery of adenovirus with LacZ and GFP reporter genes across the BBB.

Methods

Animals were anesthetized using 2% Isoflurane in an oxygen/nitrogen mixture. After a medial neck incision, a PE 10 catheter was introduced into the internal carotid artery. For ultrasound exposure, animals were placed in a stereotaxic frame and kept under inhalation anesthesia throughout the procedure. After 90 minutes of ultrasound exposure, 1ml of virus (5x10^8 titer) was injected over 10 seconds. Three animals received high dose intra-arterial mannitol injections instead of ultrasound prior to virus application as positive controls.

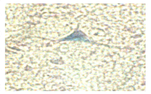
Ultrasound parameters: frequency 300 +/- 30 kHz, duty cycle 20%, pulse repetition frequency 300 Hz, duration of exposure 2 hours. Each pulse comprised four bursts of 50 cycles each. Frequency was swept from 270 kHz to 330 kHz during each burst.

After 2 or 4 days animals were sacrificed and immediately perfused with 500ml 0.9% saline solution. Brains were removed, separated into frontal, medial, posterior, and cerebellar sections, and shock frozen. Thin sections were slide mounted for staining and microscopy, other sections were used for extraction of virus DNA.

Results

Ultrasound exposed animals and controls showed GFP and LacZ expression but interpretation was difficult because of artifacts and the isolated occurrence of transfected cells. In all treated animals, reporter gene expression was found mostly in the basal ganglia. In some instances, expression could be found in the cortex. No expression was observed in the negative controls. PCR for the virus backbone showed presence of the virus in the single ultrasound treated animal that was sacrificed after two days. No significant amount of virus could be identified in negative controls or in other animals that were sacrificed after four days. Low-intensity ultrasound appears to be capable of delivering an adeno-virus across the BBB. Low transfection rates even in the positive controls indicate that passage through the BBB is not the only obstacle to transfection.

PCR of Virus DNA



Neuron with positive XGal staining after LIPUS

LIPUS increases Ad-Luc expression in rat brains

Ogden J[4], Loganathan A[4], Herken U[2], Suzuki M[4], Chiocca EA[4]

Male Sprague-Dawley rats were exposed to LIPUS using three different burst lengths, and then received Adeno virus with a luciferase reporter gene (Ad-luc) to investigate whether pulse length had an influence on BBB disruption.

Methods

Animals were anesthetized using 2% Isoflurane in an oxygen/nitrogen mixture. After a medial neck incision, a PE10 catheter was introduced into the right internal carotid artery. The catheter was continuously perfused with normal saline at a rate of 150 µl/min.

For ultrasound exposure, animals were placed in a stereotaxic frame and kept under inhalation anesthesia throughout the procedure. After 60 minutes of ultrasound exposure, 2 ml of virus was injected over 20 minutes while ultrasound exposure was continued.

Ultrasound parameters: frequency 300 +/- 30 kHz, duty cycle 10%, total duration of exposure 1 hour 20 minutes. Ultrasound bursts lengths were 300 cycles (short), 600 cycles (medium), and 1200 cycles (long), with pulse repetition frequencies of 100 Hz, 50 Hz, and 25 Hz, respectively. Frequency was swept from 270 kHz to 330 kHz, with increments of 2 kHz after 10 bursts.

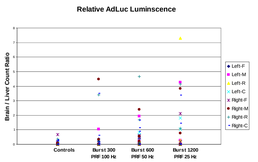
After 2 days animals were sacrificed and brains were removed, and each hemisphere was separated into frontal, medial, posterior, and cerebellar sections. Additional liver samples were collected for reference. All samples where weighed, then homogenized in tissue lysis buffer. After incubation with luciferin, relative luminescence units where determined for each sample.

Results

A large number of animals did not show luminescence of liver samples, indicating failed systemic virus delivery, and had to be excluded. We saw higher occurrence of transfection in ultrasound treated animals compared to controls, supporting the theory that LIPUS can be used to deliver viral vectors across the BBB. A high degree of variability made if difficult to clearly identify a correlation between pulse length and delivery effect. The table shows the number of animals included in each treatment group and the number of animals with a positive luminescence in at least one brain sample, the total number of brain samples per group, and the number of samples with positive luminescence.

Because of large variability in liver luminescence counts, we are showing brain luminescence for each sample as a value relative to the liver count measured in the animal. The graph shows the relative counts for each group and also indicates the location of the samples.

Pulse length	Animals	Positive	%	Samples	Positive	%
Control	11	2	18.2%	88	5	5.7%
Short	5	3	60.0%	40	9	22.5%
Medium	6	4	66.7%	48	18	37.5%
Long	6	2	33.3%	48	15	31.3%

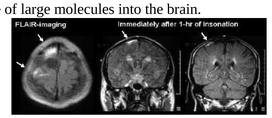


AdLuc reporter gene activity after LIPUS exposure

LIPUS accidentally causes BBB opening in human

Accidental discovery of a permeabilizing effect on the BBB occurred when non-invasive transcranial application of LIPUS to a human patient was found to cause enhanced extravasation of MRI contrast material without evidence of injury [7]. The effect at that time was considered to be a serious adverse event, but at a later time we considered the possibility that LIPUS might be used for drug delivery to the brain. Several subsequent pre-clinical studies appear to indicate that LIPUS can reversibly open the BBB to allow passage of large molecules into the brain.

Extravasation of Gd-DTPA after LIPUS exposure (from [7])



LIPUS Mediated Blood-Brain Barrier Permeabilization in Primates

Lawrence M[5] , Herken U[2], Switzer R[6] , Redmond D[5], Gullans S[5]

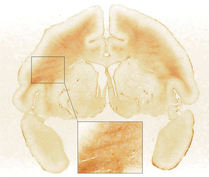
In this ongoing study, we are using African green monkeys (Chlorocebus aethiops sabeus) with a body weight of 5-6 kg to investigate LIPUS dose dependency of localized BBB opening.

Methods

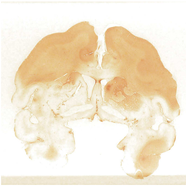
Animals are anesthetized with 1-1.5% Isoflurane, placed in a stereotaxic frame, and exposed to 60 minutes of LIPUS or no ultrasound (sham treatment). Ultrasound bursts with a length of 300 cycles are delivered at an energy defined by the peak mechanical index (MI) in situ of 0.25 or 0.35, with or without a frequency sweep. If a frequency sweep is used, ultrasound frequency is incremented in 2 kHz steps from 270 kHz to 330 kHz.

Animals are sacrificed 24 hours after ultrasound exposure and perfused with heparinized PBS, followed by a 4% paraformaldehyde solution.

Coronal brain sections are mounted on slides and incubated with an antibody against monkey IgG and then stained with a secondary antibody. The degree of regional BBB leakage is indicated by the amount of positive IgG staining. Normally only minimal amounts of IgG are present in regions outside the circumventricular organs (midline structures bordering the 3rd and 4th ventricles), which stain darkly for IgG.



Left: Regional BBB disruption after lower LIPUS intensity. Note the striated patterns. Right: Disruption pattern after higher intensity LIPUS.



Results

To date nine animals have been treated with ultrasound, and two animals served as controls. We have not observed any hemorrhages or neurological deficits in any of the animals. Controls showed only minimal amounts of IgG staining outside of the circumventricular organs, as expected.

Animals treated with the lower ultrasound dose (MI 0.25) showed uneven staining. In some regions staining appears streaked. This could indicate an occurrence of standing ultrasound waves, resulting in sufficient energy delivery and subsequent BBB opening only in those areas where antinodes occur. Animals treated with the higher ultrasound dose (MI 0.35) mostly showed pronounced regional BBB opening. The difference in outcome between lower dose and higher dose treatment could indicate a threshold effect. While the intensity required for BBB opening at the lower ultrasound dose only occurs at the antinodes of some standing wave patterns, the required dose occurs in a complete region when using the higher ultrasound dose. (This study was supported by NIH grant 1R43NS054410-01)

REFERENCES

1. Neuwelt, E.A., et al., Osmotic blood-brain barrier disruption. Computerized tomographic monitoring of chemotherapeutic agent delivery. J Clin Invest, 1979. 64(2): p. 684-8.
2. Hynynen, K., et al., Local and reversible blood-brain barrier disruption by noninvasive focused ultrasound at frequencies suitable for trans-skull sonications. Neuroimage, 2005. 24(1): p. 12-20.
3. Bandow, K., et al., Low-intensity pulsed ultrasound (LIPUS) induces RANKL, MCP-1, and MIP-1beta expression in osteoblasts through the angiotensin II type 1 receptor. J Cell Physiol, 2006. 13: p. 13.
4. Ikeda, K., et al., Effects of low-intensity pulsed ultrasound on the differentiation of C2C12 cells. Life Sci, 2006. 79(20): p. 1936-43.
5. Sena, K., et al., Early gene response to low-intensity pulsed ultrasound in rat osteoblastic cells. Ultrasound Med Biol, 2005. 31(5): p. 703-8.
6. Takayama, T., et al., Low-intensity pulsed ultrasound stimulates osteogenic differentiation in ROS 17/2.8 cells. Life Sci, 2007. 80(10): p. 965-71.
7. Reinhard, M., et al., Blood-brain barrier disruption by low-frequency ultrasound. Stroke, 2006. 37(6): p. 1546-8.

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