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QUANTITATIVE ANALYSIS OF CEUS GUIDED BLOOD-BRAIN BARRIER OPENING IN AN IN VITRO ISOLATED BRAIN PREPARATION

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ABSTRACT

US-induced BBB opening was performed on an isolated guinea pig brain maintained in vitro by arterial perfusion, a unique experimental preparation in which the BBB is morphologically and functionally preserved for several hours. The US system mounted a planar transducer with nominal frequency of 0.989 MHz. Sonications were carried out for 2 consecutive minutes with intensity from 20 to 100 mW/cm2, pulsed for 100 ms ON, 900 ms OFF duty cycle (1 kHz pulse repetition rate). MBs were used to provide the cavitating gas for US-mediated BBB disruption. A portable ultrasonographic device with a linear probe was used to obtain CEUS recordings before, during and after the procedure. Regions of interest (ROIs) were selected and peak enhancement and time intensity curves (TICs) were quantified. BBB permeabilization was assessed by quantifying at confocal microscopy the extravasation of FITC- albumin perfused after each treatment. BBB opening was effective in the hemispheres that received TUS stimulation during MBs infusion, as unilateral leakage of arterially-injected FITC-albumin into the brain parenchyma was seen only in treated hemispheres. CEUS videos were qualitatively analyzed; this preliminary analysis confirmed our hypothesis, suggesting a direct correlation between MBs concentration and the degree of BBB opening observed in histological sections.

Keywords: *guinea pig brain, US, BBB opening*

1. INTRODUCTION

Microbubbles (MB) have been extensively used as contrast agents in ultrasonography (US). In neurosurgery, contrast enhanced US (CEUS) is used to intraoperatively characterize anatomical and pathological structures in the brain, in a non-invasive and real-time fashion. [1] It has been proven that MBs concentration is not uniform and is influenced by the perfusion level of different brain areas, rather than by the tissue's intrinsic echogenicity. In addition, MBs concentration is directly correlated to US signal intensity and different anatomical structures (white matter, grey matter, basal ganglia) or lesions display a different pattern of contrast uptake. [2] The use of MBs in the central nervous system is not limited to intraoperative imaging, indeed US induces an acoustic response that leads to the controlled oscillation of the gaseous core of MBs. This mechanism, called acoustic cavitation, loosens the intercellular junctions of the vessel walls to transiently increase BBB permeability. [3-4] To our knowledge, the correlation between the dynamic distribution of MBs and the degree of BBB opening has not been investigated yet. The aim of our study is to quantitively measure the

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concentration of MBs before, during and after US-induced BBB opening to demonstrate that MBs concentration correlates with the efficacy of the procedure.

2. MATERIALS AND METHODS

US-induced BBB opening was performed on an isolated guinea pig brain maintained in vitro by arterial perfusion, a unique experimental preparation in which the BBB is morphologically and functionally preserved for several hours. [5] The US system mounted a planar transducer with nominal frequency of 0.989 MHz. Sonications were carried out for 2 consecutive minutes with intensity from 20 to 100 mW/cm2, pulsed for 100 ms ON, 900 ms OFF duty cycle (1 kHz pulse repetition rate). MBs were used to provide the cavitating gas for US-mediated BBB disruption. A portable ultrasonographic device with a linear probe was used to obtain CEUS recordings before, during and after the procedure, see Figure 1.

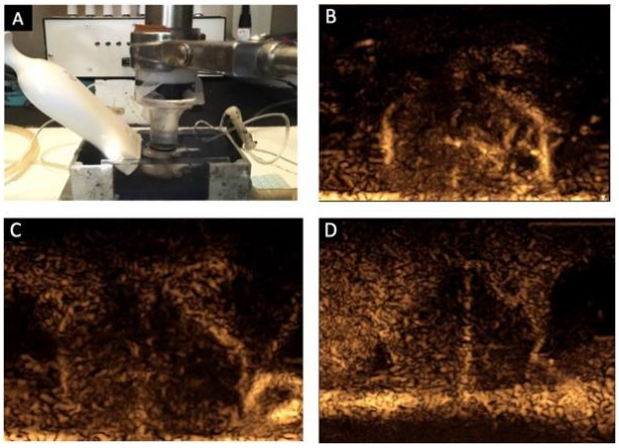

Figure 1. A: experimental setup comprising US-imaging probe, TUS planar transducer, isolated guinea pig brain. B: pre-treatment CEUS acquisition. C: during treatment CEUS acquisition. D: after treatment CEUS acquisition. The right hemisphere is the treated one, note the MBs signal reduction in the post-treatment acquisition.

CEUS videos were retrospectively analyzed using a custom image processing software created with MATLAB's App Designer for quantitative analysis of echo power. Regions of interest (ROIs) were selected and peak enhancement and time intensity curves (TICs) were quantified. BBB permeabilization was assessed by quantifying at confocal microscopy the extravasation of FITC-albumin perfused after each treatment. MB concentration in ROIs was compared to FITC-albumin extravasation in the corresponding histological section, Figure 2.

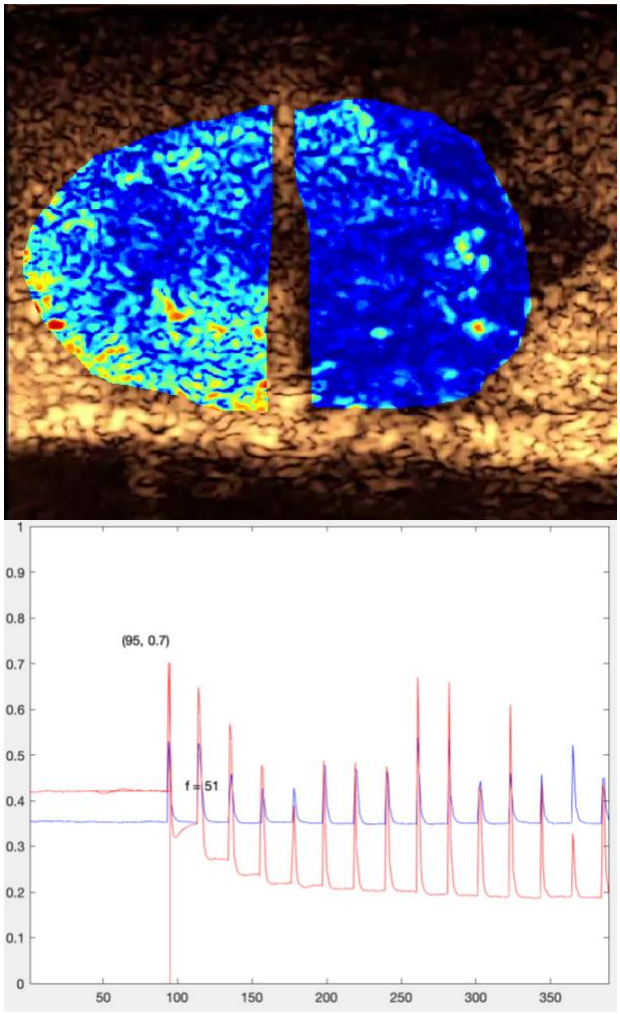

Figure 2. A: ROIs selected on the isolated guinea pig brain, corresponding to the treated hemisphere on the left and the control hemisphere on the right. B: Time-intensity curves of the treated hemisphere (red) and the control hemisphere (blue). Note the signal reduction with lower peak values in the treated hemisphere.

3. RESULTS

BBB opening was effective in the hemispheres that received TUS stimulation during MBs infusion, as unilateral leakage of arterially-injected FITC–albumin into the brain parenchyma was seen only in treated hemispheres. CEUS videos were qualitatively analyzed by a senior neurosurgeon (F.P.); this preliminary analysis confirmed our hypothesis, suggesting a direct correlation between MBs concentration and the degree of BBB opening observed in histological sections. Quantitative preliminary results showed a temporary rapid reduction of MBs signal in time intensity curves in the treated hemisphere during TUS, compared to a stable or slight reduction of signal in the control hemisphere. The lowest peak of time intensity curves was calculated both in treated and control hemispheres. Average lowest peak values were 0.16 in treated hemispheres and 0.23 in controls. The average Delta between the two groups was 0.07. These results are not statistically significant. The limitation of our model is the lack of venous drainage, impacting MBs circulation.

4. DISCUSSION

The results of this study demonstrate the feasibility of blood-brain barrier opening by means of TUS coupled with MBs in an in vitro isolated guinea pig brain. [6]
The qualitative analysis of MBs concentration suggests that the hypothesized correlation between MBs concentration and BBB opening is correct: the interaction between TUS and microbubbles leads to a reduction of microbubble signal intensity visualized on CEUS acquisitions obtained during treatment with the US portable device.
In addition, the preliminary quantitative analysis performed on CEUS acquisitions showed lower negative peak values in treated hemispheres with respect to control hemispheres, giving credit to the qualitative observation.
This reduction in MBs signal during treatment, observed both qualitatively and quantitatively, could be caused by the mechanical interaction between microbubbles and TUS, which is in turn responsible for acoustic cavitation, the mechanism underlying BBB opening.
The secondary endpoint of this study will be to compare the entity of MBs signal reduction to the entity of BBB-opening measured at confocal microscopy, to understand whether there is a statistical correlation between the degree of BBB opening and the degree of MBs signal reduction.
If a finer and statistically significant quantitative analysis confirms these preliminary results, our study would provide the first proof of principle of a correlation between the dynamic distribution of MBs and the entity of BBB opening. The knowledge of this correlation would contribute to a safer translation of said technology in neurological and neurosurgical practice.

5. REFERENCES

[1] Prada, F. et al. Intraoperative contrast-enhanced ultrasound for brain tumor surgery. Neurosurgery 74, 542–552 (2014).

[2] Prada F, Gennari AG, Linville IM, Mutersbaugh ME, Chen Z, Sheybani N, DiMeco F, Padilla F, Hossack JA. Quantitative analysis of in-vivo microbubble distribution in the human brain. Sci Rep. 2021

[3] Tung YS, Vlachos F, Feshitan JA, Borden MA, Konofagou EE. The mechanism of interaction between focused ultrasound and microbubbles in blood-brain barrier opening in mice. J Acoust Soc Am. 2011 Nov;

[4] Wang S, Samiotaki G, Olumolade O, Feshitan JA, Konofagou EE. Microbubble type and distribution dependence of focused ultrasound-induced blood-brain barrier opening. Ultrasound Med Biol. 2014

[5] de Curtis M, Librizzi L, Uva L. The in vitro isolated whole guinea pig brain as a model to study epileptiform activity patterns. J Neurosci Methods. 2016

[6] Librizzi L, Uva L, Raspagliesi L, Gionso M, Regondi MC, Durando G, DiMeco F, de Curtis M, Prada F. Ultrasounds induce blood-brain barrier opening across a sonolucent polyolefin plate in an in vitro isolated brain preparation. Sci Rep. 2022