Proceedings of Engineering and Technology Innovation, vol. 3, 2016, pp. 13 - 15

Behavioural Assessment of Blood-Brain Barrier Opening Induced by Various Ultrasound Parameters

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Received 23 March 2016; received in revised form 15 April 2016; accepted 02 May 2016

Abstract

Focused ultrasound (FUS)-induced opening of the blood-brain barrier (BBB) in the presence of microbubbles is prospective for a targeted drug delivery to the brain lesion. However, the safety of this technology has been addressed recently. The purpose of this study was to assess the behavioural changes following the FUS induced-BBB disruption (BBBD). The behavioural assessment of rats were tested using the open field and hole-board from day 1 through day 32 after undergoing BBBD induced by FUS with either a mild or heavy parameter. We found that heavy BBBD induced behavioral changes, including significantly increased locomotor activity and a longer latency to nose poke for baits. Additionally, mild BBBD resulted in significant decrease of central activity. Therefore, the behavioural changes after FUS induced BBBD should be considered before clinical application.

Keywords: behavioural evaluation, focused ultrasound, blood-brain barrier, memory

1. Introduction

Focused ultrasound (FUS) with microbubbles has offered the potential to produce BBB disruption (BBBD) noninvasively in specific regions of the brain [1, 2]. However, the greatest limitation on the use of FUS-induced BBBD in clinical practice consists of safety concerns relating to cavitation in the brain. Mechanical effects may be responsible for FUS-induced BBBD, but inertial cavitation could usually cause hemorrhaging or apoptosis in the brain tissue from the neighboring vessel [3]. Although no significant negative effects resulting from histological examination, further evaluation of brain functions following FUS-induced BBBD were still needed. Such research will increase our knowledge of the behavioral alterations that occur following BBBD and allow for a better assessment of the safety of this technique in terms of its effects on brain functions. Therefore, the purpose of this study was to examine the impact of BBBD in terms of behavioral alterations following FUS exposure in the presence of microbubbles.

2. Method

All procedures involving animals were conducted in accordance with the guidelines for the Care and Use of Laboratory Animals. The study protocol was approved by the Animal Care and Use Committee of National Yang Ming University. Male Sprague-Dawley (SD) rats weighing from 300 to 350 g were used in this study.

2.1. Ultrasound Setup and Behavioural Assessment

The ultrasound system and equipment setup were the same as used in our previous study [4]. The BBB disruption (BBBD) can be quantified based on the extravasation of Evans blue (EB), which binds to albumin. Activity in the open field was tested with the automated Flex-Field/Open field Photobeam Activity System on post-sonication days 1, 9, 18, and 32. Spatial learning ability was studied by means of a hole-board apparatus in which food rewards were used as positive motivation.

2.2. Number Synthesis

Three rats from each group were prepared for histological observation. The rats were perfused with saline and 10% neutral buffered

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formalin on days 1 and 9 after the FUS sonication. The brain slices were stained by TUNEL staining (DeadEnd Colorimetric TUNEL system, G7130, Promega, Madison, WI, USA) in order to detect DNA fragmentation and apoptotic bodies within the cells.

All values are shown as means ± SEM. The behavioral assessment data were analyzed using the Mann-Whitney U test. Other data were analyzed using the unpaired Student's *t* test. Statistical significance was defined as a p value ≤ 0.05.

3. Results and Discussion

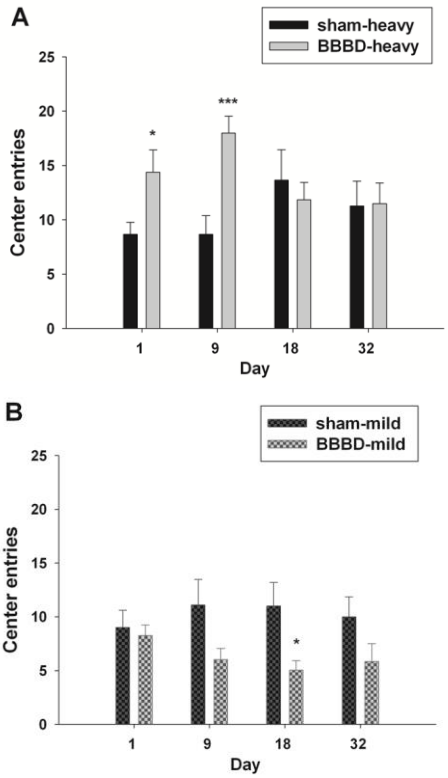

Fig. 1 Effects of rats with FUS-induced BBBD on the open field activity. (A) The number of center entries was significantly increased in the BBBD-heavy group on days 1 and 9 following sonication. (B) But the number of center entries was significantly decreased in the BBBD-mild group on day 18 following sonication. * denotes significant differences compared to the sham group at the same time points. (*, $p<0.05$; ***, $p<0.005, n = 8$)

BBBD-heavy exposed rats entered the center of the open field significantly more frequently on days 1 and 9 post-sonication compared with sham group (Fig. 1(A)). BBBD-mild exposure significantly reduced the number of center entries on day 18 post-sonication compared with sham group (Fig. 1(B)).

The BBBD-heavy group exhibited a significantly longer latency and latency to first baited hole compared with the sham group (Fig. 2(A)). No significant differences in latency and latency to first baited hole were found between the BBBD-mild group and sham group (Fig. 2(B)).

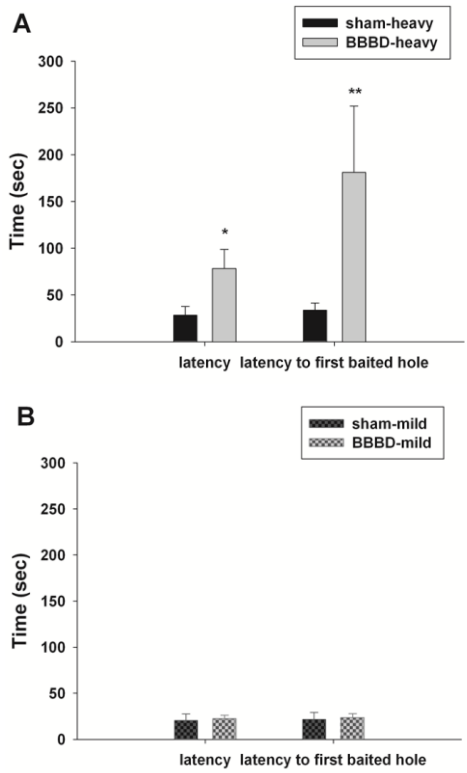

Fig. 2 Hole-board testing was performed on day 12 following sonication. The latency and latency to the first baited hole were recorded in (A) the BBBD-heavy group and (B) the BBBD-mild group. * denotes a significant difference relative to sham group. (*, $p<0.05$; **, $p<0.01, n = 8$)

To our knowledge, the current study is the first to explore the effect of BBBD induced by FUS on behavioral alterations in an animal model. The findings from this study indicate that BBBD-heavy rats produced hyperactivity relative to sham animals under open field test.

Moreover, the BBBD-heavy rats revealed spatial memory impairment in the hole-board test compared to sham group. In contrast, BBBD-mild rats only exhibited anxiety-related behaviors in the open field test.

4. Conclusions

In this paper, FUS-induced BBBD represents a major advance in the targeted drug delivery of the brain. The current study suggests the possibility that manipulation of FUS-induced BBBD might produce a variety of behavioral changes after enhanced drug delivery. Further investigations of behavioral alterations in animals following FUS-induced BBBD are needed in order to avoid abnormal brain functioning in humans following future clinical uses of FUS-induced BBBD.

Acknowledgement

This study was supported by grants from the Ministry of Science and Technology of Taiwan (no. MOST 104-2314-B-010-003-MY3).

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