Improved Drug Distribution using Rapid Short-Pulse (RaSP) Sequences In Vivo to Open the Blood-Brain Barrier

Sophie Morse, Antonios N Pouliopoulos, Julien Lin, Tiffany Chan, James J Choi

Noninvasive Surgery and Biopsy laboratory, Department of Bioengineering, Imperial College London, London, SW7 1AZ, United Kingdom

Introduction

One third of the worldwide disease burden – number of years lost due to disease – is caused by brain diseases, such as dementia, Parkinson’s and brain cancer (DiLuca and Olesen 2014). With an aging population, this burden is expected to rise. Despite the global effort to develop new treatments, there are still no effective drugs for these diseases. One of the major reasons for the lack of successful therapies is that the majority of drugs cannot enter the brain due to the blood-brain barrier (BBB) (Lu et al 2014). A promising solution to this problem is the use of focused ultrasound and microbubbles to locally and noninvasively open the BBB so that therapeutic agents can enter the brain (Hynynen et al 2001). Since its inception, ultrasound technology has been shown to deliver a range of drugs across the BBB (Burgess et al 2015). However, several underlying factors, such as concerns of efficacy and safety, have prevented its clinical translation in human patients (Baseri et al 2010, Choi et al 2007, Pouliopoulos et al 2016). We have previously shown that current ultrasound technology produces a poor drug distribution and damages arteries (Choi et al 2010). This efficacy-safety limit is a result of the use of conventional ultrasound pulse shapes and sequences, which have poor control over cavitation dynamics, generating a mixture of desired and undesired cavitation activity (Choi and Coussios 2012). We have recently developed and tested a new low pressure rapid short-pulse (RaSP) sequence in vitro, designed to promote the desired cavitation activity in the correct location (Pouliopoulos et al 2016). This new sequence will be evaluated here for its ability to improve the efficacy and safety of ultrasound-mediated drug delivery to the brain in vivo.

Methods

Rapid short-pulse (RaSP) sequences consist of short pulses emitted at high pulse repetition frequencies separated by off-time intervals in the range of microseconds. In vitro studies have shown that these sequences prolong the lifetime of microbubbles and increase their mobility during the off-time intervals, enhancing both the temporal and spatial distribution of acoustic cavitation activity (Pouliopoulos et al 2016). In this work, RaSP sequences were tested in vivo at a peak-negative pressure of 400 kPa for their ability to efficiently and safely open the blood-brain barrier (pulse length (PL): 5 cycles; pulse repetition frequency (PRF): 1.25 kHz; burst length: 10 ms). Drug delivery patterns produced using these sequences were compared against those produced by conventionally used long-pulse sequences at the same acoustic pressure (PL: 10,000 cycles; PRF: 0.5 Hz; burst length: 10 ms). Fluorescently-tagged (Texas Red) 3 kDa dextran and microbubbles were intravenously injected in mice while sonicating the left hippocampus with a 1 MHz focused ultrasound transducer. A 7.5 MHz passive cavitation detector captured the microbubble-seeded acoustic emissions. The relative dose and distribution of the drug were quantified by calculating the normalised optical density (NOD, the average increase in fluorescence in the targeted area normalised by the control) and the coefficient of variation (COV, the ratio of the standard deviation by the average fluorescent intensity in the targeted region). Safety was assessed by haematoxylin and eosin (H&E) histological staining.
Results

Despite emitting 150 times less acoustic energy, RaSP sequences delivered a dextran dose of the same order of magnitude as the long-pulse sequences (Fig.2). Moreover, the drug distribution was significantly more uniform using RaSP sequences (Fig.1), as indicated by the coefficient of variation (Fig.2). Unlike the long-pulse sequences, RaSP sequences did not produce as prominent vascular effects, where dextran accumulates in the large vessels (Fig.1c). Acoustic emissions from the short-pulses were more stable than those from the long-pulses, with the energy smoothly decreasing with time. Based on H&E analysis, the dextran delivery was considered to be safe as no tissue damage or haemorrhage was observed in the targeted region.

Conclusion

These results suggest that RaSP sequences can generate a more uniform distribution of acoustic activity in space and time, leading to an improved spatial distribution of dextran. Low pressure RaSP sequences could result in a more efficient and safe delivery of agents across the blood-brain barrier to treat diseases such as Alzheimer’s, Parkinson’s and brain cancer.

Figure 1. Comparison of the drug delivery distributions for RaSP and conventional long-pulse sequences. Fluorescent 3 kDa dextran (Texas Red) delivered to the left hippocampus (a,c) at 400 kPa peak-negative pressure using RaSP pulse sequences (a,b) and conventional long-pulse sequences (c,d). The right hippocampus was used as a control (b,d). Artefacts are present in the conventional long-pulse fluorescent control image, where folds and ventricle fluorescence is present (d).
Figure 2. Quantification of the normalised drug dose and drug distribution created using RaSP and conventional long-pulse sequences. Normalised optical density (NOD), calculated as a measure of the normalised drug dose, and coefficient of variation (COV), as a measure of the drug distribution, shown for the RaSP and conventional pulse sequences at 400 kPa peak-negative pressure (* p < 0.01; ns = not significant). The drug distribution is plotted as (1 - COV) to show the better distribution of the short-pulse sequences (RaSP).

References


