Journal of Controlled Release 192 (2014) 1–9

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

# Magnetic-resonance imaging for kinetic analysis of permeability changes during focused ultrasound-induced blood–brain barrier opening and brain drug delivery

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ARTICLE INFO

*Article history:*
Received 13 March 2014
Accepted 15 June 2014
Available online 23 June 2014

*Keywords:*
Focused ultrasound
Microbubble
Blood–brain barrier
MRI
Kinetic analysis

ABSTRACT

Focused ultrasound (FUS) with the presence of microbubbles has been shown to induce transient and local opening of the blood–brain barrier (BBB) for the delivery of therapeutic molecules which normally cannot penetrate into the brain. The success of FUS brain–drug delivery relies on its integration with in-vivo imaging to monitor kinetic change of therapeutic molecules into the brain. In this study, we developed a dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) technique for kinetic analysis of delivered molecules during FUS-BBB opening. Three kinetic parameters ($K_{trans}$, $V_e$, $K_{ep}$) were characterized dynamically to describe BBB-permeability at two FUS exposure conditions (0.4 or 0.8 MPa) over 24 h. $K_{trans}$, defined as the influx volume transfer constant from plasma to EES, and $V_e$, the EES volume fraction, were both found to be pressure-dependent. $K_{trans}$ and $V_e$ showed a peak increase of 0.0086–0.0131 $min^{-1}$ (for 0.4–0.8 MPa pressure), and 0.0431–0.0692, respectively, immediately after FUS exposure. Both parameters subsequently decreased exponentially as a function of time, with estimated half-lives of decay of 2.89–5.3 and 2.2–4.93 h, respectively. The kinetics of $K_{ep}$, defined as the efflux rate constant from the extracellular extravascular space (EES) to the plasma, were complementary to $K_{trans}$, with an initial decrease from 0.2010 to 0.1901 $min^{-1}$ followed by a significantly longer recovery time (half-life of 17.39–99.92 h). Our observations strongly supported the existence of imbalanced and mismatched kinetics of influx ($K_{trans}$) and efflux ($K_{ep}$) between the plasma and EES, indicating the existence of directional permeability during FUS-BBB opening. We further showed that kinetic change determined by DCE-MRI correlated well with the concentration of Evans Blue (EB)-albumin (coefficient of 0.74–0.89). These findings suggest that MRI kinetic monitoring may serve as an alternative method for in-vivo monitoring of pharmacokinetics and pharmacodynamics (PK/PD) change of therapeutic agents during drug delivery to the brain, and provide useful information for future optimization of FUS-BBB opening.



## 1. Introduction

A major challenge for delivering drugs to the brain is the presence of the blood–brain barrier (BBB), a structure that is composed of endothelial cell tight junctions to protect the brain from harmful substances circulating in the blood [1]. Focused ultrasound (FUS) exposure with intravascular injection of microbubbles has been reported to open the BBB in a non-invasive, transient and local manner [2–5]. Bursts of acoustic ultrasound induce microbubble cavitation in the vasculature, producing mechanical stress that causes endothelial cell deformation and temporarily disrupts tight junctions to increase BBB permeability. This effect offers a wide spectrum of opportunities for delivering large-molecule neurotherapeutic agents into the brain [6–8]. However, FUS-BBB opening is only sustained for a few hours after sonication, as observed by contrast-enhanced magnetic resonance imaging (MRI) [2,3], optical imaging [9], and immune-electron microscopy [10]. Due to the highly dynamic nature of BBB permeability, the narrow window of increased permeability needs to be well characterized in order to design optimal FUS-based drug delivery protocols.

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http://dx.doi.org/10.1016/j.jconrel.2014.06.023
0168-3659/

Previous studies demonstrated the success of medical imaging to detect FUS-induced BBB opening. MRI is one of the most reliable tools for post-operational evaluation of the degree and distribution of BBB opening, particularly by gadolinium-based contrast-enhanced T1-imaging to monitor the increase in signal intensity at the FUS exposure site [2,4,5]. Other MRI contrast agents such as superparamagnetic iron oxide (SPIO) particles are useful for distinguishing between FUS-induced opening of the BBB and brain hemorrhage in T2- or T2*-weighted images [11]. In addition, T1 or T2 contrast agents have been conjugated to microbubbles for concurrent induction of BBB opening and detection of the extent of opening from changes in image contrast [12–15].

Although FUS-BBB opening provides a unique opportunity for targeted CNS drug delivery, the efficiency of drug delivery is dominated by the specific pharmacokinetic and pharmacodynamic (PK/PD) properties of the delivered molecules. The scale and distribution of delivery of therapeutic molecules into brain is highly correlated with the level and duration of FUS-induced enhancement of blood-to-brain permeability, which directly affects PK/PD behavior. We previously demonstrated that contrast-enhanced T1-weighted MRI with Gd-DTPA can serve as a useful tool for evaluating the pharmacological endpoint in different animal models [16,17]. MR contrast-enhanced changes are known to correlate with concentrations of leaked Gd-DTPA, and MR relaxivity, which is obtained from the transfer of signal intensity from two different flip angles of T1 weighted images, can also provide a semi-quantitative measure of gadolinium deposition in the BBB opened region [16,17]. Thus monitoring of leakage of Gd-DTPA could potentially serve as an effective index to predict the dynamics of delivered drugs. Recently, DCE-MRI has also been developed to investigate the kinetics of dynamic FUS-BBB opening [18–20]. A number of studies reported the use of MRI to detect a change in the transfer rate constant caused by FUS exposure, thus verifying that FUS is capable of inducing a change in local permeability [19–24] to allow molecules with a molecular weight of ~1 kDa to leak from the blood stream into the extracellular extravascular space (EES) of CNS tissues.

In DCE-MRI kinetic modeling [25], the exchange of contrast agent between the blood plasma and EES is dominated by three major parameters [26,27]: (1) $K_{trans}$, defined as the influx volume transfer constant from plasma to EES; (2) $V_e$, defined as the volume of EES space per unit volume of tissue; and (3) $K_{ep}$, defined as the efflux (or backflux) rate constant from the EES to the plasma. $K_{trans}$ is the only index that has currently been described in DCE-MRI kinetic modeling of FUS-BBB opening. Yet, a comprehensive understanding of the kinetic of FUS-BBB opening also requires information of the volume fraction ($V_e$) as well as the efflux constant ($K_{ep}$) to fully describe permeability changes in both directions (i.e., plasma to EES, and vice-versa). Park et al. previously reported that good correlations between $K_{trans}$ and delivered therapeutic substance (doxorubicin) can be observed [24], yet there is still no report to understand the full spectrum of these three kinetic parameters during FUS-BBB opening.

The aim of this study was to conduct comprehensive MR kinetic modeling for the analysis of FUS-induced BBB opening, and to evaluate its performance in predicting the kinetics of enhanced delivery of a drug surrogate. Three key parameters ($K_{trans}$, $K_{ep}$, and $V_e$) of the compartmented model were used to characterize dynamic changes induced by FUS-BBB opening. The accuracy of MR kinetic modeling for predicting changes in BBB permeability and penetration of therapeutic molecules into the brain was confirmed by comparison with the concentration of Evans Blue dye, which served as a drug surrogate. Parametric analysis was conducted at different time points for the longitudinal and kinetic estimation of FUS-induced BBB opening. All measurements were subjected to exponential regression fitting and a linear correlation was applied to evaluate the relationship between kinetic parameters and EB extravasation.

## 2. Materials and methods

### 2.1. Preparation of animals

All animal procedures were approved by the Institutional Animal Care and Use Committee of Chang-Gung University and adhered to the experimental animal care guidelines. A total of 38 adult male Sprague–Dawley rats (250–300 g) were used in this study. Animals were divided into three groups. In group 1, 10 rats were used to measure changes in the kinetics of BBB permeability at different FUS pressures and MRI scan times. In group 2, quantification of Evans Blue dye was performed in 24 rats to confirm the correlation of changes in kinetic parameters with BBB permeability. The 4 rats of group 3 were sacrificed for histology four hours after sonication. Details of the animal experiments are summarized in Table 1.

### 2.2. Focused ultrasound equipment and sonication

Animals were shaved to remove hair growing on their scalps, and were anesthetized with isoflurane (1–2%). A catheter (PE-50; Alzet, Cupertino, CA) was inserted in the tail vein and fixed in place for IV injection. Each rat was placed directly under the $4 \times 4$ cm$^2$ window of an acrylic tank, which had been filled with deionized, degassed water and sealed with a thin film to allow penetration of ultrasound energy. Ultrasonic gel was used to fill the spaces between the animal's head and the thin-film window (Fig. 1). A FUS transducer was mounted and positioned on the water tank to generate concentrated ultrasound energy (Imasonics, Besancon, France; diameter = 60 mm, radius of curvature = 80 mm, frequency = 400 kHz, electric-to-acoustic efficiency = 70%). An arbitrary-function generator (33120A, Agilent, Palo Alto, CA; DS345, Stanford Research Systems, Sunnyvale, CA) was used to generate the driving signal fed to a radiofrequency power amplifier (150A100B, Amplifier Research, Souderton, PA, USA) operating in burst mode. The focal-zone distribution of the intensity of the ultrasound field was measured in the acrylic water tank. The free-field measured −3 dB dimension of the half-maximum pressure amplitude was 3 mm radially and 20 mm axially, and transcranial pressure loss was estimated to be about 15% (see Fig. S1).

Rat brain FUS exposures were conducted in the presence of ultrasound microbubbles, SonoVue® (Bracco, Milan, Italy). These SF6-coated microbubbles had a mean diameter of 2.0 to 5.0 μm, and were injected intravenously prior to FUS exposure with a time lapse of less than 20 s. Each bolus injection contained 2.4 μL/kg of microbubbles mixed with 0.1 mL of saline, followed by flushing with 0.01 mL heparin. After microbubble injection, burst-tone mode ultrasound was delivered at a pressure of 0.4 or 0.8 MPa (peak negative value; measured in free-field via a calibrated PVDF hydrophone [Onda, Sunnyvale, CA, USA]) to the left hemisphere of each rat with the center of the focal zone positioned at a penetration depth of 4–5 mm under the scalp (burst length = 10 ms, pulse-repetition frequency = 1 Hz, and total sonication duration of 90 s [7,17]). The targets were located 1 mm behind the bregma, 3 mm left of the midline and at a 5 mm depth from the dorsal brain surface. The control locations were selected to be the symmetrical mirror location in the right hemisphere.

### 2.3. MRI

All MRI scans used to monitor FUS-induced BBB opening were acquired on a 7-Tesla magnetic resonance scanner (ClinScan, Bruker, Germany; 7 T) using a four-channel surface coil. Animals were placed in an acrylic holder and positioned in the center of the magnet. Rats were anesthetized with isoflurane gas (1–2%) at 50–70 breaths/min during the entire MRI procedure.

In group 1, rats were immediately relocated into the MR scanning room after sonication, for measurement of maximal BBB opening. DCE T1-weighted imaging was performed to evaluate the permeability of