In vivo study of the dynamics of Blood Brain Barrier opening and closure after ultrasonic disruption. A quantitative analysis

Benjamin Marty[1], Benoit Larrat[2], Mathieu Pernot[2], Philippe Robert[3], Marc Port[3], Caroline Robic[3], Denis Le Bihan[1], Franck Lethimonnier[1], Mickael Tanter[2], and Sébastien Mériaux[1]

[1]*CEA/DSV/I2BM/NeuroSpin, Gif sur Yvette, France,* [2]*Institut Langevin/ESPCI ParisTech/INSERM U979, Paris, France,* [3]*Research Division, Guerbet, Aulnay sous Bois, France*

Background

In the last decade, it has been demonstrated that the use of low power UltraSound (US) combined with a systemic injection of lipid-shelled microbubbles enables a non-invasive and transient disruption of the Blood Brain Barrier (BBB) [1]. However, the BBB opening mechanism is not properly known, especially the maximum space between endothelial cells that is possible to obtain in safe conditions (i.e. without hemorrhages), as well as the duration of the opening after disruption. In our work, the BBB opening procedure is done under MR guidance on a rat model. We used MR contrast agents of different hydrodynamic diameters (d_H from 1 to 65nm) to estimate the maximum molecule size able to penetrate cerebral tissues. A T_1 mapping strategy was developed to quantitatively study and model the progressive closure mechanism of BBB after opening.

Materials & Methods

A 1.5MHz MR-compatible focused transducer (F/D=0.8, F=20mm) was used inside a 7T preclinical MRI scanner (Bruker). 45 Sprague Dawley rats (100-125g) had their heads shaved and were installed inside the magnet in stereotactic position under isoflurane anesthesia (2%). After acquisition of reference images, rodents were injected with 200µL of Sonovue® in the caudal vein and sonicated for 60s (3ms bursts every 100ms) with peak negative pressure of 0.45MPa. Five MR contrast agents (CA) were used: three paramagnetic gadolinium (Gd) chelates (Dotarem®: d_H ~ 1nm, P846: d_H ~ 4nm and P792: d_H ~ 7nm) and two SuperParamagnetic Iron Oxides (P904: d_H ~ 25nm and SPIO: d_H ~ 65nm). They were intravenously injected at different times after BBB opening. A MR Acoustic Radiation Force Imaging sequence (modified MSME, TE/TR = 40/1700ms, T_{acq} = 4min, R = 0.5x0.5x1mm^3) was used to localize the US focal spot and to calibrate the acoustic pressure before BBB opening [2]. A T_1-weighted sequence (for Gd-chelates) and a T_2-weighted sequence (for SPIOs) were optimized for contrast agent detection. To quantify Gd-chelate concentration, a T_1 mapping sequence was acquired prior and after injection (IR-FGE, TR_1 = 5 ms, TE = 2.5 ms, 6 segments, 60 inversion times, R = 0.2x0.2x1mm^3, T_{acq} = 12.5min). To model BBB closure mechanism, we assumed that just after disruption, endothelial gaps are opened with a size distribution given by a hemi-Gaussian function centered on 0, with a standard deviation σ_0. Then, each individual pore is assumed to return to its equilibrium position as a harmonic oscillator highly damped by fluid friction with characteristic frequency k. Under these hypotheses, the concentration at the US focal point of one CA injected at a time t after BBB opening is expressed by a function of its hydrodynamic diameter (d_H), σ_0 and k. The half closure time ($t_{1/2}$) was defined as the time after disruption when the concentration of contrast agent crossing the BBB is half the concentration obtained when the barrier disruption is maximum. After the estimation of σ_0 and k, $t_{1/2}$ is calculated as the numerical solution of an equation dependent of d_H.

Results

BBB opening was obtained *in vivo* through the skull for contrast agents of sizes up to 65nm (Figure 1). For SPIO, the penetration in cerebral tissues was very limited, suggesting that 65nm is close to be the largest gap between endothelial cells that can be obtained after a safe and reversible BBB opening. The BBB permeability duration was found highly dependent on the agent size: more than 10 hours for Dotarem® (Figure 2), less than 3 hours for P846 and around 2 hours for P792 (data not shown). We fitted our model of BBB closure mechanism on Dotarem® data exhibiting the highest reproducibility across animals: we found an initial pore typical diameter σ_0 of 1.0nm and a characteristic closure frequency k of 0.045 h^{-1} (Figure 2). From these fitted parameters, half closure time $t_{1/2}$ could be estimated as a function of molecule hydrodynamic diameter in the framework of our mathematical model (Figure 3). For a 4nm particle, the half closure time was estimated to 2.8h while for a 7nm particle this time is around 1.8h. These values match our experimental data on P846 and P792. For larger molecules, half closure times are strongly reduced (around 30 min for a 25nm particle and less than 15min for a 65nm particle). The only way to get significant amount of contrast agent crossing the BBB is then to ensure intravenous circulation at the end of US sonication.

Conclusion

Ultrasound induced BBB opening is a promising technique for local drug delivery to the brain. Our data at 7T suggest a maximum achievable opening gap around 65nm in rodents after safe BBB opening. High field MRI combined with dedicated T_1 mapping sequence enables to get very sensitive contrast agent detection and quantification. From our experimental data and BBB closure mechanism modeling, we could obtain a calibration curve predicting half closure time as a function of contrast agent hydrodynamic diameter. Those findings are valuable information in the framework of high molecular weight drug release, in order to precisely control the amount of drug delivered across the BBB after systemic injection.

References

[1] Hynynen et al., Radiology, 2001

[2] Larrat et al., Phys. Med. Biol., 2010

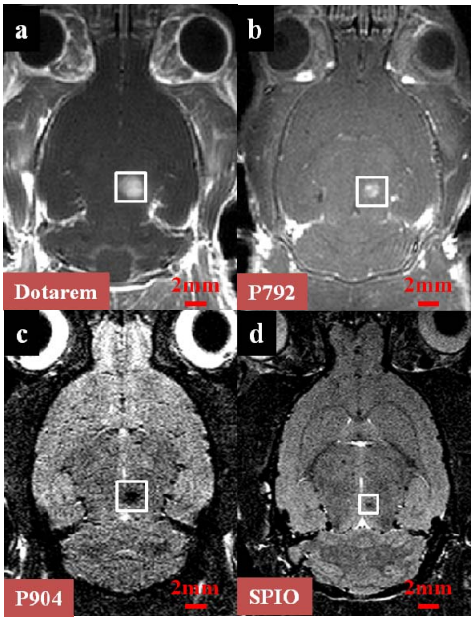

Figure 1: a-b - T_{1w} images after injection of Dotarem® and P792. **c-d-** T_{2w} images after injection of P904 and SPIO. US focal point is delimited by a white square.

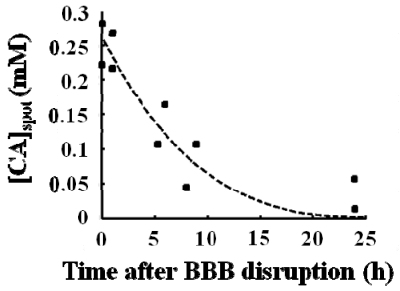

Figure 2: Dotarem® concentration at the US focal point as a function of the injection timing. Data were adjusted with a model describing BBB closure. (Dashed line, σ_0 = 1.0nm and k = 0.045h^{-1}).

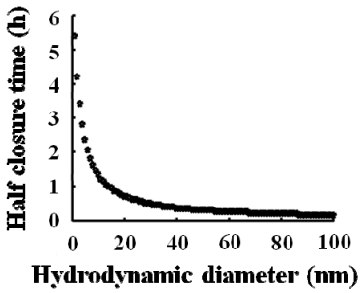

Figure 3: Theoretical half closure time derived from data modeling as a function of particle hydrodynamic diameter.