Dynamic study of tissue penetration for MR contrast agents of different sizes following ultrasound induced Blood Brain Barrier disruption in rodent models

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Background

In the last decade, many studies have shown the ability to disrupt locally and transiently the Blood Brain Barrier (BBB) with low power ultrasound (US) sonication of intravascular microbubbles [1]. However, the BBB opening mechanism is not properly known, especially the maximum space between endothelial cells that it is possible to obtain in safe conditions (i.e. without hemorrhages). In our work, the BBB opening procedure is done under MR guidance in a rodent model. We used contrast agents of different hydrodynamic diameters (d_H from 1 to 65nm) to study the progressive closure mechanism of BBB after opening and the maximum molecule size able to penetrate cerebral tissues.

Materials & Methods

Focused ultrasound protocol. A 1.5MHz MR-compatible focused transducer (F/D=0.8, F=20mm) was used inside a 7T preclinical MRI scanner (Bruker, Germany). Sprague Dawley rats (125-200g) and C57/Bl6 mice (25-30g) had their heads shaved and were installed inside the scanner in sterotactic position under isoflurane anesthesia. After the acquisition of reference images, rodents were injected with Sonovue® in the caudal vein (200µL for rats and 100µL for mice, IV) and sonicated for 30s (3ms bursts every 100ms) with peak negative pressure of 0.52MPa (inferior to the hemorrhage threshold, [2]).
MRI contrast agents (provided by Guerbet, France). Two gadolinium (Gd) paramagnetic chelates, Dotarem® (d_H ~1nm) and Vistarem® (d_H ~ 7nm), were used to study the BBB closure mechanism. Two Ultrasmall Superparamagnetic Iron Oxide Particles, USPIO A (d_H ~ 25nm) and USPIO B (d_H ~ 65nm), were injected to investigate the maximum molecule size able to cross the BBB after opening. Contrast agents were intravenously injected (300µL for rats and 100µL for mice) at different times after sonication.
Imaging protocol. 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 focal spot and to calibrate the acoustic pressure before BBB opening [3]. A T_1-weighted sequence (MSME, TE/TR = 8/300ms, T_{acq} = 6min30, R = 0.25x0.25x1mm^3) and a T_2-weighted sequence (TE_{eff}/TR = 36ms/4200ms, T_{acq} = 3min20, R = 0.12x0.12x1mm^3) were optimized for contrast agent detection. According to the agent type, either T_{1w} (for Gd chelate) or T_{2w} (for USPIO) images were acquired before and continuously after contrast agent injections to follow its distribution at the ultrasound focal point.

Results

BBB opening was obtained *in vivo* through the skull for contrast agents of sizes up to 65nm (Fig.1). For USPIO B, the penetration in cerebral tissue is very low, suggesting that 65nm is close to be the largest molecule able to cross the BBB with our procedure. The BBB permeability duration was found highly dependent on the agent size. For instance, the amount of Dotarem® crossing the BBB 2 hours after sonication (D2) is about 50% of the amount crossing the BBB just after disruption (D1) (Fig.2). Nevertheless its injection 10 hours after sonication still exhibits a detectable enhancement on T_{1w} images. For Vistarem®, the BBB is open at least 10 minutes after sonication (V2) but a complete closure was observed for a 2 hours delayed injection (V1) (Fig. 2). These findings suggest that the BBB closure speed is much higher at short times after tight junctions disruption than at late stages.

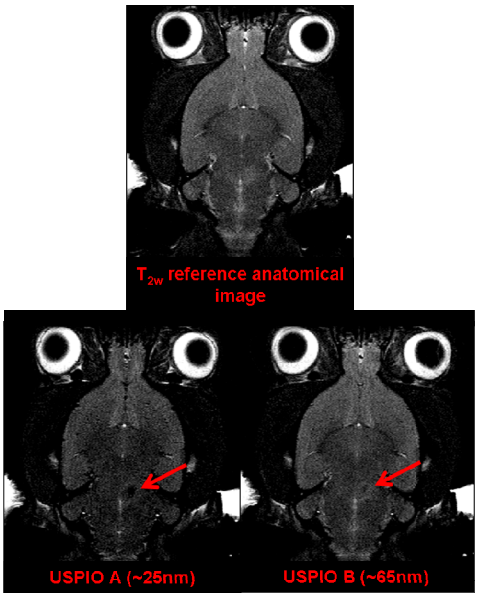

Fig.1. T_{2w} images before and after injection of USPIO A and USPIO B revealing the BBB disruption in rat brain at US focal point (red arrow)

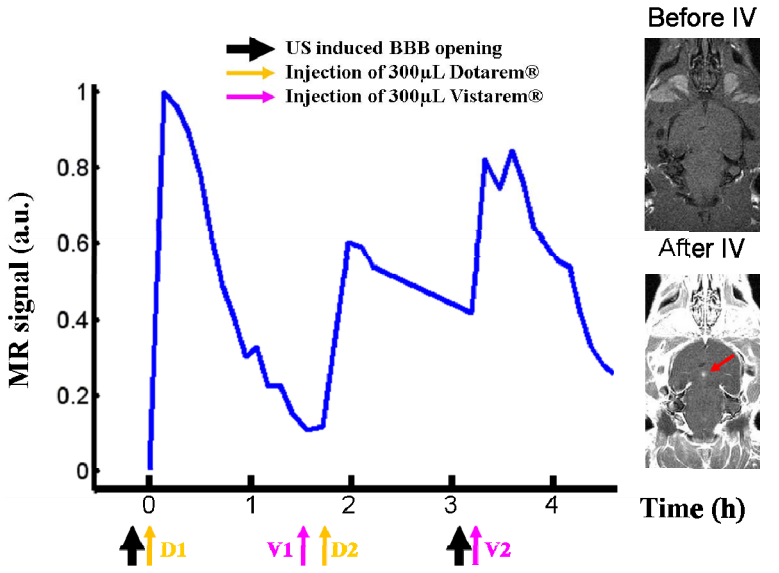

Fig.2. Signal enhancement on T_{1w} images at the US focal point (red arrow) at different times after injection of Dotarem® or Vistarem® in rats

Conclusion

Ultrasound induced BBB opening is a promising technique for local drug delivery. High field MRI combined with dedicated sequences enables to get very sensitive contrast agent detection. This is a prerequisite to rigorously study the penetration of molecules in brain tissues. To reach even higher sensitivity to contrast agent detection, the whole BBB opening set up is being transferred on a 17T preclinical scanner. Our data at 7T suggest a maximum achievable opening gap of 65nm in rodents using a dedicated protocol. BBB closure dynamics was studied using contrast agents of different sizes showing a fast initial closure of largest gaps. Those findings are valuable information in the framework of high molecular weight drug release, either for molecular imaging or therapeutic drug delivery.

References

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