Molecular MRI of thrombus in a rat ischemic stroke model

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Introduction: Ischemic stroke is the leading cause of morbidity and the third most common cause of mortality in USA. The identification of the culprit thrombotic lesion, micro-emboli and the source of the embolus are all critical to patient management. EP-2104R [1], a fibrin-specific molecular MR agent, was previously shown to enhance arterial and venous thrombi in large animal models (swine, rabbit) [2,3] and in humans [4]. Those studies were performed on clinical scanners and focused on thrombi outside the brain. Here we use EP-2104R for the first time in a rodent model, to characterize thromboembolic stroke in a rat at high field (4.7T).

Materials and Methods: The T1 of EP-2104R in fibrin gels was measured by inversion recovery and relaxivity determined from the slope of 1/T1 vs EP-2104R concentration. For thrombus imaging, autologous rat blood was allowed to clot in PE50 tubing and aged 24 hrs. The aged clot (25 mm) was inserted in the right internal carotid artery via catheter and placed at the level of the middle cerebral artery. Imaging sequences included time of flight (TOF) angiography to demonstrate reduced blood flow, diffusion weighted imaging (DWI) to verify the presence of an ischemic lesion, T1-weighted spin echo imaging to assess potential leakage of the probe into the brain infarct, and high resolution flow-suppressed 3D T1-weighted gradient echo imaging (TR/TE/flip=40/5.8/75º, NEX=4, FOV=5.8x2.9x2.9 cm, matrix=300x150x150, resolution=0.193 mm/pixel and scan time=1 h) to observe the clot. After baseline MRI, 10 µmol/kg of EP-2104R (n=6) or 200 µmol/kg of GdDTPA (external control, n=5) was injected via femoral vein catheter and the imaging repeated. Some animals also received a simultaneous dose of 40 µmol/kg of YDTPA (n=3), an MRI-silent version of GdDTPA. Animals were sacrificed immediately after imaging and the tissues analyzed for Gd and Y concentration by ICP-MS. Images were analyzed using Osirix. Regions of interest were drawn in the thrombus, adjacent blood, adjacent brain, and air outside the animal and the signal intensity (SI) and its standard deviation (SD) measured. Contrast to noise ratios (CNR) comparing tissue A to tissue B were calculated as $CNR_{(A:B)}=[SI_A - SI_B]/SD_{(air)}$.

Results: The relaxivity of EP-2104R per Gd (and per EP-2104R molecule) was 16.3 (65.2) at 1.4T and 10.0 (40.0) $mM^{-1}s^{-1}$ at 4.7T, 21°C. In all animals, TOF angiography demonstrated restricted flow in the right internal carotid artery and DWI showed the presence of a hyperintensity confirming ischemic lesion. Thrombi were plainly visible in the EP-2104R-enhanced 3D T1-weighted images (Fig. B), but were not apparent prior to EP-2104R injection (Fig. A) or if animals were administered GdDTPA. The CNR for clot to blood and clot to brain post-injection of EP-2104R was significantly greater than for the baseline pre scan ($p<0.0001$); however, that was not the case for GdDTPA injection ($p=0.96$) (Fig. C). Ex vivo tissue analysis (80 min post injection) showed that the average concentration of Gd in the clot for animals injected with EP-2104R was 7 times that of GdDTPA (Fig. D) or YDTPA, the internal control. On the other hand, the blood concentration of EP-2104R was similar to that of GdDTPA (Fig. D).

Conclusions: This is the first rodent study using EP-2104R as an imaging probe for thrombus. The efficacy of EP-2104R to image clot in a thrombo-embolic stroke model was successfully proved in this study. Although the relaxivity of EP-2104R is lower at 4.7T as compared to 1.5T, it is still sufficient to detect thrombi with high contrast relative to blood and surrounding tissue. The high CNR for clot to blood correlates well with the ex vivo ICP-MS analysis of gadolinium in these tissues.

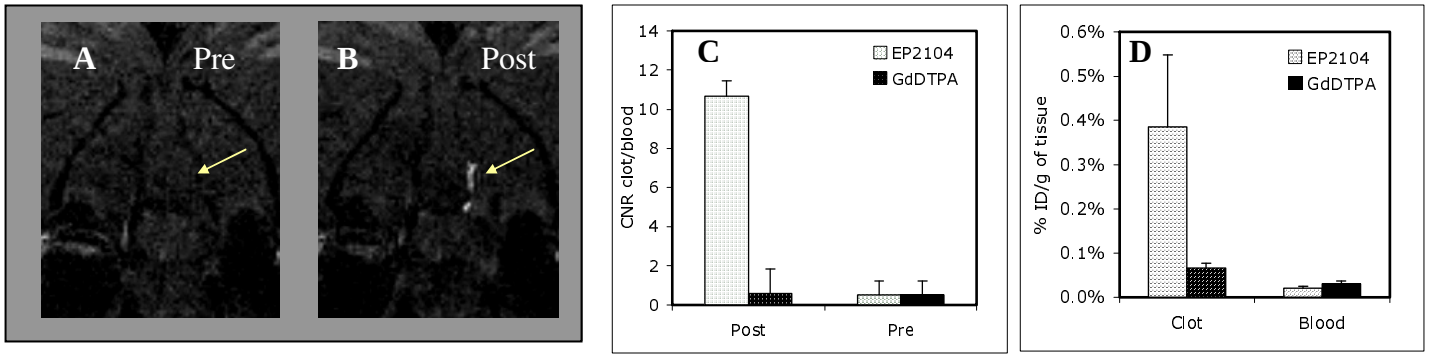

Figure: 3D T1-weighted image post injection of EP-2104R (B) clearly identifies the thrombus (arrow) in the rat brain that was not visible on the pre image (A). (C) Clot to blood CNR comparing pre-injection to post-injection for EP-2104R quantifies the considerable clot enhancement, while no increase in CNR is seen for GdDTPA. (D) The ex vivo Gd concentration in the clot, is 18 times higher than in the blood for EP-2104R; however, the values for clot and blood are similar in the case of GdDTPA injection.

References: [1] Overoye-Chan K. et al *J. Am. Chem. Soc*. **2008**, *130*, 6025. [2] Spuentrup, E. et al *Circulation* **2005**, *111*, 1377. [3] Sirol, M. et al *Circulation* **2005**, *112*, 1594. [4] Spuentrup, E. et al *Eur. Radiol.* **2008**, *18*, 1995.