Overhauser-enhanced MRI as a non invasive probe of BBB breakdown and redox state following ischemia/reperfusion

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PURPOSE: The development of acute reperfusion therapies in ischemic stoke has changed stroke care. Nevertheless, these treatments remain limited by a short therapeutic window due to the risk of reperfusion injury and hemorrhage within the infarct. MRI-based biomarkers for visualizing and quantifying the progressive disruption of the BBB at hyperacute stages of stroke are presently unavailable, and although contrast from exogenous relaxation-based MRI contrast agents such as Gd-DTPA is correlated with hemorrhagic transformation of an infarct, it is not sensitive enough to probe more mild BBB disruption (1). Detection of early and mild BBB disruption is an unmet need in acute stroke diagnosis and management (2). Free-radical-sensitive Overhauser-enhanced MRI (**OMRI**) is a promising technique for imaging the distribution and dynamics of free radicals, and a recently developed fast high-resolution OMRI methodology (3) offers new perspectives for the imaging of free radicals in living organisms. We describe here an OMRI-based method to probe hyperacute BBB breakdown following ischemic stroke using OMRI in conjunction with an injected stable free radical.

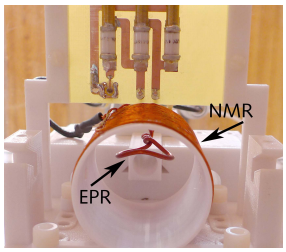

Figure 1: Probe for OMRI rat imaging at 6.5 mT: NMR@: 276 kHz, ESR@141 MHz.

TEMPOL (4-hydroxy-TEMPO), a small molecule with a stable unpaired electron spin, is detected by OMRI with very high sensitivity. In a normal physiological state, TEMPOL does not cross the BBB (4). Because of its small size (172 Da) however, it may be able to cross the BBB under pathological circumstances associated with early BBB opening (e.g. ischemia), and act as an OMRI-detectable tracer. The use of TEMPOL as a small, exogenous OMRI agent would allow monitoring BBB disruption in stroke at the hyperacute stage, potentially much earlier than the traditional relaxation-based MRI contrast agents that rely on the leakage of larger molecules (such as Gd-DTPA) across the BBB.

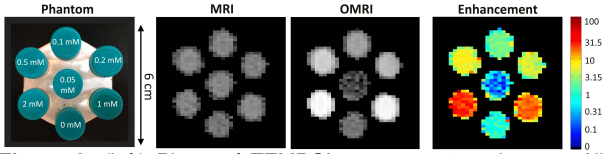

Figure 2: (left) Photo of TEMPOL concentration phantom All seven vials have very similar **MRI** magnitudes. **OMRI** demonstrates marked image-based free radical sensitivity.

METHODS: A custom built, low-field MRI scanner with a biplanar 6.5 mT electromagnet and biplanar gradients equipped was used in these experiments (5). 3D OMRI was performed using an optimized sequence based around b-SSFP as described in (3). Sensitivity of b-SSFP-based OMRI to free radical concentration was performed using the NMR/ESR coil setup of (3) using vials containing TEMPOL in concentrations from 50 μM–2 mM, and a control containing only water. A rat model of cerebral ischemia/reperfusion was used to test the ability of our technique to detect injected TEMPOL free radicals crossing the BBB *in vivo*. *In vivo* experiments were performed using with a single loop ESR coil inside a solenoid NMR coil (Figure 1). Under anesthesia, MCAO occlusion was performed in a 3 month old Wistar rat by insertion of filament via external carotid artery. Following 75 min MCAO and 60 min reperfusion, 3.6 μl/gbw of 300 μM TEMPOL was injected into the carotid artery after which the animal was sacrificed and OMRI imaging begun.

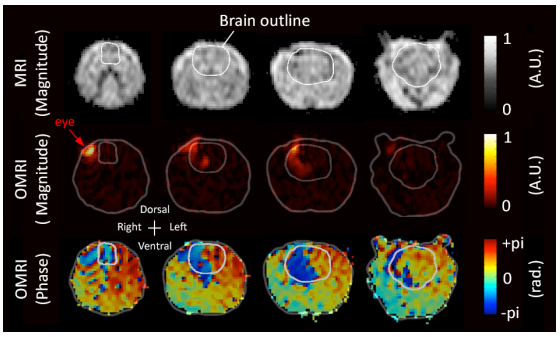

Figure 3: OMRI magnitude and phase images acquired from a rat at 6.5 mT following 75 min right MCAO and 60 min reperfusion. Four coronal slices from 10 slice data set shown. OMRI (NA=10) imaging time was 195 seconds. Low-resolution anatomical MRI (NA=80) was acquired in the OMRI scanner at 6.5 mT with DNP pulses disabled. MRI imaging time was 17 min. All images, voxel size: 1.1 x 1.6 x 8 mm^3, TE/TR: 18/36 ms, Matrix: 128 x 35 x 10

RESULTS: The sensitivity of b-SSFP-based OMRI to free radical concentration is shown in Figure 2. Conventional MRI offers no window into the presence of free radicals in the test object, and as a result all seven vials have very similar image magnitudes. The OMRI scan demonstrates marked image-based free radical sensitivity. The OMRI enhancement image is computed from the ratio of OMRI to MRI magnitude.

In vivo OMRI signal enhancement in the frontal lobe and eye ipsilateral to the ischemic site is clearly visible in the OMRI images (Figure 3) following reperfusion. As the Overhauser-enhanced signal has phase opposite to that of the thermal signal, the phase of the OMRI image in Figure 3 provides very sensitive contrast even in cases where the radical concentration is very low and the Overhauser enhancement may be small.

DISCUSSION & CONCLUSION: We have imaged TEMPOL at low concentrations with OMRI methods *in vitro*, and crossing the BBB following ischemia/reperfusion *in vivo*. The use of OMRI in conjunction with the stable free radical TEMPOL as an exogenously administered probe in hyperacute stroke is a new and novel approach, and this study suggests that TEMPOL may be a suitable probe for observing early BBB breakdown following reperfusion in rodent I/R models. Additionally, as TEMPOL reduction has been used as a functional probe to study redox status in tissue (6), we hypothesize that temporally resolved OMRI may be used to indicate the redox status of the ischemic tissue.

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