Dynamic in vivo free radical imaging with Overhauser-enhanced MRI

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PURPOSE: 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 (1) offers new perspectives for the imaging of free radicals in living organisms. This method has been used to probe BBB breakdown following ischemic stroke in rats in conjunction with an injected stable free radical (2). Here we explore if OMRI can be used to acquire free radical images with sufficient spatial and temporal resolution to probe oxidative stress status in the brain. We present time-resolved *in vivo* measurements following a single injection of TEMPOL. TEMPOL (4-hydroxy-TEMPO), a small molecule with a stable unpaired electron spin, is detected by OMRI with very high sensitivity. According to the literature, TEMPOL does not cross the BBB in a normal physiological state but does pass into brain parenchyma under pathological circumstances associated with oxidative stress and disease (3). As TEMPOL reduction has been used to non-invasively monitor tissue redox status in animal models (4), we hypothesize that time-resolved OMRI may be a tool to elucidating the redox status of brain tissue in neurologic diseases in which oxidative stress plays a significant role such as reperfusion injury (5), head trauma (6), Alzheimer's dementia (7) and multiple sclerosis (8).

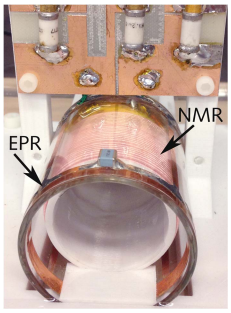

Figure 1: Probe for OMRI imaging at 6.5 mT: NMR: 276 kHz, ESR: 141 MHz.

METHODS: A custom built, low-field MRI scanner equipped with a biplanar 6.5 mT electromagnet and biplanar gradients was used in these experiments (9). 3D OMRI was performed using an optimized sequence based around b-SSFP as described in (1). *In vivo* experiments were performed using a modified Alderman-Grant ESR coil outside of a high sensitivity solenoid NMR coil wound from 5/39/42 Litz wire that fits the rat head (Figure 1). Under isoflurane anesthesia, the right carotid bifurcation of a male Sprague Dawley rat was dissected and the pterygopalatine artery ligated. Retrograde cannulation (Micro-Renathane MRE40, Braintree Scientific) of the right carotid bifurcation was performed through the external carotid artery. The catheter, filled with saline 0.9% with heparin 50U/ml, was then tunnelized to the back of the animal and connected to the injection system. Oxygen saturation, temperature, and cardiac and respiratory rates were continuously monitored.

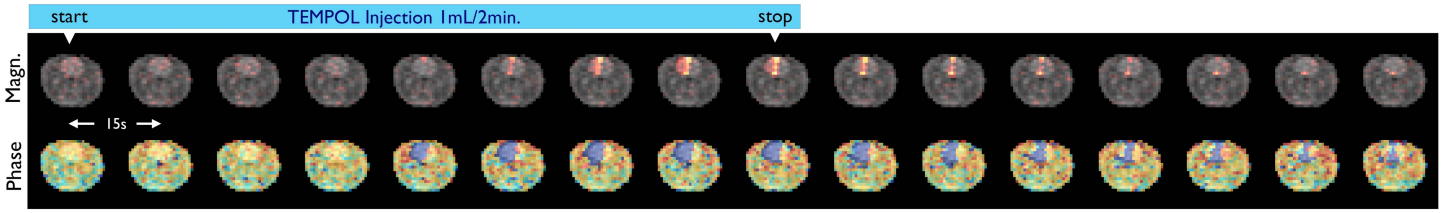

Figure 2: Dynamics of one slice from 3D OMRI dataset acquired from a rat at 6.5 mT: OMRI (color) magnitude and phase images (NA=1) are superimposed on anatomical MRI (grey), acquired in the OMRI scanner with DNP pulses disabled (NA=30). Anatomical imaging time was ~5 min. All images, voxel size 1.1×1.6×8 mm^3, TE/TR=25/50 ms, Matrix 128×35×11. TEMPOL (1.4 µl/gbw) was injected into the right ICA over 2 minutes.

Following acquisition of two reference scans (one with EPR off and one with EPR on), 1 ml of 150 mM TEMPOL was injected over 2 min with an infusion pump (GenieTouch Kent Scientific). OMRI imaging began at the same time as the infusion, and continued for 105 seconds after the injection was complete. A full 11 slice OMRI acquisition was acquired every 9 seconds, followed by a 6 second delay. This was repeated 16 times. Total imaging time was 240 s.

RESULTS: The internal carotid artery is responsible of the vascularization of its ipsilateral hemisphere via its terminal branches. The Circle of Willis enables some cross vascularization of one hemisphere from the contralateral side. Therefore, one would expect the cerebral distribution of a compound injected directly into the ICA to reflect the ipsilateral hemisphere and to a lesser degree the contralateral hemisphere. This is consistent with our dynamic *in* vivo OMRI results shown in Figure 2 and Figure 3, where marked DNP enhancement is seen in the hemisphere ipsilateral to the TEMPOL injection. As the Overhauser-enhanced signal has phase opposite to that of the thermal signal, the phase of the OMRI image in Figure 2 provides very sensitive contrast even in cases where the radical concentration is very low and the Overhauser enhancement may be small.

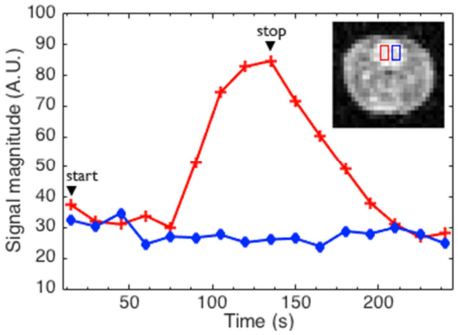

Figure 3: Mean OMRI magnitude from two brain ROIs: ipsilateral (red) and contralateral (blue) to the TEMPOL injection over the 240 s OMRI acquisition.

DISCUSSION & CONCLUSION: We have imaged the free radical TEMPOL with time-resolved OMRI methods *in vivo*. The use of fast bSSFP-OMRI in conjunction with an exogenously administered free radical molecule to probe redox status is a novel approach. As a method, temporally resolved OMRI may be used to study redox status of brain tissue made permeable to TEMPOL from oxidative stress. We note here that the long clearance times seen in some regions of the brain in Figure 2 may indicate TEMPOL in the brain parenchyma.

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