Manganese-Enhanced MRI (MEMRI) and Diffusion-Weighted MRI Mismatch in the Absence of a Specific Neuronal Stimulus

D. Bennett[1], N. Henninger[2,3], M. Fisher[4,5], and C. Sotak[4,6]

[1]Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA, United States, [2]Neurology, University of Massachusetts Medical School, Worcester, MA, United States, [3]Neurology, University of Massachusetts Memorial Healthcare, Worcester, MA, United States, [4]Radiology, University of Massachusetts Medical School, Worcester, MA, United States, [5]Neurology, University of Massachusetts Memorial Health Care, Worcester, MA, United States, [6]Biomedical Engineering and Chemistry and Biochemistry, Worcester Polytechnic Institute, Worcester, MA, United States

Introduction: Manganese (Mn^{2+}) has proven to be a useful MR contrast agent for functional imaging[1] and neuronal tract tracing[2]. The paramagnetic properties of Mn^{2+} provide localized contrast on a T_1-weighted (T_1W) MR image after cellular sequestration via voltage-gated calcium channels[1]. Many MEMRI experiments rely on the delivery of a significant volume of Mn^{2+} to the brain parenchyma via an osmotically-disrupted blood-brain barrier (BBB). Neuronal activity associated with stroke[3] and cortical spreading depression[4] can also result in localized T_1-enhancement. For example, in an effort to detect ischemic brain regions using MEMRI Aoki et al.[3] coined the term "MEMRI-DWI mismatch" to categorize the difference between T_1-enhanced brain regions and regions with an ADC below $0.5x10^{-3}$ mm^2/s after permanent occlusion of the middle cerebral artery in the rat. The T_1-enhanced regions in Aoki's study were suggested to represent stroke core. However, mechanisms other than stroke, such as brain edema and the paramagnetic effects of manganese, were not discussed with respect to ADC deficits in the hemisphere of interest. Here, using an experimental protocol similar to that of Aoki et al.[3], we show significant ADC reductions in brain regions infused with hyperosmotic solution, manganese chloride, and Evans Blue as well as non-specific T_1-enhancement and DWI contrast due to Mn^{2+} accumulation. Additionally, 2,3,5-triphenyltetrazolium hydrochloride (TTC) staining showed signs of tissue injury in areas with significant ADC deficit. These studies suggest that when $MnCl_2$ is delivered via an osmotically-disrupted BBB, careful assessment of baseline MEMRI-DWI mismatch regions should be performed prior to any subsequent neuronal stimulus or ischemic intervention.

Methods: Eight male Sprague-Dawley rats (280-350 g) were anesthetized with 2.0% isoflurane mixed with breathing-quality air during surgery. Prior to each injection, the external carotid (ECA) and pterygopalatine artery were ligated and the common carotid (CCA) was temporarily clamped (~10 minutes) during catheterization of the ECA with PE-10 tubing. 1.6M arabinose solution (5 ml/kg; Sigma) was infused into the internal carotid artery over 55 seconds. During the arabinose infusion, the CCA was clamped to ensure flow towards the brain. Immediately after arabinose infusion the CCA clamp was removed. Two (N=3) and ten (N=4) minutes after arabinose infusion manganese chloride ($MnCl_2 \cdot 4H_2O$, MW = 198; Sigma), dissolved to 10 mM in isotonic saline, and Evans Blue (EB, Sigma), dissolved to 2% in $MnCl_2$ solution ($MnCl_2$+EB = 2 ml/kg), was infused into the internal carotid artery at 4.5 ml/hr. $MnCl_2$+EB and arabinose solutions were filtered with 0.45 μm syringe filters (Nalgene). MR images were acquired using a Bruker Biospin 2.0T/45 cm imaging spectrometer operating at 85.56 MHz for ^1H and equipped with ±20G/cm self-shielded gradients. DW images were acquired with six b-values (23, 92, 207, 828, 1126, and 1471 s/mm^2), 64x64 matrix zero-filled to 128x128 before Fourier transformation, FOV=3x3cm, six 2-mm slices, TR/TE = 2000/51.0ms, Δ= 6.0ms, δ = 24.31ms, and NEX=8. T_1W MRI was performed as follows: TR/TE = 500/10.8ms, FOV = 3x3cm, matrix = 128x128, six 2-mm slices with 0.1mm gap and NEX = 8.

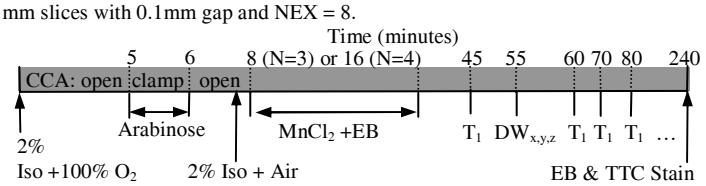

Figure 1: Timeline of infusions, imaging and histological staining.

Results: Animals infused with $MnCl_2$+EB 2 minutes after arabinose showed a T_1W signal increase of 39±8% covering 43±7% of each infused hemisphere. Animals infused with $MnCl_2$+EB 10 minutes after arabinose showed a T_1W signal increase of 40±4% covering 50±19% of each infused hemisphere. Figure 2 shows images from three animals that include the average signal intensity of DW images, corresponding ADC maps, T_1W images, EB and TTC stains. Images were acquired according to Figure 1. Figure 2A and 2B show regions of bright T_1-enhancement corresponding to regions with dark EB staining (white arrows). Figure 2A and 2B also show regions of DW hypointensity due to Mn^{2+} accumulation (white arrow 2A, center of hemisphere in DW image 2B). Red arrows in Figure 2A, 2B, and whole hemisphere in 2C demonstrate regions of MEMRI-DWI mismatch. Non-T_1-enhanced regions shown in Figure 2A and 2B do not show correlated (gross) EB staining but do correspond to areas of tissue injury as verified by TTC stains (red arrows). Data shown in Figure 2C are from an animal infused with 1.6M arabinose at 50 ml/hr ($MnCl_2$+EB 10 minutes after arabinose) to control for brain edema and non-specific T_1-enhancement due to infusion rate of arabinose. For the slice shown, brain water changes are apparent and may reflect severe edema due to hyperosmotic shock. Brain edema may be responsible for reducing blood flow and delivery of $MnCl_2$+EB to this region. Figure 3 shows percent area of non-T_1-enhanced regions with ADC deficit, percentage ADC deficit in non-T_1-enhanced regions and percent area of the $MnCl_2$+EB-infused hemisphere with a MEMRI-DWI mismatch. T_1-enhancement was defined as voxels in infused hemisphere whose signal intensity exceeded the mean +2 SDs in the non-infused hemisphere. ADC deficit was defined as voxels in the infused hemisphere with ADC values less than the mean plus 2 SDs in the non-infused hemisphere. MEMRI-DWI mismatch size was defined as the area of ADC deficit within regions without T_1-enhancement compared to the area of the entire hemisphere. Calculations and regions of interest were made using imageJ (NIH).

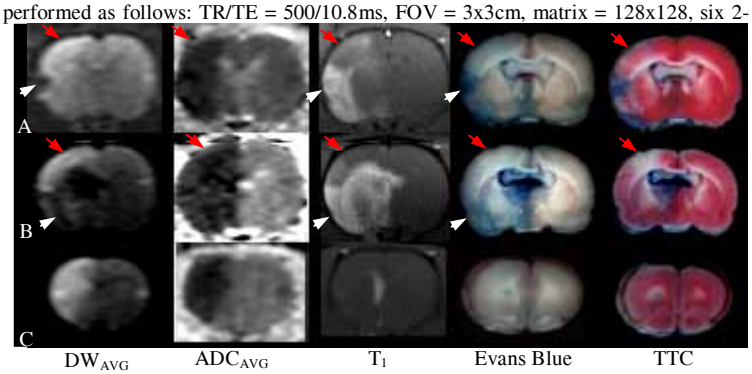

Figure 2: Averaged signal intensity of DW images acquired in x,y, and z directions ($b=1126$ s/mm^2) , average ADC map, T_1W, EB, and TTC images from three animals. MR images are from animals infused with $MnCl_2$+EB A) 2 minutes and B), C) 10 minutes after arabinose bolus. Red arrows in A) and B) point to regions of MEMRI-DWI mismatch and tissue damage suggestive of low blood flow to these regions possibly due to severe edema. White arrows indicate EB-stained regions that match T_1-enhancement and DWI hypointensity. C) One animal infused with 5ml/kg 1.6M arabinose at 50 ml/hr shows a complete MEMRI-DWI mismatch in this slice. ADC deficit and apparent hemispheric volume changes (EB and TTC slices) in C) suggests severe edema which may explain lack of T_1-enhancement and EB stain in this slice.

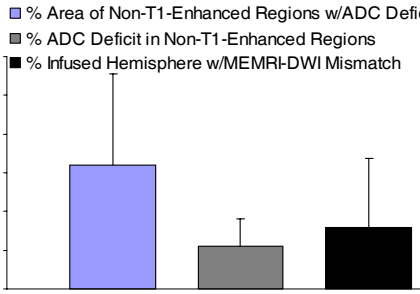

Figure 3: % Area of non-T_1-enhanced regions with ADC deficit (blue bar), % ADC deficit in non-T_1-enhanced regions (gray bar) and % of $MnCl_2$+EB-infused hemisphere with a MEMRI-DWI mismatch (black bar). Each bar represents average ±SD across all 6 MR slices of all animals infused with $MnCl_2$+EB 10 minutes after the 1.6M arabinose bolus.

Discussion: Many MEMRI experiments rely on the delivery of Mn^{2+} to the brain parenchyma via an osmotically-disrupted BBB prior to specific neuronal stimulus[1,3,4]. For these studies, non-specific T_1-enhancement and DWI contrast is undesirable and may obscure the expected T_1-contrast and ADC changes. Although ischemic conditions were not intentionally induced in these experiments, our data suggests that hyperosmotic shock itself can give rise to non-specific brain T1 and ADC changes (permanent, Figure 2B) under these conditions. These observations are consistent with previous studies that provided evidence for brain cell and endothelial cell injury after hyperosmotic shock[5,6,7] and for ADC changes as a result of brain edema[8,9]. Our data does not, however, permit us to rule out T_1-enhancement, DWI contrast and edema formation by an anesthetic effect. Isoflurane, specifically, has been shown to increase blood plasma glutamate levels in humans[10] and exacerbate brain edema in brain-injured rats[11]. Although we did not use a combined non-volatile and volatile anesthetic regimen as described by Aoki et al.[3], this approach may be helpful in reducing or eliminating any non-specific T_1-enhancement under these conditions. While insufficient anesthetic depth may contribute to non-specific T_1-enhancement, the MEMRI-DWI mismatched regions shown here may also reflect a brain response (functional, volumetric, or both) to the hyperosmotic disruption of the BBB alone. The results of these experiments stress the need for careful assessment of baseline MEMRI-DWI mismatch prior to any subsequent neuronal stimulus or ischemic intervention; in order to ensure that the osmotic disruption of the BBB itself does not contribute significantly to any non-specific brain T1 or ADC changes.

References: [1] Lin et al. *Mag. Reson. Med.* **38**:378-88 (1997). [2] Aoki et al. *Neuroimage* **22**:1046-59 (2004). [3] Aoki et al. *Mag. Reson. Med.* **50**:7-12 (2003). [4] Henning et al. *Mag. Reson. Med.* **53**:851-857 (2005). [5] Suzuki et al. *J Neurosurg* **69**:421-428 (1988). [6] Lossinsky et al. *J Neurocytology* **24**:795-806 (1995). [7] Richmon et al. *Brain Research* **780**:108-118 (1998). [8] Baird et al. *JCBFM* **18**: 583-609 (1998). [9] Barzo et al. *J Neurosurg* **87**:900-907 (1997). [10] Stover et al. *Acta Neurochir (Wien)* **147**:847-853 (2005). [11] Stover et al. *Acta Neurochir Suppl.* **76**:375-378 (2000).