MRI Detects Oxidative Stress Induced by Methaemoglobin

G. Leung[1], and A. R. Moody[1]
[1]Medical Imaging, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada

Introduction: Multiparametric MRI is able to identify significant features of plaque complication such as intraplaque haemorrhage (IPH).IPH produces a focal hyperintensity on T1 weighted MR images when signal from both fat and flowing blood are suppressed (Figure 1A). IPH uniquely identified patients who had plaque growth[1,] differentiated patients who were at increased risk of future stroke[2], and predicted future events in a patient population with >50% carotid stenosis. This evidence suggests that MRI can be used as a tool to identify patients at risk for future clinical events using IPH as a surrogate marker. Red blood cells within IPH may contribute to increased plaque progression through two mechanisms. Firstly, haemoglobin (Hb) within the red blood cell contains haeme and molecular iron the latter being a potent -generator of free radicals[3]. These free radicals are capable oxidising low density lipoprotein (LDL), which is accepted as the cause then driver of plaque development[4]. Secondly, the red blood cell membrane contains high concentrations of LDL that contribute to the plaque lipid core. Both of the components necessary to drive progression are delivered to the plaque during IPH. We hypothesise that the vessel wall T1 hyperintensity detected on MRI is associated with an environment that is a potent oxidiser of LDL. We aim to demonstrate differential T1 relaxivity for blood in different oxidation states and show that these oxidation states have divergent oxidation effects on LDL.

Methods: Whole blood was drawn into vials and different oxidation products were prepared by bubbling O2 (oxyhaemoglobin, oHb) N2 (deoxyhaemoglobin dHb) or NO (methaemoglobin, mHb). All samples were placed in a 3T Philips Achieva system (Philips Healthcare, Best, Netherlands) in a transmit/receive quadrature head coil. To -estimate R1 a single slice, 2D Look-Locker technique was used to measure signal after inversion recovery. Pulse sequence parameters include TR/TE/ɵ = 19ms/5ms/5°, slice thickness of 10mm and a delay of 20ms between each gradient echo readout. From 4 samples, r1 was calculated as the slope of the best fit line. Assaying for lipid oxidation was performed using the fluorescent LDL analog cis-parinaric acid (PnA). Fluorescence activity is lost when the chromophore is cleaved during oxidation of PnA. Samples were loaded into a clear cuvette and emission data from a 305nm laser were summed every 2nm from 410 to 460nm.

Results: Figure 2 shows the cumulative fluorescence of PnA when incubated with haeme products in intracellular and extracellular oxidation states. Small decreases in PnA fluorescence are seen after the addition of 1 mM of oHb and dHb. Intracellular ferric Hb shows a larger decrease in PnA fluorescence compared with the ferrous iron products and extracellular ferric Hb exerts a significant effect on PnA fluorescence. Figure 3 shows the calculated r1 values for intra and extracellular haeme products in their ferrous and ferric states. A small increase in the r1 value is seen for both dHb and extracellular dHb over oHb and extracellular oHb; however, both intra and extracellular mHb have approximately a 10 times higher relaxivity than either oHb and dHb.

Discussion:It has been suggested that there is a correlation between a - low T2* value, indicative of intracellular blood products, and poor patient outcome[5]. The evidence presented in this paper however would suggest the contrary, as intracellular products appear significantly less harmful than extracellular blood products owing to the barrier effect of the cell membrane against lipid oxidisation. The differentiation of intracellular and extracellular components using T2* likely reflects intact RBCs indicating fresh plaque haemorrhage, defining the start of a destabilisation event. In conclusion, we have demonstrated that ferric haeme is significantly more pro-oxidant than ferrous haeme. Additionally, we have shown that this ferric haeme has a much higher relaxivity than its ferrous counterparts. This evidence in tandem suggests that the MR detected T1 hyperintensity within the vessel wall is an endogenous biomarker of an intraplaque environment that is highly pro-oxidant and pro-atherogenic.

References: 1. Takaya et al, Circulation 2005;111(2)1:2768-75 2. Altaf et al Stroke 2007;38(5):1633-5 3.Alayash Antioxid. Redox Signal 2001;3(2):313-27 4. Nishi et al Atherosclerosis 2002;160(2):280, 5 Raman et al, JACC Cardiovasc Imaging 2008;1(1):49-57

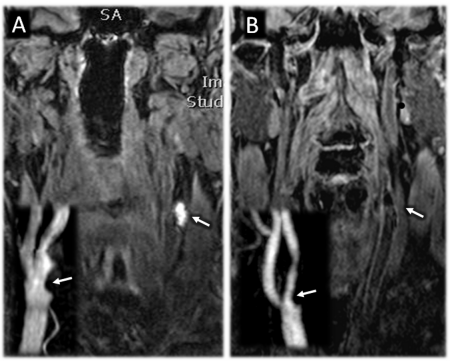

Figure 1: MR Detected Intraplaque Haemorrhage Identifies Patients with Increased Risk of Cerebrovascular Events Independent of Stenosis Panel A shows a coronal slice of a T1 weighted three dimensional, spoiled gradient echo volume that is fat and flow suppressed. Focal T1 hyperintensity, denoted by the arrowhead, appearing in the left carotid bifurcation is indicative of intraplaque haemorrhage in the arterial wall. Furthermore, natural history trials have correlated this presentation to an increased risk of future clinical events. Inset is a bright blood MR angiogram showing stenosis at the carotid bifurcation of the same artery. This level of stenosis is similar to that of the stenosis seen in the inset of panel B. However, despite having similar stenosis, the absence of a T1 hypertintense signal is suggestive of stable plaque morphology.

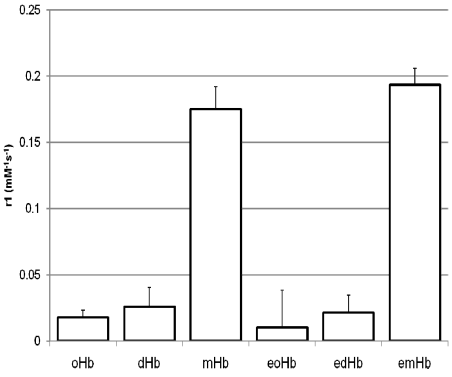

Figure 2: Ferric Hb quenches more PnA Fluorescence than Ferrous Hb
In the absence of haemoglobin (Hb) products, no cis-parinaric Acid (PnA) fluorescence is lost. However, upon addition of ferrous Hb, there is an increasing amount of fluorescence lost. Substantial fluorescence quenching is seen on the addition of extracellular mHb, with significantly less using an intracellular product. This suggests that the cellular membrane is an important inhibitor of lipid oxidation via Hb sequestration. Error bars are standard deviation of the mean from three separate trials. PnA is a mixture of LDL and cis-parinaric acid, H2O2 is hydrogen peroxide, X is the PnA, LDL and H2O2 solution, oHb is oxyhaemoglobin, dHb deoxyhaemoglobin and mHb methaemoglobin. The e prefix indicates extracellular.

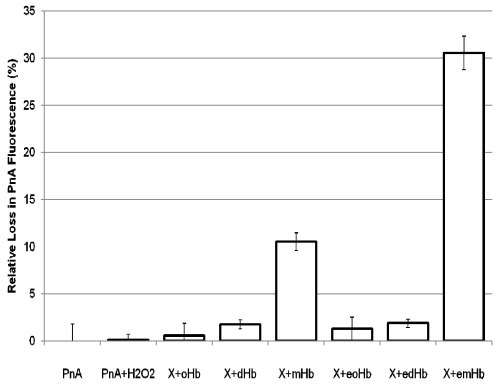

Figure 3: Ferric Hb has Greater Relaxivity than Ferrous Hb In its ferrous oxy and deoxy forms, haemoglobin is not a strong T1 relaxation agent, as shown by the low calculated r1 values. However, on conversion to its ferric met form, Hb generates significant T1 relaxation. No significant differences were found between the intra and the extracellular forms. oHb is oxyhaemoglobin, dHb deoxyhaemoglobin and mHb methaemoglobin. The e prefix indicates extracellular.