Haptoglobin Phenotype Modulates MRIPH Signal

G. Leung[1,2], H. Cheung[2], S. E. Chiu[1], B. Wong[3], D. Cole[3], and A. R. Moody[1,2]
[1]Medical Biophysics, University of Toronto, Toronto, Ontario, Canada, [2]Medical Imaging, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada, [3]Division of Biochemistry & Genetics, Sunnybrook Health Sciences Centre

Introduction. It has been shown that the signal hyperintensity associated with magnetic resonance detected intraplaque hemorrhage (IPH) is related to an environment of increased oxidative stress[1]. Furthermore, IPH also contributes to the growth of the necrotic core[2], increases oxidative stress within the vessel wall[3] and delivers iron to the plaque core in the form of haeme[4]. Many endogenous protection mechanisms have evolved to modulate the effects of free iron. Haptoglobin (Hp), originally described as anti-haemoglobin (Hb), appears to specifically bind extracellular Hb[5]. This binding appears to modulate the reactivity of Fe^{3+}. The Hp gene is polymorphic in humans with two common alleles, Hp-1 and Hp-2. These two alleles appear to offer differing degrees of cardiovascular protection; those individuals with the Hp-2 allele appear to be at greater risk of cardiovascular events, especially when paired with diabetes[6], compared to individuals with only the Hp-1 allele. Additionally, the Hp-2-2 phenotype is associated with an increased oxidative stress load within atherosclerotic plaque[7]. Together, this data seems to indicate that patients possessing an Hp-2 allele are at increased risk to cardiovascular disease.

The Hp protein sequesters Fe^{3+} from the surrounding environments, modifying its ability to react with lipids. In the context of the Fe^{3+} associated MR signal hyperintensity, this sequestration may alter the ability of water to interact with the paramagnetic Hb-Fe^{3+} core, changing the MR properties. Therefore, we studied the MR signal behaviour of Fe^{3+} in the presence of the different Hp proteins. We hypothesise that the very large Hp proteins may alter the ability of surrounding water molecules to approach the paramagnetic core and therefore modulate the detected MR signal.

Methods Informed consent was obtained from N=6 volunteers to collect 30mL of fresh blood in this study approved by the local research ethics board. Hb-Fe^{3+} was prepared by incubating washed and lysed red blood cells with 50 mg nitric oxide donor diethylamine-NONOate. Hp1-1 and Hp2-2 were reconstituted in phosphate buffered saline immediately prior to each experiment and were used to create a 10.5μM stock solution. To compare the protection efficiency of Hp, Desferrioxamine (DFO) (Novartis) a specific Fe^{3+} chelator was used as a control. To determine the lipid oxidation ability of Hb-Fe^{3+} in the presence of Hp-1-1 and Hp-2-2 the assay for thiobarbituric acid reactive species (TBARS) was used. Samples were prepared in a 96 deep well plate with well volumes of 600μL. To determine the effect of Hp-1-1 and Hp-2-2 on lipid oxidation, 1.7μM to 5.25μM concentrations of each were prepared and 1mM of Hb-Fe^{3+} solution and 1mM hydrogen peroxide were then added to this solution. MR relaxometry parameters were measured in a 3T Philips Acheiva (Philips Medical Systems) using a 2D Look-Locker technique.

To evaluate whether the effects seen *in vitro* also hold *in vivo*, N=46 patients consented to have their Hp phenotype correlated with their MR imaging in a study approved by the local research ethics board. Individuals were recruited consecutively from patients with prior diabetes volunteering for a neurovascular MRI between December 2008 and June of 2010. These patients had no previously known vascular disease and were otherwise asymptomatic for neurovascular symptoms. All study patients were imaged using a 3T Philips Acheiva scanner using an MRI pulse sequence previously validated to detect intraplaque haemorrhage.

Results Figure 1 shows the results of the TBARS assay, indicating that Hp-1 is more effective at preventing TBARS formation than Hp2. Furthermore, figure 2 shows that Hp-1 protein decreases relaxivity whereas Hp-2 increases protein relaxivity with increasing concentration. Finally, Table 1 shows that Hp-2 appears to be positively correlated with MRIPH presentation in a cohort of patients.

Discussion We have shown that the two most dichotic phenotypes of Hp have different abilities to inhibit lipid oxidation with the Hp-1-1 phenotype appearing to be more effective than Hp-2-2. Additionally, these phenotypes differ in their appearance when observed using a clinical 3T MRI system with the Hp-1-1 phenotype inhibiting Fe^{3+} enhancement and Hp-2-2 phenotype increasing Fe^{3+} enhancement. This data supports the hypothesis that patients with MRI detected IPH have an Hp phenotype that is less able to prevent iron mediated lipid oxidation thus increasing plaque vulnerability.

Additionally, in a small group of patients, the appearance of hyper intense plaque haemorrhage was seen only in patients with an Hp-2 allele and not seen in patients presenting with the Hp1-1 phenotype. While this patient study is not statistically significant, it has a trend that would support the hypothesis that patients with Hp1-1 phenotype do not develop an MR detectable IPH.

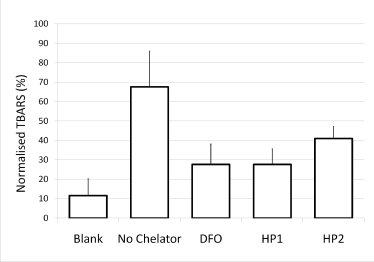

Figure 1: Normalised Thiobarbituric Acid Reactive Species (TBARS) Assay for Various Iron Binding Molecules
Upon addition of Desferrioxamine (DFO) a significant drop in TBARS is seen ($p<0.0005$) – this holds true for both Haptoglobin proteins (Hp1 and Hp2) ($p<0.005$). However, the decrease in TBARS production is not as dramatic for the Hp-2-2 protein as is for the Hp-1-1 protein, which is not statistically different from the addition of the DFO molecule.

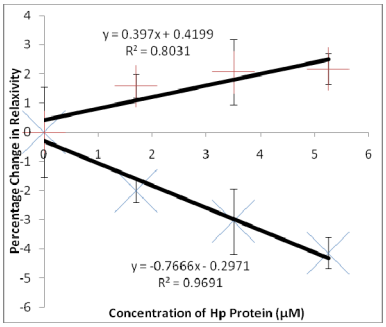

Figure 2: Percent Relaxivity Change in the Presence of Haptoglobin Proteins
Data are normalised to the samples without haptoglobin (Hp) protein. Increasing Hp concentrations have dichotic effects on the MR relaxivity – as more Hp-2-2 protein is added (red +), the relaxivity increases. Conversely, increasing concentrations of Hp1-1 protein (blue x) decrease the measured relaxivity. Hp protein samples were added to a 1mM concentration of Hb-Fe^{3+}.

	MRIPH +	MRIPH -	Totals
Hp 2	4	33	37
Hp1 Allele Only	0	9	9
Totals	4	42	46

Table 1: Haptoglobin Phenotype Distribution with respect to MRIPH status
This 2x2 contingency table shows the Haptoglobin (Hp) status of patients and their MR status, either positive IPH signal detected (MRIPH+) or not (MRIPH-). Of the n=46 patients, only 4 of these were MRIPH positive, however all four had an Hp-2 allele (either Hp2-2, or Hp1-2). The remaining 33 patients presenting with a Hp-2 allele were negative for MRIPH, suggestive that Hp-2-2 status alone is not sufficient for plaque haemorrhage. However it appears to be one risk factor that determines which patients may go on to haemorrhage.

References. [1] Moreno PR Curr Mol Med 2006;6(5):457-77 [2] Levy, Curr Mol Med 2006;6 479-488 [3] Alayash Antioxid Redox Signal 2001;3(2):313-327 [4] Sasazuki T, Immunochemistry 1971;8(8):695-704 [5] Bowman Adv Hum Genet 1982;12:189-261 453-45 [6] Suleiman Diabetes 2005;54(9):2802-2806 [7] Kalet Litman Atherosclerosis 2010;209(1):28-31