MRI Characterisation of a Rat Endothelial Injury Model

K. K. Cheung[1,2], M. J. Ramirez[3], P. Lehtolainen[3], D. L. Thomas[1], D. G. Gadian[2], R. J. Ordidge[1], A. M. Taylor[2], and M. F. Lythgoe[2]

[1]Department of Medical Physics, University College London, London, United Kingdom, [2]RCS Unit of Biophysics, UCL Institute of Child Health, London, United Kingdom, [3]Centre for Cardiovascular Biology, University College London, London, United Kingdom

Introduction – The vascular endothelium is critical in maintaining normal biological functions such as haemostasis and inflammatory responses. This layer is often impaired and becomes dysfunctional in cardiovascular diseases, and therefore the regeneration of a functional endothelium may play a crucial role in end-organ survival. We have established an animal model to investigate angioplasty-induced endothelial injury in the common carotid artery (CCA). This study aims to characterise the development of injury in this model by monitoring changes in lumen area using MRI, in order to provide an initial platform for studies incorporating stem cell therapy in the future.

Methods – ***Study Design:*** Seven male adult Sprague-Dawley rats were scanned before balloon surgery and on days 2, 7, 14, 21 and 28 after surgery.
***Surgery Protocol*:** Animals were anaesthetised with Midazolam (5mg/ml) and Fentanyl (0.315mg/ml), and maintained with 2L/min O_2. A 2F embolectomy catheter was inserted into the left CCA. The balloon was inflated and withdrawn with rotation to denude the endothelium.
MRI Evaluation: Multislice, transverse spin-echo 2DFT images of the CCA were obtained using a 2.35T SMIS system, with the first slice positioned immediately proximal to the bifurcation of the CCA. (TR = 1000; TE = 30; FOV = 25mm; 9 slices; slice thickness = 1.5mm; 256x256 pixels, scan time = 43mins). The lumen areas of the left CCA in all nine image slices were measured and averaged to obtain the left mean lumen area (MLA); this was repeated for the control side (right CCA).
Histological Methods: At day 28 the LCCAs were extracted, transversely-sectioned and H&E stained. The intima-to-media (I:M) ratio and the ratio of lumen loss due to neointimal hyperplasia (LLR) were calculated.
Data Analysis and Statistics: *i) MRI:* The ratios between the left and right MLAs (L:R ratio) were calculated for each acquisition and were compared to the pre-injury ratio by paired t-test. ii) *MRI-Histology comparison:* The MRI index (L:R ratio of individual image slices) and histological indices (I:M ratio and LLR) from day 28 were compared on a slice-to-slice basis (n=40).

Results – Figure 1 shows typical MR images at various timepoints. The mean L:R ratio from all animals plotted against time suggest three stages of luminal change (Figure 2):

1) Acute lumen gain within two days post-surgery, which may be the direct result of balloon expansion, as well as production of vasodilating compounds in response to endothelial denudation[1].
2) Progression of lumen loss after initial gain, with greatest loss at around 14 days post-surgery possibly due to neointimal hyperplasia[2].
3) Gradual recovery of lumen patency after day 14.

Figure 3a and 3b show the correlations between the *in vivo* MR index (L:R ratio) and *ex vivo* histological indices (I:M ratio and LLR). Correlations were significant and moderate (L:R vs I:M– r=-0.44; L:R vs LLR– r=0.56; both $p<0.05$). The MRI L:R ratio and histological LLR both measured the effects of neointimal hyperplasia on the lumen size, as opposed to the I:M ratio which does not take the lumen size into account; this may explain why the MRI L:R ratio has a stronger correlation to the LLR than to I:M ratio. It should be noted that as the MRI and histological indices were obtained from *in vivo* and *ex vivo* environments respectively, factors such as vascular compensatory mechanisms in response to the injury *in-vivo* and variations caused by tissue preparation may all contribute to the observed difference in measured parameters.

Conclusion – We have characterised the pattern of lumen changes of the CCA in this endothelial injury model over a period of 28 days using MRI, in which three distinct stages of balloon injury development were observed. This study shows the feasibility of MRI for assessing the effect of balloon injury by monitoring changes in lumen size *in-vivo*, which has the advantages of providing a non-invasive repeated *in vivo* assessment of vascular injury. This method would be incorporated in our future studies to investigate the therapeutic effects of stem cells in this model.

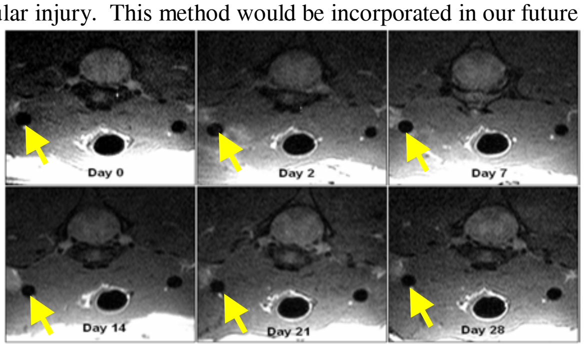

Figure 1: Typical MR images. (LCCAs indicated by arrows)

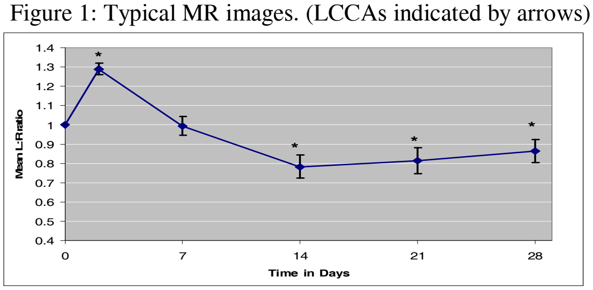

Figure 2: Diagram showing the mean L:R ratio plotted against time in days. Error bars are +/- SE. ($p<0.05$ where indicated by *)

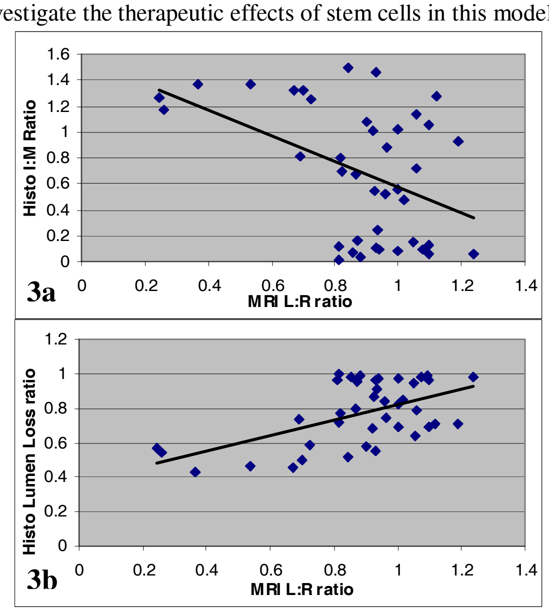

Figure 3: In-vivo MR L:R ratio plotted against histological indices (3a– I:M ratio; 3b– LLR), with r=-0.44 and 0.56 respectively. ($p<0.05$)

References – [1] Joly GA et al., Circulation Research 1992; 71:331-8 [2] Gabeler EEE et al., BMC Cardiovasc. Disorders 2002; 2:16-28