CHARACTERISATION OF VASCULAR INJURY RESPONSES FOLLOWING BALLOON INJURY OF THE RAT CAROTID ARTERY USING COMBINED *IN VIVO* IMAGING AND *EX VIVO* MICROSCOPY

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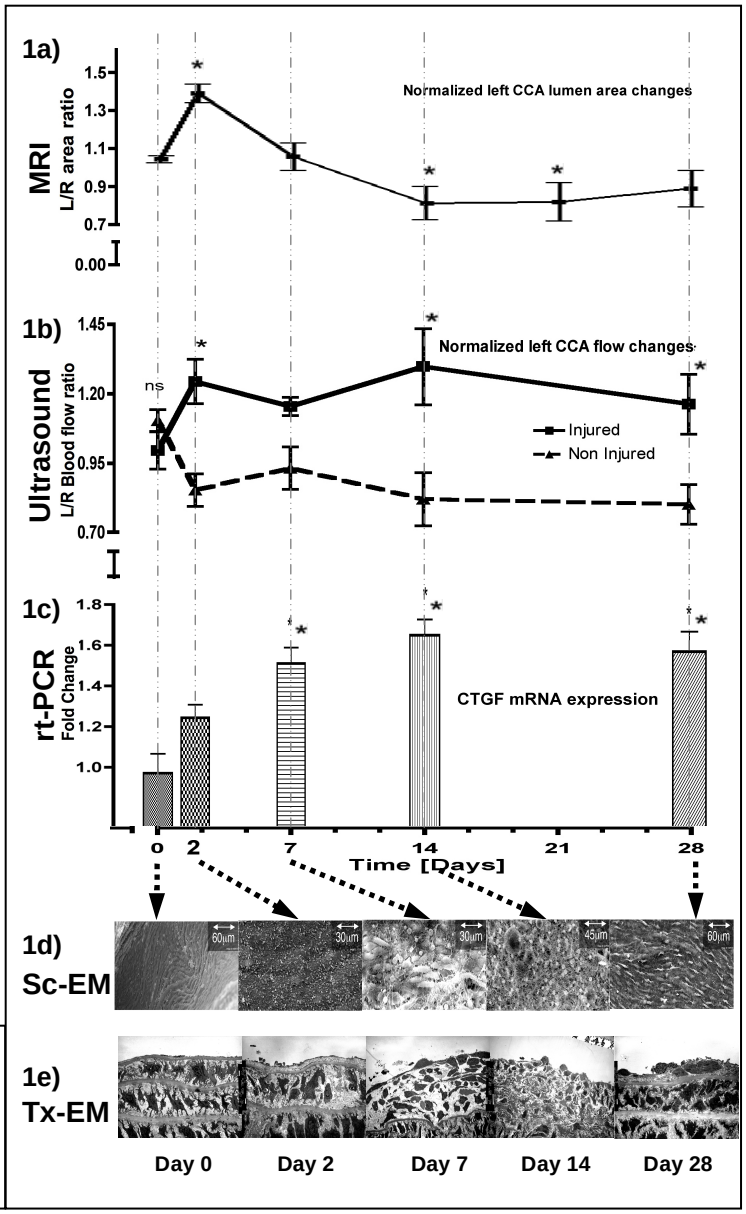
Introduction – The vascular endothelium is critical in maintaining normal biological functions such as haemostasis and inflammatory responses. This layer is often impaired and becomes dysfunctional in cardiovascular diseases, and paradoxically, following revascularisation therapy such as angioplasty. Understanding the evolution of vascular remodelling is therefore of great importance and may provide insights for the development of novel interventions. Here we present the use of *in vivo* MRI and vascular ultrasound in combination with *ex vivo* electron microscopy for extensive characterisation of the events involved in vascular remodelling following balloon angioplasty of the rat carotid artery over a 28 day follow-up period.

Methods – ***Animal Model:*** Male Sprague-Dawley rats were anaesthetised and underwent balloon surgery using a well-described method[1]. In brief, a 2F embolectomy catheter was inserted into the left common carotid artery (CCA), inflated and withdrawn with rotation to denude the endothelium. ***In vivo MRI and ultrasound imaging:*** Animals were imaged at five time points: 24 hours prior to surgery (baseline) and 2, 7, 14 and 28 days post-surgery. For MRI (n=7), nine transverse image slices were acquired using a spin-echo 2DFT sequence on a 2.35T SMIS system. Lumen areas of the left and right CCA were measured by ImageJ software. For ultrasound and Doppler studies (operated n=6; sham-operated n=6), anatomy of both CCAs were investigated using 2D, colour Doppler and M-mode sonography (14MHz transducer/Vivid 7, GE Healthcare). Pulse wave Doppler was applied in the mid-segment of CCAs, approximately 1cm before the bifurcation. Lumen diameters, pulse rate, velocity time integral and blood flow were acquired. Both MRI and ultrasound data obtained from the left CCA was normalised to the non-operated right CCA to control for growth. ***Ex vivo electron microscopy:*** CCA samples were extracted at the stated time points, dissected longitudinally and fixed in glutaraldehyde for analysis. ***CTGF mRNA expression:*** cDNA was synthesised following RNA isolation, and real-time PCR was performed (LightCycler 2.0). Relative quantification of gene expression was performed using the comparative ΔΔCT method. Data were normalised with GAPDH.

Results – MRI evaluation showed a significant increase of lumen area on day 2 post-surgery, followed by a significant decrease below baseline (time point 0) at day 14 and 21 (Fig. 1a). Ultrasound demonstrated a consistent and significant increase in blood flow of the left CCA in the injured group post-surgery compared to the sham group (Fig. 1b). EM images confirmed denudation of endothelium and showed sustained platelet aggregation 2 days post-surgery. This was followed by leukocyte aggregation, fibrinous deposits and neointimal hyperplasia on day 7. Neointimal hyperplasia peaked at day 14; this related well with the MRI data, as high degree of neointimal hyperplasia may be reflected by severe loss of lumen areas. This is further supported by analysis on CTGF mRNA expression, a marker for collagen deposits and cell proliferation, where expression progressively increased to a maximum also at day 14 ($p<0.05$ vs day 0). By day 28, a reduced degree of neointimal hyperplasia was observed by EM, and endothelial regeneration appeared to be complete (Fig. 1d & 1e).

Conclusion – In this study, we have presented an extensive characterisation of events related to vascular remodelling in a rat carotid balloon injury model. *In vivo* MRI and ultrasound imaging provided the means for repeated, non-invasive assessments of the physiological responses to vascular injury, while the basic mechanisms that underlie vascular remodelling were further investigated by *ex vivo* techniques. Specifically, the progression of lumen changes revealed by *in vivo* MRI was in good agreement with the morphological and functional changes as demonstrated by EM and rt-PCR. The successful combination of methods presented in this study provides a platform for future experiments including tracking of labelled cell populations to assess their impact on the complex interactions involved in vascular remodelling and endothelial regeneration.

Reference – Fishman JA et al, Lab Invest 1975; 32:338-51



Figures 1a) MRI data showing normalised LCCA lumen changes over time (*$p<0.05$ vs day 0); **1b)** Ultrasound data showing normalised LCCA flow changes (*$p<0.05$ vs time & injury); **1c)** Real-time PCR data showing CTGF mRNA expression at different time points (*$p<0.05$ vs day 0); **1d)** Surface scanning EM (Sc-EM); **1e)** Transmission EM (Tx-EM).