In-vivo imaging of macrophage accumulation in symptomatic carotid atheroma

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Background

It has long been accepted that the risk of thrombo-embolic stroke in the presence of symptomatic carotid stenosis is high[1]. Indeed studies over the years have shown a definite benefit of surgery in those patients with stenosis of 70% or more of the carotid lumen. Conventional clinical measures of "plaque load" are derived from simple angiographic measures of stenosis and pay no attention to the composition of the plaque and can often underestimate degree of stenosis due to the phenomenon of vascular remodelling. It has been shown that "vulnerable" plaque is that which has a thin fibrous cap, extensive lipid core and an associated inflammatory infiltrate.[2] This inflammatory infiltrate has been impossible to detect *in-vivo* until recently.

High resolution MR imaging allows accurate quantification of plaque components, thereby improving risk stratification in these patients and the use of the novel contrast agent Sinerem, an Ultrasmall Super-Paramagnetic Iron Oxide (USPIO) which is taken up by the macrophage population *in-vivo* has allowed the direct imaging of plaque inflammation at 1.5T visible in FSE T1,T2 and T2* sequences.[3] Studies using USPIO, thus far, have used very limited numbers.

Methods

30 Symptomatic patients were recruited to the study through the neurovascular department. They were all scheduled for endarterectomy and were imaged prior to this. Patients were imaged at 1.5T using our carotid protocol (including T1,T2,T2*,FatSat and STIR sequences) pre and post Sinerem infusion. The time from USPIO infusion to endarterectomy ranged from 40 hours to 18 days (mean ± S.D, 6.9 ± 4.8 days).

At the time of surgery, the plaques were retrieved, fixed, sectioned and underwent histological analysis using a variety of stains and immunohistochemistry. One stain (Perls) was used to determine presence of USPIO under light microscopy. Antibodies, identifying macrophages were also used (MAC387).

MR images and histology sections were validated for presence of plaque and quality of image/specimen. All deemed to be of sufficient quality to proceed to further analysis were matched histology slice to MR slice by two experienced readers and were analysed for the presence of macrophages and Perls on histology and post USPIO signal drop on imaging.

Results

Patient demographics were 22 males and 8 females overall with a median age of 70yrs and severe internal carotid artery stenosis (median ICA stenosis 77%). All had risk factors consistent with severe atherosclerotic disease.

Comparison between the pre- and post-USPIO infusion image pairs resulted in 128 (60%) post-infusion MR images being described as positive for USPIO enhancement. (evidence of a new signal loss) from a total of 26 individuals.

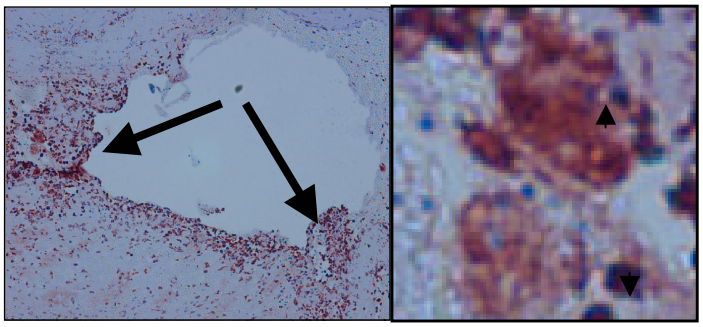

Figure 1. Localisation of macrophages to fibrous cap. (a) CD68+ macrophages accumulating in shoulder regions of the plaque (x4) and (b) high power view (x 80) showing intra-cellular accumulation USPIO particles (black arrowheads).

On histological analysis, there were 97 (47%) sections from 23 plaques that demonstrated Perls positivity. This was seen in distinct areas of macrophage accumulation, adjacent to areas of plaque haemorrhage which were not stained positively with the reagent. In particular, at higher magnifications, Perls staining appeared to co-localise to macrophages in shoulder regions of the fibrous cap (Figure 1).

Qualitative MR image analysis was highly sensitive (92.5%) and moderately specific (64%) for detection of USPIO particles within atheromatous plaques. There was, in addition, good agreement between MR imaging and histology in the location of the Perls stain and USPIO signal effect. However, the agreement between MR and histology for characterising the nature of the USPIO signal effect (ie focal, multi-focal or diffuse) was only moderate (Cohen's kappa=0.47, p<0.05).

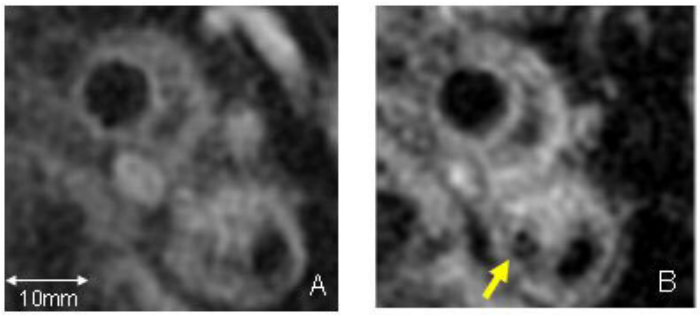

Figure 2 Axial MR images pre-(A) and post-USPIO(B) (T2*W TE=5.6ms) showing USPIO focal enhancement (yellow arrowhead)

Conclusions

We were able to demonstrate USPIO enhancement in 26/30 symptomatic individuals. Thus, USPIO enhanced MR examination of carotid atheroma allows *in-vivo* demonstration of macrophage accumulation within the plaque. Now that this technique has been validated for symptomatic patients, further studies looking at risk stratification of asymptomatic patients and those with moderate symptomatic stenoses are needed to better risk stratify these groups and inform subsequent therapy.

Acknowledgements

The authors wish to thank GlaxoSmithKline for funding the study.

References

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