E-selectin targeted USPIO enhancement of atherosclerotic plaques visualizes treatment response and mechanism of action

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Introduction

In atherosclerotic plaque development inflammatory processes play a key role. E-selectin, a cell surface adhesion molecule, is induced exclusively on activated endothelium and mediates the adhesion of inflammatory monocytes, which infiltrate the plaque, and become macrophages.

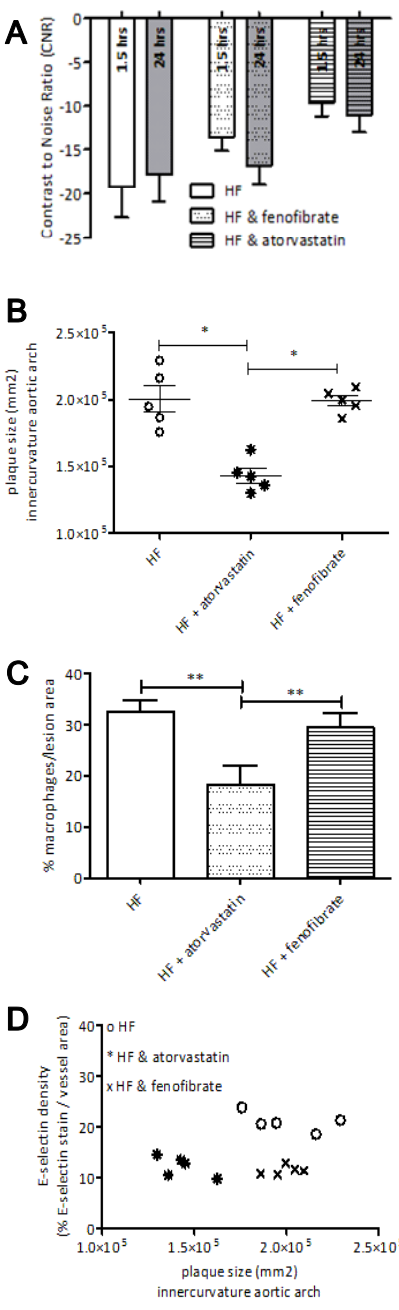
Anti-atherosclerotic treatment is based on either reducing the macrophage pool, or influencing the macrophage M1/M2 differentiation. Statin treatment reduces the attraction of monocytes, thereby reducing macrophage accumulation in the plaque. In contrast, fibrate treatment activates PPAR-α, which shifts the monocyte differentiation from M2 to M1 polarization, and to a more stable plaque phenotype.

The aim of this study was to test whether E-selectin targeted USPIOs could visualize the treatment-specific effects of statins or fibrates in ApoE$^{-/-}$ mice.

Methods

A **peptide ligand** for E-selectin (sialyl Lewis X) was conjugated to an ultra small iron oxide (USPIO).[1] **ApoE-/-mice** were exposed to a western diet with or without atorvastatin (0.1 g/kg BW) or fenofibrate (0.1 g/kg BW) for 4 weeks. **MRI experiments** were done after treatment at a 9.4T vertical bore Bruker system. Retro-spectively gated T2*W MRI was performed on the ascending aorta before and 1.5 hours and 24 hours after i.v. injection of E-selectin-USPIO. These two time points were chosen based on earlier pilot experiments to provide measures of specific endothelial USPIO targeting (1.5 h) and non-specific macrophage related USPIO uptake (24 h).[2] Contrast-to-noise ratios (CNR) were determined based on regions of interest drawn in the vessel wall of the aortic arch and muscle.

Histology was performed on the aortic arch and carotid arteries to determine plaque size and extent of the macrophage pool using F4-80 and MoMa2. M1 and M2 cells were classified according to their staining for MCP-1, CD54,iNOS, CD206, and arginase-1. **Statistical analyses** were done in SPSS 17.0.2 using one-way analysis of variance (ANOVA), followed by a Bonferroni correction.



Results & discussion

T2*W MRI at 1.5 h after E-selection-USPIO showed a significant decrease in endothelial enhancement at this time point for both treatment, though more pronounced in the statin group (Fig. A). At 24 hours after injection, USPIO uptake in the plaque is dominated by passive uptake in all groups.[2] Passive uptake was highest in the untreated animals; passive USPIO accumulation was significantly reduced by statin treatment, but not by fibrate treatment (Fig. A). Histology of the aortic arch confirmed the stipulated treatment mechanisms. Plaque size, largely dependent on the macrophage pool (Fig. C) was reduced only for the statin treatment, not for fibrates (Fig. B). In contrast, endothelial expression of E-selectin is reduced for both treatments compared to the untreated group (Fig. D). The MRI results at both imaging timepoints reflect the different treatment effects: endothelial activation is reduced with both treatments, resulting in a lower specific endothelial targeting (CNR) at 1.5 h. The plaque macrophage pool is only reduced with statin treatment, resulting in lower passive USPIO uptake at 24 hours, whereas the CNR after 24 h is unchanged for the fibrin-treated group. However, histology did show a shift from M2 to M1 polarized macrophages (data not shown), indicating that fibrate treatment did result in a more stable plaque phenotype.

Conclusion

The combined imaging of E-selectin and the macrophage pool allows to monitor treatment outcome of antiatherosclerotic drugs and provides insight into the treatment mechanism. With the fibrate treatment, we show that plaque size alone is not sufficient to assess treatment response.

References

1. Boutry, S.*et al.* Magn Reson Med. 2005 Apr;53(4):800-7. 2. Den Adel B. *et al.* Proc. of the ISMRM 2012, 4356.