p38 MAPK Inhibition Reduces Aortic Ultrasmall Superparamagnetic Iron Oxide Contrast Agent Uptake as Assessed by MRI in an Atherosclerotic Mouse Model

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Introduction

Ultrasmall superparamagnetic iron oxide (USPIO) has been used as a contrast agent for non-invasive MRI assessment of atherosclerotic plaque inflammation both in humans (1) and WHHL rabbits (2) but has not been extensively investigated in mouse atherosclerotic models. Daugherty et. al. (3) showed that the addition of Angiotensin II (Ang II) to apoE$^{-/-}$ mice promotes a marked increase in the number of macrophage present in the atherosclerotic lesions. The p38 mitogen-activated protein kinase (MAPK) pathway has been shown to play an important role in the atherosclerosis lesion development process, particularly in endothelial /monocyte interactions. In this study we examined whether it would be possible to non-invasively monitor alteration of plaque inflammation using a p38 MAPK inhibitor in Ang II infused apoE$^{-/-}$ mice

Methods

ApoE$^{-/-}$ mice (n=30) were subjected to a subcutaneous infusion of either saline or Ang II for 21 days via Alzet osmotic minipumps. Animals were assigned to one of three groups: saline, Ang II infusion (1.44 mg/kg/day) or Ang II + p38 MAPK inhibitor (SB239063, 150 mg/kg/day, dietary). Dosing of the p38 MAPK inhibitor was initiated one week prior to Ang II administration. After 21 days, USPIO MRI contrast agent (Combidex®) was administered i.v at 1000 μmol/kg on two consecutive days prior to scanning on a 9.4 Tesla Bruker μimaging system. During each imaging session a series of transverse, sagittal, and coronal scout images through the heart (FLASH sequence, TR/TE = 50/2.7 ms, FOV = 3×3 cm, matrix = 128 × 128, slice thickness = 0.5 mm, number of averages = 4) were acquired. An oblique 2D TOF image slice was positioned such that the image slice captured the entire aortic arch from the root to the left subclavian artery. A final high resolution image was acquired in CINE (FLASH sequence, TR/TE = 12/2.8 ms, FOV = 2.5×2.5 cm, matrix = 256 × 256, slice thickness = 1.0 mm, number of averages = 8). At the end of the final imaging sequence, the entire aorta and heart was removed following in situ formalin fixation. Cathepsin-S immunohistochemical staining for macrophage and Perl's iron staining for USPIO were performed on sections through the aortic valves. Absolute iron content was measured in the aortic arch by ICP-MS analysis. Body weights, food consumption, blood pressure, urinary isoprostane, plasma lipids and cytokines were analyzed.

Results

Plasma chemokines MCP-1 and MIP-1b were significantly elevated in the Ang II, but not in the Ang II+SB-239063 group. *In vivo* USPIO uptake in the aortic arch was observed by MRI as focal regions of signal loss in all animals (Fig 1). The normalized signal loss was increased in the Ang II administered apoE$^{-/-}$ mice (Fig 2) while treatment with SB239063 decreased normalized signal loss in the apoE$^{-/-}$ mouse arch. However, while the Ang II group had significantly higher absolute iron content (↑103%, P<0.001) in the arch compared with the saline group, the Ang II+SB-239063 group did not (↑6%, NS). There was a significant positive correlation between the absolute iron content of the aortic arch and the normalized *in vivo* MRI signal intensity loss present in the aortic arch (r=0.79, P<0.001). Immunohistochemical localization of iron (Perl's) and macrophages (Cat-S) within the atherosclerotic lesion can be seen in Fig. 3. Perl's staining demonstrated that iron accumulation is mainly associated with the macrophages.

Conclusion

The present study demonstrates that non-invasive assessment of USPIO uptake, as a marker for inflammation in mouse atherosclerotic plaque, is feasible and that p38 MAPK inhibition attenuates the pro-inflammatory uptake of USPIO in the aorta of Ang II infused apoE$^{-/-}$ mice.

Fig 1

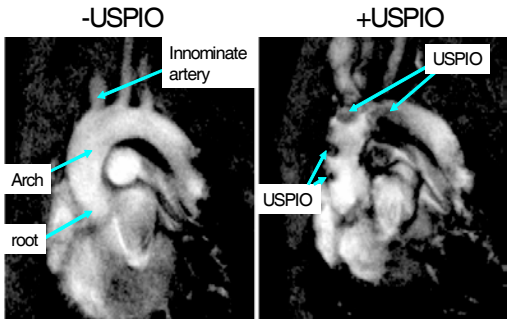


Fig 2

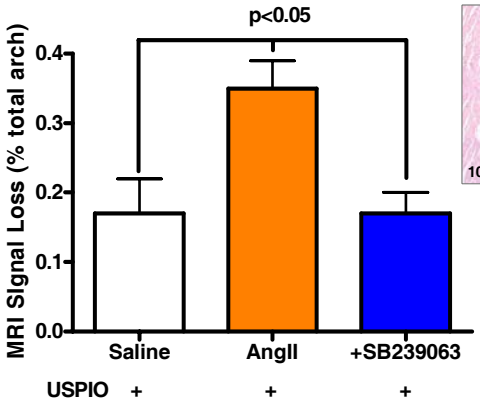
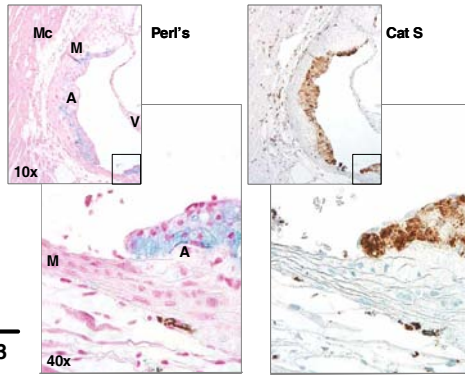


Fig 3



References:

1) Kooi et al. Circulation 107:2453, 2003
2) Ruehm et al. Circulation 103:415, 2001
3) Daugherty et al. JCI 105:1605, 2000