

Pharmacological Inhibition of CCR2 Decreases Macrophage Infiltration in the Aortic Root of the huCCR2ki / apoE^{-/-} mouse: MRI Assessment

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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) and has recently been used to show pharmacological inhibition of macrophage phagocytosis in apoE^{-/-} mice (3). The purpose of this study was to assess whether pharmacological intervention with a CCR2 antagonist could reduce macrophage infiltration into atherosclerotic plaque as monitored using USPIO contrast agent in an apoE^{-/-} mouse.

Methods

Mice (huCCR2/apoE^{-/-}, 22-24 wks, n=40) were subjected to a subcutaneous infusion of Ang II (1000 ng/kg/min) for 5 wks via an Alzet osmotic minipump. Animals were assigned to one of three groups: Ang II (vehicle); Ang II + CCR2 inhibitor (GSK1344386B, 10 mg/kg/day, dietary); control (-Ang II). After 35 days of dosing, USPIO MRI contrast agent (Combidex®) was administered i.v. at 1000 μ mol/kg on two consecutive days prior to scanning on a 9.4 Tesla, Bruker μ imaging 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 = 3x3 cm, matrix = 128 \times 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 = 2.5x2.5 cm, matrix = 256 \times 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. Ex vivo high resolution MRI of the aortic root was performed prior to MOMA immunohistochemical staining for macrophage and Perl's iron staining for USPIO. In a subset, absolute iron content was measured in the aortic root by biochemical analysis. Body weights, food consumption, thioglycollate induced peritonitis, plasma lipids and cytokines were analyzed.

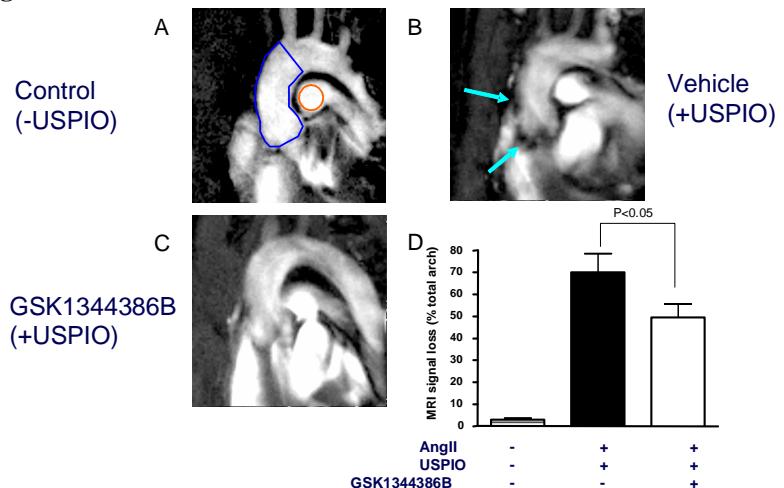
Results

Following 5 weeks of dietary dosing, there were no significant differences between groups in body and liver weight, or in plasma cholesterol. The peritonitis experiment demonstrated 92% inhibition of monocyte recruitment in the GSK1344386B group. In vivo MRI revealed a 30% reduction in signal intensity attenuation in the ascending aorta following USPIO administration in the GSK1344386B group (Fig 1). Ex vivo MRI reflected a decrease in aortic root plaque area (0.81 ± 0.05 vs 0.69 ± 0.03 mm², p<0.05) and decrease in aortic root plaque R2* (0.13 ± 0.01 vs 0.10 ± 0.01 ms⁻¹, p<0.001) while the absolute USPIO uptake (Fe content) in the aortic root was reduced (65.3 ± 22.0 vs 8.3 ± 1.3 μ g/g, p<0.05) in the GSK1344386B group. Furthermore, there was a 30% decrease in Mac-2 staining of the aortic roots in the GSK1344386B group.

Conclusion

This is the first description for the use of a selective CCR2 antagonist to decrease the rate of atherosclerotic plaque progression. Additionally, the application of USPIO contrast agent was used to assess decreased macrophage infiltration into the plaque non-invasively.

Fig 1



References:

- 1) Kooi et al. Circulation 107:2453, 2003
- 2) Ruehm et al. Circulation 103:415, 2001
- 3) Morris et al. ATVB 28:265, 2008