Molecular Imaging of Inflammation in Atherosclerosis Plaque Using Functionalized MRI Contrast Agent

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Introduction: The pathogenesis of atherosclerosis contains an important inflammatory component and can be described by a series of specific cellular and molecular responses [1]. Functional parameters such as vessel wall transport between blood and artery wall play an important role in early stages of atherosclerosis and appear to change progressively with the evolution of the disease [2]. The apolipoprotein E knockout ($ApoE^{-/-}$) mouse is well known to develop atherosclerotic lesions that have similar characteristics, evolution and distribution to those found in humans. In this study, we propose to evaluate the arterial wall contrast enhancement with a new macromolecular gadolinium agent functionalized to target inflammation in $ApoE^{-/-}$ mice.

Methods: The CMD-A2-Gd-DOTA (P717, Guerbet, France) was modified into a functionalized product (CM8S) for targeting of the adhesion molecule, P-Selectine [3]. The P717F have a high relaxivity r_1= 11.7 $mM^{-1}(Gd).s^{-1}$ (60 MHz). Each product (P717 and CM8S) were marked with rhodamine. MRI experiments were performed on a 2 Tesla magnet and half-birdcage coil. P717 and CM8S are evaluated in $ApoE^{-/-}$ and C57BL/6 mice by pre and post contrast MRI after injection of 60µmol Gd/kg. After an axial and coronal scout images of the abdominal aorta, pre and post contrast images were acquired. For MR angiography (MRA), a 3D-fast gradient echo was used in the coronal plane with the following parameters: TR, 13 ms; TE, 3 ms; flip angle, 30°, and pixel size of 211×211×234 μm^3. Axial T1 weighted images of the abdominal aorta were obtained above and below the renal arteries with a 2D multislice spin echo sequence (13 slices): TR, 450 ms; TE, 18 ms; 1 mm slice thickness, in plane resolution of 90 µm and four signal average. Signal enhancement was measured from pre- and post-contrast T_1 weighted images by Creatools, a software dedicated to arterial wall analysis [4]. The data obtained by MRI were then correlated to histo-pathology and immunohistochemistry (P-selectin, CD68 and PSGL1, ligand of P-Selectine). Immunofluorescence (IFC) (fluorescein and rhodamine filters) was performed to co-locate the target molecule and the marker.

Results: Measurement show a major effect of the CM8S in $ApoE^{-/-}$ (increase in signal > 40%) (Figure 1) compared with $ApoE^{-/-}$ mice injected with P717. The presence of P-selectine in the lesion at the same level of the aortas is shown by immunohistochemistry and is confirmed by fluorescein in IFC (Figure 2) in $ApoE^{-/-}$ mice but not in control mice (C57BL/6). CM8S presents a different spatial distribution compared to P717: a specific enhancement is shown by MRI and IFC whereas the non specificity of P717 is confirmed.

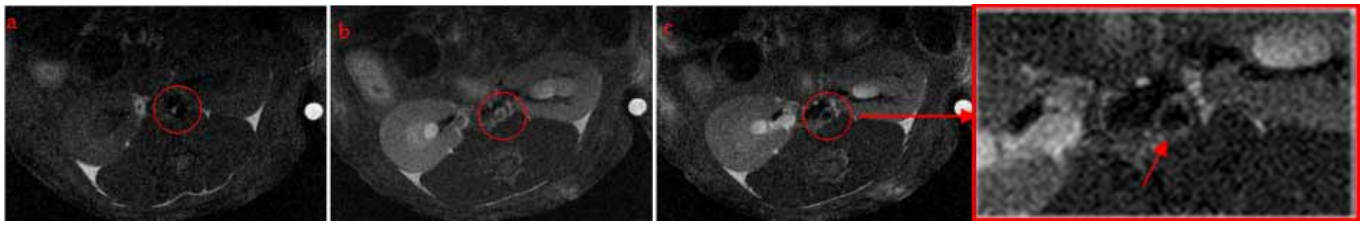

Figure 1: Axial T1 weighted images of the abdominal aorta from an $ApoE^{-/-}$ mouse, 28 weeks old, acquired before (a) and 15 (b), 30 min (c) after CM8S injection. Circle indicates the aorta.

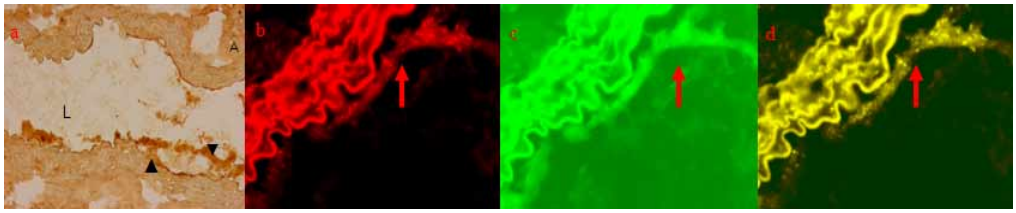

Figure 2: The P-Selectine as shown by immunohistochemistry (a, arrowhead) and green fluorescence IFC (c, arrow). Red fluorescence (b) is for rhodamine and yellow for the colocalisation (d) in the same $ApoE^{-/-}$ mouse as Figure 1 injected with CM8S.

Conclusion: This study demonstrates the efficacy of the functionalized product CM8S tested in vivo in the mouse to mark the inflammatory lesion compared to non specific P717.

References

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