

Inflammation Imaging of Atherosclerosis: MRI of Iron Oxide-Labelled Macrophages Trafficking in ApoE2 (KI) Mice Lesions

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The inflammatory process is a crucial event in atherogenesis in which macrophages play an important and determinant role but their insight evolution remains unknown [1,2]. The chemotaxic response through gamma interferon (INF γ) provide their accumulation into inflammatory sites[3] and high resolution MRI permits the detection of their dynamic recruitment [4,5]. Our purpose is the MRI characterization of plaque inflammation and macrophages trafficking to aortic arch in ApoE2 (K.I) mice using iron oxide Anionic Magnetic Nano Particules (AMNP) labelled macrophages.

Method: Bone Marrow cells were cultured for 8 days in Teflon flasks at 10^6 cells/ml in presence of macrophage clone stimulating factor (M-CSF) and Fms-related tyrosine kinase 3 ligand (Flt3-Ligand) (10 ng/ml). Macrophages were activated by INF γ (10ng/ml) during 3 days. Macrophages were labelled for six hours in a 0.54mM AMNP solution [6]. 10^7 cells per mouse were injected after baseline MRI and followed during 3 days. ApoE2 ki mice (mean age 56 ± 6 weeks) under a high fat diet have been used in the study. C57BL/6J mouse were used as control. A high resolution multi-contrast MRI protocol was performed on a 4.7 Tesla Bruker: a proton density and a T2 weighted spin echo (SE) sequences, two multi TE gradient echo (GE) sequences for standard negative contrast imaging of iron and a positive contrast sequence (gradient compensation method). Mice were euthanized by cervical dislocation. The ascendant aortic arch was taken for histological Prussian blue + DAB enhanced, for iron and immunohistochemistry (IHC) analysis using monoclonal antibodies directed against CD11b, F4/80, and MHC class II molecules for macrophages (figure 1).

Results:

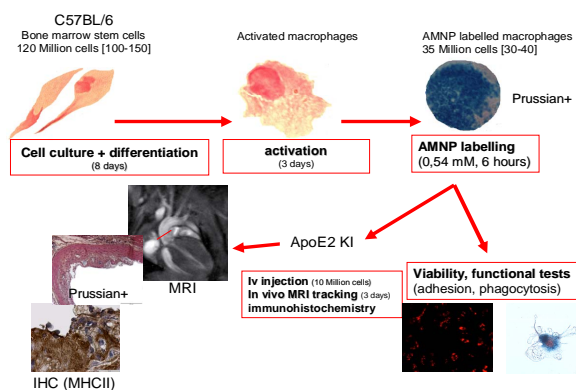


Figure 1: Experimental protocol.

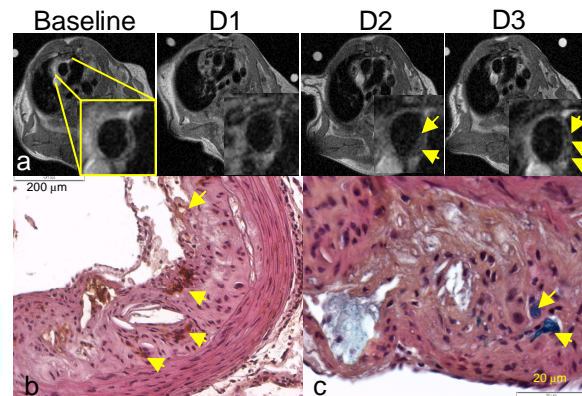


Figure2: MRI signal loss (arrows) (a) DAB enhanced (+) and prussian blue(+) staining, iron distribution corresponding (b and c).

After cell culture, we obtained more than 10^8 macrophages. This number reduced to 3.10^7 to 4.10^7 after macrophages labelling, with 14% lightly, 30% moderately and 56% strongly labelled. MRI shows a local signal loss inside the vessel wall, (mean signal over the 3 days is -27% to -37% $n=6$), which is confirmed by co-localization with prussian blue positive cells and activated macrophages (MHCII) (figure 2). More over a dynamic intra-plaque trafficking was observed during the 3 days with intimal infiltration and/or adventitial, confirmed also by prussian blue and MHCII.

Discussion:

MRI macrophage labelling allows to assess specifically atherosclerotic plaque inflammation status in-vivo. As ApoE2 Ki mice is an excelent model for plaque inflammation this method can be used to follow-up in-vivo treatment accuracy and may provide a new index of the plaque evolution (i.e, migration and intimal/adventitial distribution.

References:

[1] F.K.Swirsky 2007, [2] D.B.Weinreb 2007, [3]A.Mantovani 2002, [4] B.Qiu 2007,[5] M.F.Kircher, [6] C.Wilhelm 2008