Intravenous injection of fluorescent iron-oxide nanoparticles for in vivo loading and tracking of monocytes to myocardial infarction by MRI and optical imaging

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Introduction

Inflammatory cells are involved in numerous pathologies, including myocardial infarction. In the case of myocardial infarction the majority of the infiltrating cells are monocytes/macrophages. So, the aim of the study was to evaluate the feasibility of in vivo monocytes/macrophages loading by intravenous injection of fluorescent iron oxide nanoparticles and tracking of these cells in the very same animal to myocardial infarction by MRI and optical imaging. Hence, monocytes/macrophages were in vivo loaded with i.v. injection of fluorescent iron-oxide nanoparticles before inducing an inflammatory lesion in rats. Once the nanoparticles have been cleared from the plasma, an ischemia-reperfusion episode of the heart was realized to induce an acute and localized inflammation. The monocytes/macrophages infiltration of the MI was then tracked in vivo by MRI in the very same animal and cell infiltration confirmed by ex vivo MRI, reflectance fluorescence and histology.

Materials and Methods

Experiments were approved by the institutional animal care committee. Rats were injected with fluorescent iron oxide nanoparticles (10 mg/kg) (n=12). After disappearance of the nanoparticles from the blood, myocardial infarction was induced. Monocytes/macrophages were then tracked in the very same animal by MRI and optical imaging. Control groups were 1) non injected animals (n=12), 2) injected animals associated to a sham operation (n=4). All images were acquired on a clinical scanner at 1.5T with a dedicated microscopic surface coil (47 mm diameter) (Intera, Philips Medical System, Best, NL). Cine imaging was realized using a prospective ECG-triggered segmented turbo field echo (TFE) cine sequence, TR/TE 12/4.9 ms. Flip angle (FA) 30°, 288x288 matrix sampled on a Cartesian grid, 80 mm field of view (FOV) and 2 mm slice thickness yielding an in plane resolution of 280x280 µm. Eleven to twenty cardiac phases where acquired per R-R cycle depending on the heart rate. Two short axial plane, as well as 2 and 4 cavities views were acquired for each animal. Fast Field Echo (FFE) sequence was used for iron oxide particles detection; TR/TE1/TE2 350/7/15 msec. FA 50°, 395x608 matrix sampled on a Cartesian grid, 120 mm FOV and 2 mm slice thickness with a negative gap of -1mm yielding an in plane resolution of 200x300 µm. Multi-echo spin echo sequence was used to calculate T2 relaxation time; TR/TEmin/TEmax 520/5.9/94.4 msec with 16 echoes in-between, 256x256 matrix sampled on a Cartesian grid, 80mm FOV and 3 mm slice thickness yielding an in plane resolution of 310x310 µm. T2 values were calculated according to the equation: signal intensity = M0 (e-TE/T2), where M0 is the initial magnetization, TE is the time of echo.

Ex vivo fluorescence imaging: The heart was explanted, rinsed in NaCl 0.9% and imaged with an IVIS-200 system (Calipers live science AG, Switzerland) using filters of 500-550 / 575-650 (abs/em). Images were digitally acquired as 16-bit images on a cooled-CCD camera.

Ex vivo MR imaging: The heart was placed inside a test tube containing NaCl 0.9% and scanned again with a surface microscopic coil of 23 mm diameter utilizing the same FFE sequence as in vivo, but at higher resolution (100x100 µm and a slice thickness of 2 mm).

Results

The presence of iron-loaded cells can be detected by magnetic resonance imaging in vivo. Here, we showed that the detection of inflammatory cells in vivo correlated well with ex vivo imaging (MRI and reflectance fluorescence) and histology (see figure 1, 2).

Conclusion

This study demonstrates that monocytes/macrophages can be loaded in vivo by a simple i.v. injection of iron-oxide nanoparticles and then tracked in vivo by MRI in the very same animal in a rodent model of MI. Ex vivo imaging (MRI and fluorescence) as well as histology correlated well with in vivo results, with the estimated number of macrophages in vivo by MRI showing less than 3% error compared to histological analysis.

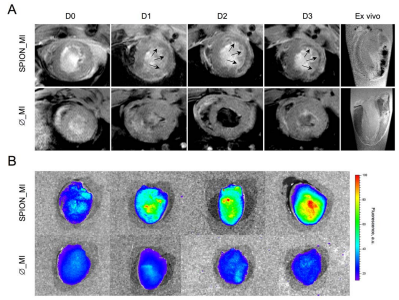

Figure 1: In vivo MRI of the infarcted groups is presented in (A). The first line corresponds to a rat, who had received iron oxide nanoparticles and clearly shows the appearance over time (day [D] 0 to day 3) of a hypointense signal in the myocardial infarction area (arrows). The second line corresponds to a rat, who hac not received iron oxide nanoparticles and does not show any hypointense signal. Ex vivo MRI (last column) confirmed the in vivo results. Ex vivo reflectance fluorescence (B) also confirmed the MR results. A representative image of the injected group shows a clear increase in fluorescence over time, whereas the non-injected group does not show any fluorescent signal.

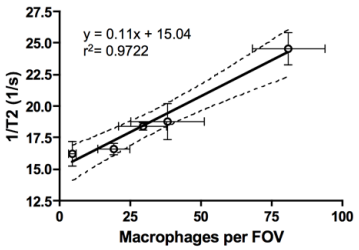

Figure 2:
Correlation between the numbers of iron-oxide loaded macrophages counted in immunohistochemistry and the 1/T2 values. Hence, quantitation of the monocytes/macrophages infiltration of MI in vivo by MRI provides an excellent estimate of the number of macrophages, with an error < 3%. Dashed line corresponds to 95% confidence interval.