Preferential magnetic labeling and in vivo tracking of bone marrow derived macrophages after their intravenous administration in COPD animal model

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Abstract:
Noninvasive imaging of macrophages activity has raised increasing interest for diagnosis of different diseases, which make them attractive vehicles to deliver contrast agents for diagnostic or drugs for therapeutic purposes. Coupled with the use of super-paramagnetic iron oxide (SPIO) nanoparticles, MR Imaging of macrophages offers a promising noninvasive approach for an early and better assessment of the pathological and physiological impairments in chronic obstructive respiratory diseases (COPD). However, before developing such targeted delivery mechanism, the effect of their magnetic labeling need further investigation. Inside the body, different environmental conditions will orient macrophages to have either a pro-inflammatory (M1) or immuno-modulator (M2) profile. In this study, the effect of SPIO PEGylation (addition of Polyethylene Glycol) and their further surface modification with carboxylic (-COOH) or amine (-NH2) groups on M1 and M2 bone marrow derived macrophages (BMDM) phenotype, labeling efficiency and toxicity was first investigated. Then, macrophages biodistribution and noninvasive tracking were assessed after their intravenous administration in a COPD animal model.

Bone marrow cells issued from tibiae and femora of donor Balb/c mice were first differentiated into M1 or M2 polarized macrophages [1]. They were then labeled with either SPIO, SPIO-PEG, SPIO-PEG-COOH or SPIO-PEG-NH2 nanoparticles (100 nm) at extracellular iron concentration of 2 mM (100 µg/ml). Labeling conditions (i.e., nanoparticles size and concentration, incubation time, culture medium …) were based on preliminary study performed on non-polarized macrophages labeled with SPIO showing a best compromise between labeling efficiency and biocompatibility [2]. M1 and M2 labeling efficiency was determined using two independent techniques: Ferrozine-based spectrophotometry and magnetophoresis (or Cell Tracking Velocimetry) assays.
Interestingly, both techniques showed statistically similar iron concentration in all labeled cells as the used magnetic nanoparticles have equal magnetization value. A higher uptake by M2 macrophages compared to M1 subsets was observed for all the different SPIO nanoparticles (Figure1a), which is in line with their role as debris scavengers. An enhanced labeling efficiency for both carboxylic and amine modified PEGylated SPIO was measured for M1 and M2 BMDM subpopulations. Along with their polarization flexibility, this will certainly pave the way for easier coupling of positively or negatively charged magnetic nanoparticles with specific antibody targeted to a particular subpopulation of macrophage and offer a promising strategy for an early and better diagnosis of inflammatory diseases using noninvasive MRI.

Magnetic nanoparticles biocompatibility was then evaluated using MTT cell growth assay for cell viability, JC-1 fluorescence kit for mitochondrial membrane potential as a hallmark of apoptosis and 2',7' dichlorofluorescein diacetate (DCFDA) fluorogenic dye for Reactive oxygen species generation. No variation in cell viability and mitochondrial membrane potential was detected after labeling M1 and M2 macrophages with the different magnetic nanoparticles (Figure1b), which confirms the safety of the used dextran-coated SPIO nanoparticles and their further surface modifications. However, a statistically significant increase in ROS generation was measured mainly with plain SPIO nanoparticles and to a lower extent with carboxylic and amine modified PEGylated SPIO. At low levels, ROS generation appears to be involved in regulating normal cell functions.
On the other hand, macrophages membrane receptor expression assessed using flow cytometer and iNOS and Arginase1 activity measurement were performed to characterize the phenotype of M1 and M2 BMDM before and after their magnetic labeling. M1 and M2 macrophages was found to maintain their respective and characteristic polarization profile. However, a minor attenuation in surface membrane receptor expression and additional release of nitrite as iNOS activity marker was detected probably due to ROS release.

Finally, to evaluate the biodistribution of intravenously injected macrophage subpopulations in COPD, whole-body MRI investigation was performed on mice receiving 1 million of M1 or M2 amine modified PEGylated SPIO (SPIO-PEG-NH2) macrophages using a Bruker 4.7T scanner. Mice were first intrapulmonary exposed to a bacterial lipopolysaccharide (LPS) liquid suspension using a microSprayer aerosolizer to develop a COPD inflammatory model with the most effect observed 48 hours post exposition chosen as macrophages injection time point. Susceptibility-weighted gradient echo sequence

was used to evaluate the biodistribution of macrophages subpopulations in organ of interest (i.e., liver, spleen and kidneys) and ultra-short (UTE) radial sequence was used to detect the migration of macrophages subpopulations to the lung [3].
As expected, M1 and M2 labeled macrophages were mainly detected in the spleen and to a lower extent in the liver with the maximum signal attenuation observed 2 hours post injection (Figure1c). No variation in M1 and M2 macrophages biodistribution was observed in the systemic organs of interest. However, a higher detection for M2 macrophages subsets was detected in the lung of COPD group. This preferential homing of M2-polarized macrophages to the LPS-induced inflammation site in the lung is in line with their proposed immuno-modulating functions.

In conclusion, carboxylic or amine modified PEGylated SPIO nanoparticles have been shown to be safe and have a higher labeling efficiency while not affecting neither the polarization nor the biodistribution of macrophages sub-populations. A preferential migration of magnetic labeled M2 macrophages was detected in the lung of COPD animals. This strategy will certainly pave the way to evaluate, using a noninvasive imaging modality, the role of macrophages and their polarization in the orchestration and resolution of inflammation.

References

[1] Al Faraj et al., Contrast Media and Molecular Imaging, Volume 8, Issue 2 (2013), pages 193–203.
[2] Al Faraj et al., International Journal of Molecular Sciences. Accepted manuscript
[3] Al Faraj et al., Radiology, Issue 263 (2013), pages 169-78

Figure1: (a) Quantitative analysis of iron content in M1 and M2 macrophages after labeling with either SPIO, SPIO-PEG, SPIO-PEG-COOH or SPIO-PEG-NH2, assessed using either spectrophotometer (upper row) or magnetophoresis (lower row). (b) Percentage of viability (assessed by MTT), ratio of red fluorescence divided by green fluorescence (assessed by JC-1 for mitochondrial membrane potential), and reactive oxygen species generation (assessed by ROS assay) of M1 labeled macrophages (upper row) or M2 labeled macrophages (lower row), compared to control non-labeled macrophages. (c) MR images of lung acquired using UTE radial sequence (upper row), liver (middle row) and spleen and kidneys (lower row) acquired using susceptibility-weighted gradient echo sequence pre- and post-injection of SPIO-PEG-NH2 labeled M2 macrophages.