Targeted iron oxide probes for enhanced macrophage visualization by MRI and PET

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Objective

To develop and evaluate targeted iron oxide probes for improved plaque-localized macrophage activity visualization.

Introduction

Atherosclerosis is a progressive inflammatory disease that is responsible for more deaths in industrialized countries than any other condition [1]. It is the rupture of atherosclerotic plaque that leads to major events such as stroke and myocardial infarction [2]. Currently, it is difficult to localize locations of vulnerable plaques prior to major events, leading to considerable interest in strategies for early detection of these structures. Iron oxide (IO) nanoparticles have been used for MRI of plaque macrophage content, which are considered critical for plaque progression. However, uptake of current IO particles is non-specific, relying on phagocytosis. We have developed a method to coat iron oxide nanoparticles with sulfated dextran (SDIO). The increased negative charge of these particles' surface enhances their uptake via the SR-A receptor. Here, we evaluated the *in-vitro* and *in-vivo* functionality of the SDIO compared to dextran coated IO (DIO). For clinical application, it is desirable to quickly target the particle signal with a highly sensitive marker (e.g. with PET) before high resolution MRI. To pursue this concept, we added DOTA functionality to the SDIO. ^{64}Cu-DOTA-SDIO was synthesized and used for PET/MR imaging in a mouse model of carotid plaque formation.

Methods

SDIO synthesis: DIO were synthesized as previously described [4]. DIO was sulfated by heating with 2-methyl-2-butene SO_3-pyridine complex under argon for 2 hours. DOTA-SDIO was prepared by attaching DOTA-NCS to SDIO, then ^{64}Cu into the DOTA for PET. *In-vitro studies*: Uptake of the SDIO was compared to DIO by measuring T_2 relaxivity of P388D1 cell lysates after 2 hours of incubation using a 1.4T Bruker Minispec mq60. Specificity of SDIO was tested by competitive incubation with dextran sulfate. Toxicity of SDIO was evaluated using a resazurin assay.
Imaging: The atherosclerotic mouse model was induced by ligation of one of the carotid arteries in 12 week old ApoE -/- mice. Images were acquired in a 7T Bruker Biospec. **MR-only**: 2 scan protocols were investigated: a) RARE TR/TE = 4000/22ms matrix size 128 x 128, FOV= 35.35x35.35mm^2 b) FLASH, TR/TE=1000/5ms, F.A. = 35, matrix size = 200 x 200, FOV= 35.35x35.35mm^2, pre-injection, 4 and 24 hours post i.v. injection of 30mg/kg of DIO or SDIO. **PET/MR**: Images were obtained at 4 hour and 24 hours post ^{64}Cu-DOTA-SDIO injection. PET/MR images were acquired simultaneously with an MR-compatible PET insert [5]. After visualization of the PET signal, localized MRI/PET was acquired (PET = 1200s (4/24 hours) MRI: FLASH, TR/TE=500/5ms)

Results

Purified SDIO showed an r_2/r_1 = 5.4. Successful sulfation was verified by the presence of S=0 peaks by IR. *In vitro* assays verified the specificity of SDIO uptake. There was >95% cell viability for cells incubated from 4-24 hours. MR images show darkening in the region surrounding the ligated carotid, maximal at 4 hours (Figure 1). PET/MR image dataset for a mouse is shown in Figure 2. Initial scout scans showed PET signal in the head and neck region, which guided MRI localization. MRI showed signal darkening at the carotid site ipsilateral to the ligation side, corresponding to T_2/T_2* effects of iron oxide accumulation. This corresponded with PET signal accumulation, suggesting the presence of the dual labeled particle. At 4 hours post injection, the PET and MR signal were seen across multiple slices of the image set. By 24 hours, accumulation of signal was more focal; corresponding approximately with the region of ligation.

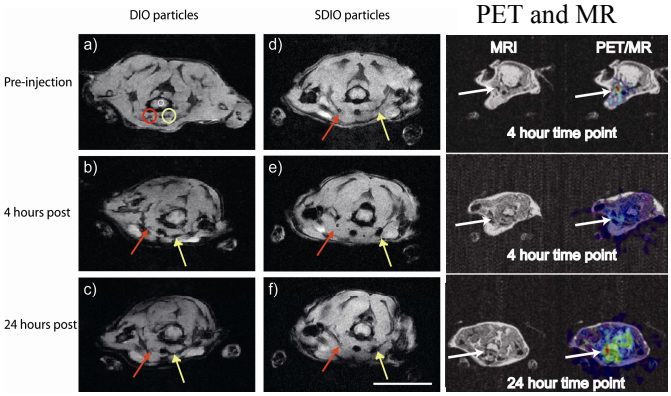

Figure 1: In vivo MRI of SDIO (yellow is ligated carotid)

Figure 2: PET/MR of ^{64}Cu-DOTA-SDIO. (arrow is ligated carotid)

Discussion

A targeted iron oxide particle to probe macrophage localization was synthesized and evaluated *in-vitro* and *in-vivo*. SDIO showed decreased T_2 compared to DIO. SDIO cell uptake was inhibited by dextran sulfate, suggesting a targeted uptake mechanism. *In-vivo* images at 4 and 24 hour showed increased T_2 signal accumulation at the ligated carotid site, which was higher for SDIO. This suggests the enhanced efficacy of the SDIO to target plaque macrophage content. This uptake pattern was also seen for ^{64}Cu-DOTA-SDIO. Simultaneous PET/MR allowed rapid analysis of PET signal in the context of MR images. PET uptake then guided more specific MR imaging at the regions of interest. PET/MR signal localized to carotid regions ipsilateral to the site of ligation, suggesting particle infiltration into inflammatory plaque sites. Further studies are underway to verify these *in-vivo* results. Nevertheless, this study demonstrates the promise of SDIO and its dual modal variants for effective imaging of vulnerable plaques.

References

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