In vivo imaging T cells in the Rheumatoid arthritis with nano-sized iron oxide particles by MRI

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Introduction

T-cells are a major cell population involved in rheumatoid arthritis (RA). The activation of T-cells is believed to correlate with the pathogenesis of the condition. Understanding the initiation, migration, and distribution of the T-cells provides important perspectives regarding RA, and may also help the development of therapeutic interventions. Bone marrow is an important source for hosting both naïve and mature T-cells. Observation of T-cells in the bone marrow and the lesioned site may reveal important information with respect to the turnover of T-cells in RA. To carry out such monitoring noninvasively and repeatedly, an in vivo labeling technique using a new nanoparticle contrast agent, IOPC and IOPC-CD3 was employed. The contrast agent was injected into the bloodstream, and presumably T-cells would be one of the major cell populations that uptake the nanoparticles. The IOPC had a longer blood circulation time that allowed uptake by cells over a longer duration. Immunohistological corroboration was carried out to verify the results.

Materials and Methods

IOPC particles were prepared with iron particles coated with polyethylene glycol (PEG), containing a terminal carboxyl group on the surface [2]. The PEG coating rendered the nanoparticles with a longer blood circulation time due to minimal nonspecific adsorption of proteins onto the nanoparticles and thus reducing uptake by the liver. Relaxivity measurement was performed by a 0.47T mini-spectrometer (Bruker, Billerica, MA). A rat RA model, collagen-induced arthritis (CIA), was used to simulate the pathology and associated T-cell activation. This model was induced by immunization of female Lewis rats (aged 6-8 weeks old, weighted 150g) with an emulsion of incomplete Freund's adjuvant (IFA) and type II collagen (CII)[1], followed by a booster injection on day 7. Erosive polyarthritis typically developed 10 -14 days after the primary immunization. Sham controls received IFA /saline injection. At the onset of RA, MR images were perform on a 4.7T MR scanner (Bruker, Ettlingen, Germany) with a volume coil of 72-mm inner diameter as the transmitter and a quadrature surface coil as the receiver on the surface of the knee joint. T2 weighted imaging (T2WI) was obtained repeatedly before RA, at different intervals after RA with a fast spin echo sequence with TR=4000ms, TE=60ms, echo train length=8, slice thickness=0.5mm, FOV=3x3cm, NEX=10, and Matrix: 256x128. IOPC was administrated intravenously at 3 mg/kg. The knees were harvested for immunohistochemical staining with antibodies to detect PEG and CD3 (specific to T cell) and Hematoxylin & Eosin staining.

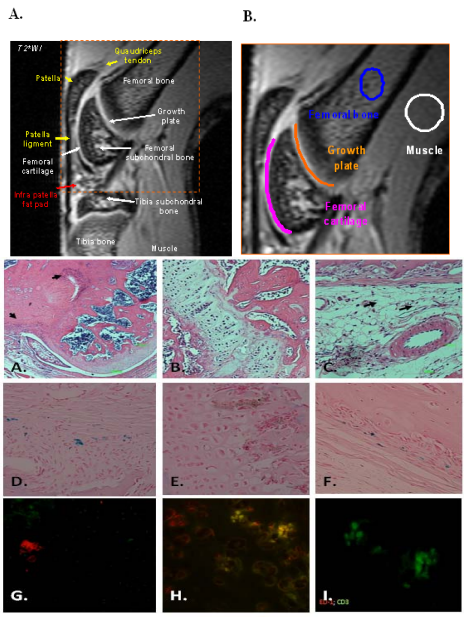
Results and Discussion

The transverse relaxivity (R_2) value of IOPC particles was 224.34 $s^{-1}mM^{-1}$ with an average particle size of 70nm. T2WIs of the knee from the sham and RA rats were acquired before and after RA. The selection of regions of interest (ROI) is shown in Fig.1(A,B), including the femoral bone marrow (FB), and the growth plate (GP). However, CIA rats were infused instead with IOPC or IOPC-CD3 particles showing a strong accumulation in the inflammatory bone tissue. Quantification of the % signal change relative to the baseline in the FB and GP is illustrated in Fig.2(A,B), where noticeable differences between sham and RA were seen. While CIA rats was infused with IOPC, SNR at the femoral bone (52%±8%) and growth plate (82%±8%) decreased on day 1 (Fig. 2 A). In addition, with the IOP-CD3 administration on day 8, the SNR at femoral cartilage (78%±5%) and growth plate (56%±7%) decreased. The contrast agent continued SNR to decrease at growth plate (Fig. 2B) with time. The decreased SNR at GP (72%±6%) may be due to the migration of IOPC-CD3 particles targeting T-cells. The IOPC-CD3distribution in the FB and GP was verified by immunostaining against PEG (Fig. 3(G-H)). CD-3 immunostaining merged with the PEG immunoreactivity Fig.3(H), and H&E staining shown in Fig. 3(A-C) revealed the T cell infiltration into the GP with IOPC in the RA rat.

Conclusion

Our results suggested that IOPC-CD3 particles are powerful and potential specific cellular imaging that can be used to label non-phagocytic T-cells for *in-vivo* MRI cell trafficking studies, which can contribute widely to the field of regenerative medicine and inflammatory diseases.

Figure 1. Assessment of CIA abnormality by *In vivo* MRI. The anatomy located by T2 weighted imaging (a). The ROI were shown on T2WI as reference for signal to noise ratio (SNR) measurement. GP: growth plate; FB: femoral bone marrow (b).



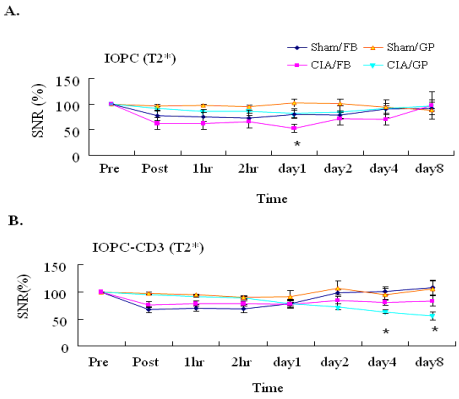


Figure 2. The changes of SNR (%) were monitored according to T2WI.T2*WI showed the rat knee of sham and CIA before (pre) and after (post) I.V administration of IOPC and IOPC-CD3. The ROI were shown on T2*WI as reference for signal to noise ratio (SNR) measurement. GP: growth plate; FB: femoral bone marrow.

Figure 3. Histopathological analysis. (a-c) H&E staining shows massive mononuclear cell infiltration into the corresponding section of the bone tissue and blood vessels. Prussian blue staining was carried out for iron detection (d-f). Immunofluorescence analysis of the bone tissue (g-i), after intravenously infusing IOPC-CD3 into RA onset rat

References

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