Nano-size MR probe detects T cells infiltration in bone marrow and growth plate in rat model of rheumatoid arthritis

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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, 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, immediately after IOPC injection, 1 hour, 2hours, and 1 day post-IOPC injection, as shown in Fig.1 (A). The selection of regions of interest (ROI) is shown in Fig.1(B), including the femoral bone marrow (FB), and the growth plate (GP). Before the injection of the contrast agent, RA caused a lesion in the GP that was detectable by T2WI as a hypointense area (Fig.1 (A), the Pre column), which was also demonstrated in Fig. 2(A) by histology. Moreover, the patella ligament thickness of RA rat increased with time (data not shown here). After injection of IOPC, the nanoparticles accumulated in bone marrow vastly in both sham and RA rats. However, with time, the IOPC induced hypointense signals gradually differed between the sham and RA animals, as shown in Fig. 1(C). Quantification of the % signal change relative to the baseline in the FB and GP is illustrated in Fig.1(C), where noticeable differences between sham and RA were seen. The GP of RA rats continued to exhibit lower signals with time, which may suggest the infiltration of IOPC-labeled T-cells into the area. Moreover, the hypointensities in FB was less obvious in the sham control one day after IOPC injection, but remained unchanged in the RA rat. The IOPC distribution in the FB and GP was verified by immunostaining against PEG (Fig. 2(A)). CD-3 immunostaining (Fig.2(B) merged with the PEG immunoreactivity (Fig.2(C), and H&E staining shown in Fig. 2(D) revealed the T cell infiltration into the GP with IOPC in the RA rat.

Conclusion

T-cell infiltration into the growth plate of RA rat was observed by MRI using IOPC as the in vivo labeling agent. In the future, IOPC conjugated with a T-cell specific antibody, CD3, will be developed for targeted molecular imaging.

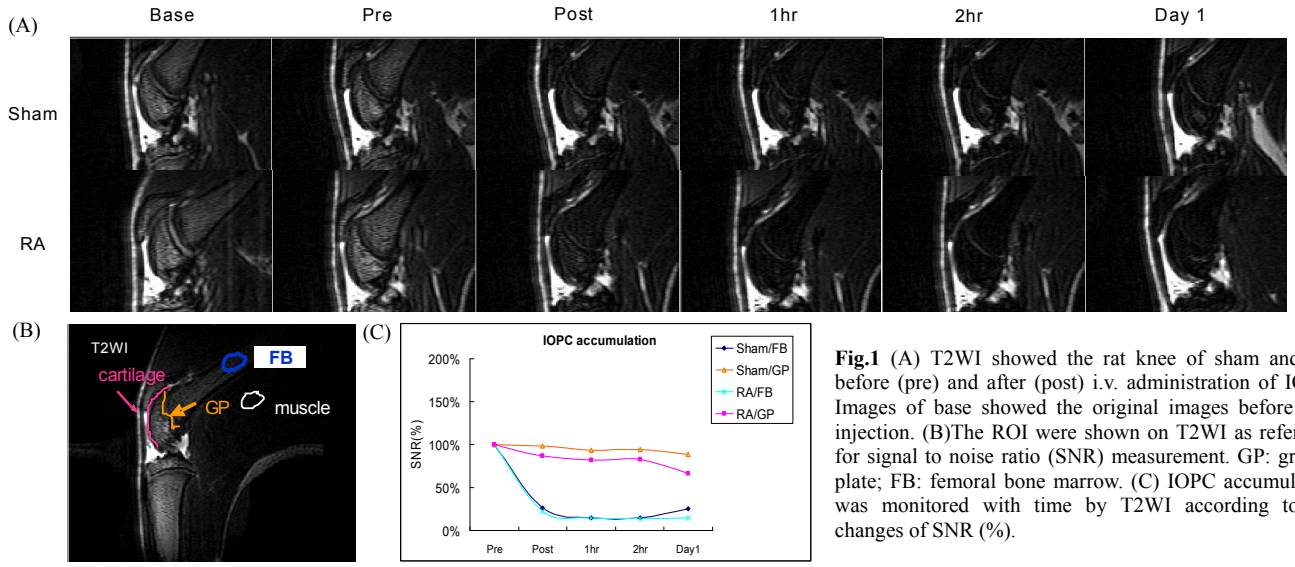

Fig.1 (A) T2WI showed the rat knee of sham and RA before (pre) and after (post) i.v. administration of IOPC. Images of base showed the original images before CIA injection. (B)The ROI were shown on T2WI as reference for signal to noise ratio (SNR) measurement. GP: growth plate; FB: femoral bone marrow. (C) IOPC accumulation was monitored with time by T2WI according to the changes of SNR (%).

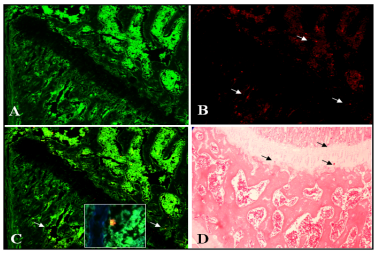

Fig. 2. Immuno-fluorescent staining on paraffin embedded knee section of RA rat. The results showed staining of (A) mouse anti-PEG mAb (with anti-mouse IgG-FITC) and (B) PE-conjugated anti-CD3 (C) an overlay image of (A) and (B). (D) Optical micrograph of tissue section stained with H&E.

References

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2. Chen CL, *et al.* (2010) *Mol Imaging Biol* (in Press).