Detection of Immune Cell infiltration and Visualization of Inflammatory Dilation on Myocardial Disorder Using Fluorescent Magnetic Nanoparticles

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Introduction: Myocarditis is defined as inflammation of myocardial tissue with characteristic inflammatory cellular infiltration into myocardium. Limited availability of non-invasive and biologically precise diagnostic tools poses a challenge for the evaluation and management of patients with myocarditis.

In this study, we investigated whether PEGylated fluorescent magnetic nanoparticles (MNP) could detect the inflammatory areas in experimentally induced autoimmune myocarditis (EAM) rats with in vivo cardiac MRI. In addition, we examined the MNP's possibility if we apply to clinical research by exploring the inflammatory dilation and therapeutic effect.

Materials and Methods: Myocardial inflammation was induced in twenty five 7-week-old male Lewis rats as the induction method of autoimmune myocarditis described previously.[1] We used home-made magnetic nanoparticles (MNP) T2 contrast agent that enable the detection of both their fluorescence and magnetic properties in cells and tissues.[2]

To observe the inflammatory dilation by the passing of the days, MNP was administered two times differentiating the fluorescent dye into five individual EAM rats. We acquired the *in vivo* pre-MNP CMR images using EAM rats at 15 D post-immunization (PI), and then 5 mg Fe/kg of MNP synthesized with FITC was intravenously injected. Post-MNP cardiac MR (CMR) images were acquired at 16 D and 20 D PI. After acquiring post-MNP CMR images at 20 D PI, then same dose of MNP, coated with RITC, was injected as same method once again. Then, post-MNP CMR imaging was performed at 21 D PI.

For tracing the therapeutic progress, the 1 mg and 5 mg/kg of cyclosporine were treated every day beginning from first immunization induction day into 20 EAM rats.[3] The MNP were administrate at 12 D and 19 D PI, then post-MNP MRI was performed 24 hrs after. MRI measurement was performed at a 4.7 T MRI system (BioSpec 47/40; Bruker, Germany) with dual ECG and respiratory gating (SA Instruments, Stony Brook, NY, USA). T2*-weighted MR images were obtained with a fast low-angle shot gradient-echo (FLASH) sequence (TE/TR = 6/130 ms). After finishing the series of CMR imaging, the EAM rats were sacrificed and the extracted hearts were suffered histological analysis and fluorescence microscope imaging. We acquired the FITC and RITC fluorescence microscope images at same tissue slides.

Results and Discussion: We could observe that the inflammatory areas were grown as days passed from MNP-enhanced CMR images (Fig. 1). At 24 hr post-MNP CMR images, there were shown the negative-contrast areas in various places. The negative-contrasted areas in the 16D and 21 D post-MNP CMR images and merged fluorescent signal areas are good correlated with inflammatory areas in H&E stain results. Most of all, MNP negative-contrasted areas and fluorescent signal areas are matched with immune cell infiltration areas very well. These results indirectly reflect that the MNP negative-contrasted areas are originated from the MNP engulfed by infiltrated immune cells. Furthermore, MNP-enhanced MRI effectively visualized the therapeutic progress of immunosuppressant (Fig. 2). We could see that the inflammatory areas couldn't increase any more or the inflammation didn't occur, although inducing myocarditis.

From our study, we could verify that MNP-CMR could effectively visualize myocardial inflammatory cellular infiltrates, and monitoring the dynamic evolution of myocardial inflammation in a preclinical model of EAM. We showed that MNP-CMR could be a highly efficient tool for visualizing myocardial inflammation in the early course of the disease with a potential for quantifying the response to specific therapies for myocarditis. Furthermore, this MRI method along with histopathological analysis of the MRI-guided biopsies will assist in the diagnosis of the focal manifestations of myocarditis.

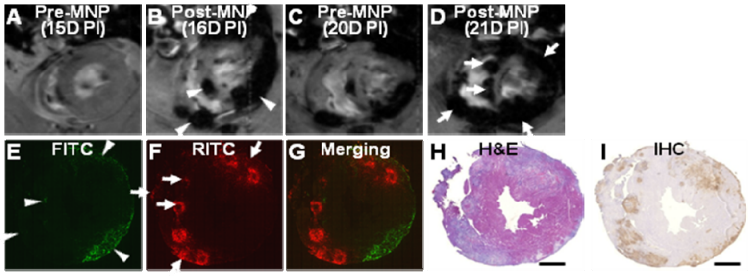

Figure 1. The observation results of inflammatory dilation using MNP with *in vivo* cardiac MRI. We injected the MNP twice into same individuals. We could clearly distinguish the inflammatory areas at 1st MNP (FITC) injection and extended inflammatory areas after 2nd MNP (RITC) injection. The FITC signal areas were good correlated with the negative contrasted areas at 1st post-MNP (FITC) CMR images (arrow heads). Like the FITC results, RITC signal areas coincided `with the arisen negative contrasted areas at the 2nd post-MNP (RITC) CMR images (arrows). Moreover, we could find that FITC signal enhanced areas and RITC signal enhanced areas don't overlap in almost areas from fluorescence microscope images. Scale bar: 2mm.

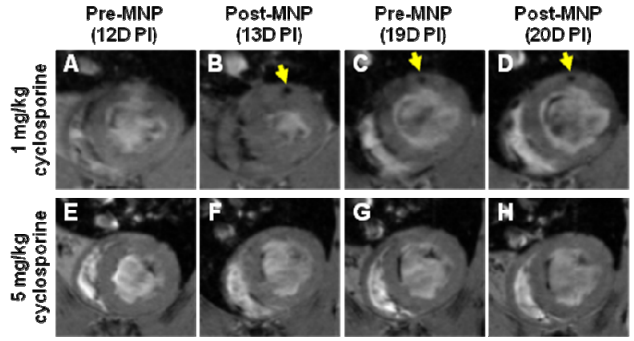

Figure 2. The observation of therapeutic progress on two individuals. They were administrated 1 mg/kg (A-D) and 5 mg/kg (E-H) cyclosporine each per day. From 1 mg/kg cyclosporine administered EAM rat, we could see the inflammatory areas at 1st post-MNP (13D PI) CMR images and remained until to 19 D PI. Furthermore, we could find out that this area size didn't increase from 2nd post-MNP CMR image (20 D PI). These results reveal that the inflammatory areas could not extend because of therapeutic effect by cyclosporine. Because of preventing effect of cyclosporine, there was no inflammatory area at 1st post-MNP CMR images and 2nd post-MNP CMR images in case of 5 mg/kg cyclosporine administration.

References

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