Noninvasive Visualization of Myocardial Inflammation using Magnetofluorescent Nanoparticle-contrasted MRI in Rat Autoimmune Myocarditis

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Introduction: Myocarditis is defined as inflammation of the myocardium, mostly caused by viral infection, which leads to autoimmune activation against the host's own myocardial tissue [1]. Endomyocardial biopsy is still considered to be the gold standard for diagnosing Myocarditis, however it is invasive and low sensitive because of sampling error and high inter-observer variability [2]. Therefore, a novel diagnostic modality to detect the inflammation of myocardium through noninvasive means is needed. Here we investigated whether nanoparticle-contrasted cardiac MRI would be feasible and effective in detecting the status, and discriminating the grade of inflammation in a rat model of experimental autoimmune myocarditis (EAM).

Materials and Methods: EAM was induced in thirty male 7-week old Lewis rats, as previously described [3]. We used here bifunctional magnetofluorescent silica nanoparticles (MFSNs) that enable detection of both fluorescence in cells and tissues and magnetic properties by MRI, as described earlier [4]. All rats were intravenously injected with 12.5 mg Fe/kg of MFSN and then MRI was performed up to 48 hours in control (n=5), 10 days (n = 5), 14 days (n = 5), 17 days (n = 5), 21 days (n=5), and 40 days post-immunization rats (n = 5) according to the phase of inflammation. MRI was performed on a small animal 4.7 T MRI system (BioSpec 47/40, Bruker, Germany) with dual ECG and respiratory gating (SA Instruments, Stony Brook, NY, USA) for cardiac MR imaging. Gradient-echo (FLASH) sequence was used to evaluate the susceptibility effect of the contrast agent (TE/TR=6/150 ms). Serial cardiac MRI was conducted before, 10 minutes, 30 minutes, 24 hours and 48 hours after the injection of MFSN in control and three groups of EAM rats with different phases. Eight and six ROIs were selected from the myocardium and the pectoral muscle, respectively. Contrast-to-noise ratios (CNR) of the myocardium to the pectoral muscle were calculated for plotting a time course curve. For histological evaluations as compared to MRI results, all hearts were stained with hematoxylin and eosin (HE) for general morphology and with Sirius red for collagen content. Confocal laser scanning microscope (CLSM; LSM 510 Meta, Zeiss, Germany) was done to identify the accumulation of MFSN in the inflamed myocardium.

Results: Representative slice images from control, 10d, 14d, 17d, and 40d models before and 30 min, 24 hours, and 48 hours after injection are shown (Figure 1). The CNRs were measured from eight ROIs relative to the mean value from six ROIs in the skeletal muscle, then normalized CNR (nCNR) were calculated relatively by the CNR before injection. At 30 min after injection, the nCNRs of the five animal models are almost same (0.30 ± 0.05). On 24 hours images, the nCNR for control was almost recovered to the pre-injection state, however, the myocardia for the diseases models are still negative-contrasted (10d and 40d, 0.75 ± 0.07; 14d and 17d, 0.55 ± 0.05). The similar data are shown depending on the disease stages (control, 0.97 ± 0.05; 10d and 40d, 0.79 ± 0.06; 14d and 17d, 0.61 ± 0.06) at 48 hours also (Figure 2). Figure 2 shows that the nCNRs for 10d and 14d are recovered more to the initial value compared with those of 14d and 179d, and the stage dependence of the nCNRs at 48 hours are shown in Figure 2. It is likely to have a maximum negative contrast (40%) on days 14 and 17, the stage dependence of nCNR shows that there is a maximum concentration of the MFSNs in myocardium on days between 14 and 17. In histological study, HE and Sirius red staining of the rat hearts presented macrophage infiltration consistent with MR results. Confocal laser scanning microscope (CLSM) images showed MFSNs were in the macrophages at 48 hours after the injection of the nanoparticles.

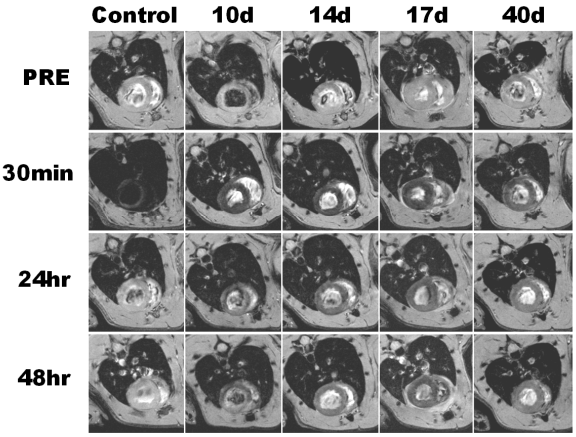

Figure 1. Images of control rat show relatively faster recovery than others. At 24 and 48 hours, strong negative enhancement is still shown in myocarditis rat on days 17.

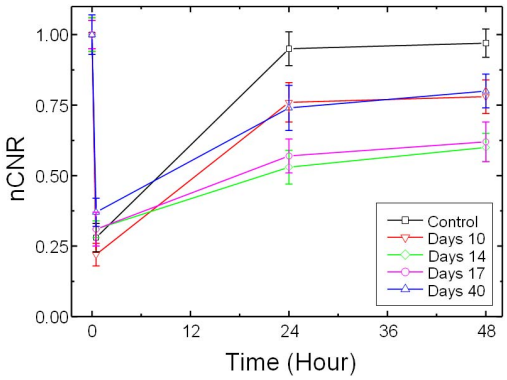

Figure 2. Time course of nCNR values. The similar nCNR values were observed in rats on days 10 and 40 at 24 and 48 hours following MFSN injection, and similarly on days 14 and 17.

Conclusion: We report noninvasive approach using *in vivo* MRI with iron-oxide-based contrast agent to detect the amount of macrophages in myocardium of myocarditis rats. We present a feasibility to discriminate the grade of inflammation in subacute stage of experimental autoimmune myocarditis rat model.

References

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