Non-invasive in vivo imaging of inflammation in experimental viral myocarditis by 19F cellular MRI

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Introduction

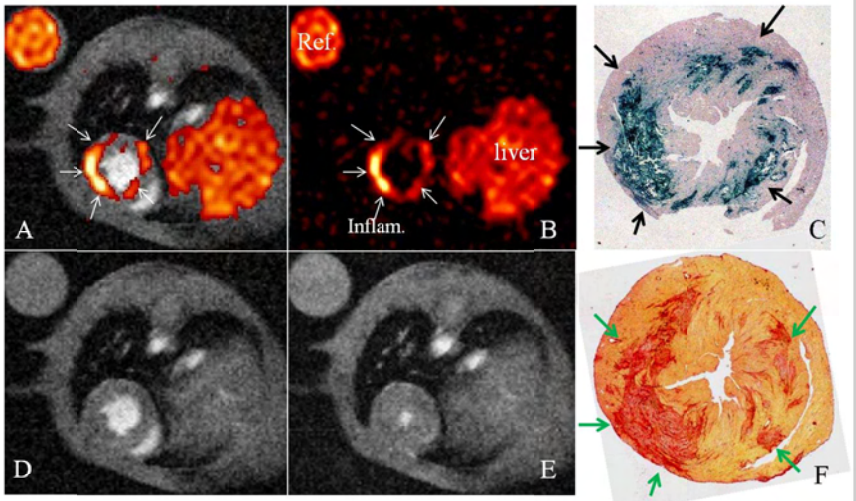
Myocarditis, most often caused by common viral infections such as enteroviruses, is a challenging disease for diagnosis and treatment. Its clinical presentations are non-specific. The gold standard to diagnose myocarditis is histopathology and immunohistochemistry to define cardiac injury and inflammatory cells in the myocardium within endomyocardial biopsies. However, this invasive procedure is often insensitive to detect myocarditis[1]. A non-invasive, specific and sensitive method is needed for early detection of myocarditis. Thus, a non-invasive imaging technique based on fluorine-19 (^{19}F) cellular MRI was applied in this study, to detect inflammation of viral myocarditis in a mouse model *in vivo*.

Methods

Viral myocarditis was induced by infecting A.BY/SnJ mice (n=5) with coxsackievirus B3 (CVB3). PFC emulsion (150µl, concentration 20% v/v) was injected *i.v.* 13 days post-infection. As control two non-infected mice received the same dose of PFC. The labeling efficiency by PFC was examined by flow cytometry in blood samples after injecting fluorescence labeled PFC emulsion.

All MR measurements were performed at a 7T small animal scanner. *In vivo* ^{1}H/^{19}F-MRI was acquired by a ^{1}H/^{19}F birdcage coil. ^{19}F MRI was superimposed on ^{1}H-Cine images allowing localization of inflammation in myocardium. *In vivo* ^{19}F images were acquired by TSE sequence with spatial resolution (SR) 0.5×0.5×0.5mm. ^{1}H-Cine was acquired by FLASH sequence.

H&E, Sirus red staining and immunohistochemistry applying Mac3 and CD3 antibodies were applied to examine myocardial injury, collagen deposition, macrophage recruitment and T cell infiltration in myocardial tissue, respectively.



In vivo ^{19}F/^{1}H-MRI and histopathology of murine CVB3-myocarditis. (A): Hybrid image of *in vivo* ^{19}F (B) and ^{1}H-Cine at end diastole (D) unambiguously visualized inflammation (hot color, indicated by white arrows) in myocardium. (E): ^{1}H-Cine at end systole showed mild impairment of regional contractility. (C): Mac3 immunohistochemistry showed that ^{19}F signal (white arrows in A and B) in the heart matched well with the distribution of macrophages (stained blue, indicated by black arrows) in the myocardium. (F): Sirius red staining visualizes collagen deposition (stained red, indicated by green arrows) in necrotic areas. (*^{19}F signal is in hot color. Inflam.: inflammation in myocardium. Ref.: external ^{19}F reference.*)

Results

PFC emulsion preferably labeled CD11b+ cells (mainly monocytes and neutrophils) in blood after intravenous injection (data not shown). ^{19}F-MRI detected patchy ^{19}F signals in myocardium exclusively in infected mice (Figure A&B) but not in controls. The ^{19}F signal was primarily found in the left ventricular wall, especially in basal segments. The contractile function of infected myocardial segments appeared only mildly impaired. The global contractile function was largely preserved (Figure D&E). Mac3 immunohistochemistry (Figure C) and H&E staining revealed that the distribution of ^{19}F signal is spatially correlated with presence of macrophages and myocardial necrosis and fibrosis as shown by sirus red staining (Figure F).

Discussion and conclusion

^{19}F-MRI was able to unambiguously detect inflammation in the myocardium of CVB3-infected mice. The ^{19}F signal was due to macrophages labeled by PFC within cardiac lesions. If clinically approved PFC emulsion is available for MRI cell tracking, this method has potential to be translated to clinics to assist early, non-invasive diagnosis of viral myocarditis. In addition to T cells, macrophages are reported to contribute significantly to the development of extensive postviral fibrosis, which finally may lead to dilated cardiomyopathy[2]. Thus, this noninvasive ^{19}F-MRI cell tracking technique might be valuable to improve the management of patients suffering from acute and chronic viral myocarditis by localization and quantification of macrophage infiltrates, also in follow up studies.

Reference: 1. Baughman, K. L. (2006). Circulation. 2. Szalay, G. et al. (2009) Circulation Research

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