Biomaterials 68 (2015) 67–76

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Visualizing myocardial inflammation in a rat model of type 4 cardiorenal syndrome by dual-modality molecular imaging

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ARTICLE INFO

Article history:
Received 3 February 2015
Received in revised form
27 July 2015
Accepted 31 July 2015
Available online 1 August 2015

Keywords:
Type 4 cardiorenal syndrome
Myocardial inflammation
Cardiovascular imaging
Nanoparticle
Iron oxide nanoparticle
Optical imaging

ABSTRACT

Type 4 cardiorenal syndrome (CRS) is a life-threatening world health problem in which chronic kidney disease leads to progressive cardiovascular disease. In type 4 CRS, cardiac inflammation is an excellent target for both detection and therapy; however, this progression was underestimated by previous studies due to the lack of effective detection methods. To noninvasively visualize cardiac inflammation and monitor therapeutic efficacy of anti-inflammatory treatment in type 4 CRS, we here synthesized a dual-modality magneto-fluorescent nanoparticle (MNP) by combining ultrasmall superparamagnetic iron oxide nanoparticle and Rhodamine B for both magnetic resonance imaging (MRI) and optical imaging. This dual-functional MNP exhibited excellent performance such as high r2 relaxivity coefficient (283.4 mM^{-1} s^{-1}), high magnetism (96.7 emu/g iron) and a near neutral surface charge to minimize the reticuloendothelial system uptake. *In vivo* cardiac MRI showed significant negative contrast in the type 4 CRS rats, and the signal intensity on optical imaging was significantly higher in the type 4 CRS group compared with sham-operated and drug-treated groups. The specific targeting profile of MNPs to monocyte-macrophages was proven by histopathological analysis. Taken together, we demonstrate that this dual-modality strategy is feasible for noninvasively assessing myocardial inflammation and monitoring therapeutic efficacy in type 4 CRS.

1. Introduction

The rising prevalence of chronic kidney disease (CKD) and its associated cardiovascular morbidity has aroused increasing public concerns [1]. Numerous studies propose that patients with CKD are more prone to die of cardiovascular complications rather than kidney failure [2]. Recently, the relationship between initial CKD and the following chronic cardiovascular disease (CVD) has been emphasized and defined as type 4 cardiorenal syndrome (CRS) [3]. The generic cardiovascular pathological changes in response to CKD in type 4 CRS include coronary atherosclerosis, chronic myocardial inflammation and cardiac remodeling (cardiac hypertrophy and fibrosis) [4]. Experimental and clinical data support the findings that in type 4 CRS there is increased production and enhanced release of proinflammatory cytokines, chemokines and growth factors, which increase cardiac stiffness to reduce compliance, impair heart function and alter cardiac electrical activity [5,6]. In heart failure following myocardial infarction, monocyte-macrophages have been recognized as the key player of cardiac damage and remodeling [7]. However, in type 4 CRS, cardiac inflammation was largely underestimated in previous studies [8,9], which was most probably due to the lack of effective and noninvasive detection methods.

An increasingly number of studies propose that macrophage-mediated cardiac inflammation is an essential mechanism in the progression of CRS [10,11], which represents an ideal target for both noninvasive detection and therapeutic evaluation. However, direct histopathological evidence of cardiac inflammation is difficult to obtain in patients with type 4 CRS. Endomyocardial biopsy is the

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http://dx.doi.org/10.1016/j.biomaterials.2015.07.050
0142-9612/

gold standard for identifying CVDs, but invasive complications and sampling errors limit its clinical use [12]. Recently, the application of nanotechnology-based molecular imaging has achieved rapid advancements in animal and clinical studies [13,14]. Among all the available imaging techniques, magnetic resonance imaging (MRI) possesses high spatial resolution and the ability to dynamically visualize tissue without limitations of depth and angle. In addition, optical imaging is widely used in recent studies for its sensitivity and high lesion detection rate [15]. Thus, a dual-modality strategy with a combination of MRI and optical imaging will achieve improved diagnostic accuracy via MRI and high sensitivity via optical imaging, which could guide therapy and surgery in clinical application.

Gadolinium (Gd) and iron oxide nanoparticle are the two most widely used MR contrast agents in cardiovascular imaging. Although Gd-based contrast agents (GBCAs) are excellent in identifying myocardial infarction based on leakage into damaged myocardial capillaries [16], they cannot adequately distinguish diffuse cardiac inflammation with intact cell membranes in type 4 CRS [17]. Compared with GBCAs, iron oxide nanoparticles have increasingly been used to detect tissue macrophage infiltration in diseases such as atherosclerosis [18] and tumors [19]. The specific uptake of iron oxide nanoparticles by infiltrated macrophages, facilitated by their targeted profile with high sensitivity and strong negative contrast, can enable a noninvasive visualization of tissue cellular infiltration in macrophage-based diffuse inflammation, such as myocarditis [20]. Moreover, because iron oxide nanoparticles have been conventionally used as iron-replacement therapy in patients with renal deficiency, they have a unique safety profile, even in patients with CKD [21]. Rhodamine B is a frequently used fluorescent dye that possesses favorable spectral properties in the visible region (excitation = 550 nm, emission = 573 nm) with high usability and low cost.

Hence, the purpose of this study was to establish a dual-modality magneto-fluorescent nanoparticle (MNP) that uses MRI and optical imaging to noninvasively diagnose myocardial inflammation in a rat model of type 4 CRS and evaluate its utility in assessing the curative effect of anti-inflammatory therapy.

2. Materials and methods

2.1. Synthesis and characterization of the MNPs

Detailed synthesis and characterization of the nanoparticles were reported in the Data supplement.

2.2. Rat model of type 4 CRS

All experimental protocols were approved by the Institutional Animal Use and Care Committee of Southeast University, Nanjing, China. Sixty male Sprague–Dawley rats weighing 180–220 g were used in this study. Subtotal nephrectomy (SNx) was performed in rats to induce initial kidney injury by first resecting the entire right kidney and subsequently resecting two-thirds of the left kidney one week later under deep anesthesia administered by isoflurane inhalation (Keyuan Pharmaceutical, China). A group of sham-operated rats (sham, n = 20) were used as the controls. All rats were allowed 4 weeks to recover and form a stable condition of CKD. Then, the SNx rats were randomly divided into 2 groups and intragastrically administered vehicle (Veh), which contained 0.5% methylcellulose (SNx + Veh, n = 20), or ramipril (Ram), a standard angiotensin-converting enzyme (ACE) inhibitor that used as an anti-inflammatory drug (1 mg/kg/d, SNx + Ram, n = 20) for an additional 12 weeks.

2.3. MRI

In vivo cardiac MRI was performed in a 7.0 T MR Scanner (Bruker PharmaScan, Germany) with respiration and electrocardiograph double-gated at week 16. A gradient-echo T2*-weighted FLASH sequence was performed in the axial direction of each heart before MNP administration (pre-MNP) and 24 h after MNP injection (post-MNP) at a dose of 10 mg Fe/kg body weight through the tail vein. The contrast-to-noise ratio (CNR) and the changes in the CNR (ΔCNR = CNR pre-MNP minus CNR post-MNP) were calculated. A detailed description is provided in the Data supplement.

2.4. Optical imaging

After MRI all rats were sacrificed and their hearts (n = 6 per group) were removed and subjected to optical imaging using the Maestro *In-Vivo* Optical Imaging System (excitation = 550 nm, emission = 573 nm; Caliper Life Sciences, MA). Images were acquired with the Maestro 2.4 software, and the fluorescence signal of Rhodamine B was extracted from the autofluorescence spectra using the multi-excitation spectral imaging system (Caliper Life Sciences). To measure the fluorescence intensity, regions of interest (ROIs) were placed on the entire left ventricular myocardium, and signals were normalized by the exposure time and area of the ROI (scaled counts/s), as previously described [22].

2.5. Histopathology

Heart sections were acquired and stained with hematoxylin & eosin (H&E), Masson trichrome, and incubated with rabbit anti-rat antibodies against CD68, TNF-α, IL-6 and MCP-1 (Abcam, UK), followed by a goat anti-rabbit secondary antibody (Sigma–Aldrich, USA). To assess the phagocytosis of MNPs by inflamed cardiac macrophages, consecutive cardiac slices were first stained with Prussian blue and CD68 immunohistochemistry (IHC). Next, immunofluorescence staining was performed with a rabbit anti-rat CD68 antibody, followed by staining with a goat anti-rabbit Alexa Fluor 488 polyclonal antibody (Life Technologies, USA) and nuclear staining with DAPI (Sigma–Aldrich, USA). Confocal laser scanning microscope (Olympus, Japan) was used to acquire immunofluorescence images. For quantification, 10–20 randomly selected microscopic fields were imaged for each section, and the quantification work was conducted using Image Pro-Plus6.0 (Media Cybernetics., USA) by 2 blinded readers.

2.6. Real-time PCR, western blot and transmission electron microscopy (TEM)

Fresh cardiac tissues were acquired for the quantitative real-time PCR analysis of TNF-α, IL-6 and MCP-1 mRNA (Life Technologies, USA) and the western blot analysis of CD68, TNF-α, IL-6 and MCP-1. To detect the ultrastructure of nanoparticle distribution in the cardiac macrophages, TEM (H-600; Hitachi, Japan) was performed in fresh cardiac tissues at 120-kV accelerating voltage. A detailed description is provided in the Data supplement.

2.7. Statistical analysis

Data are reported as the mean ± standard deviation (SD). Statistical comparisons were performed using a one-way analysis of variance (ANOVA). Post hoc analysis with appropriate Bonferroni correction was conducted, and two-sided testing was used. The correlation analysis was performed using the Pearson correlation coefficient. All statistical tests were performed using SPSS software