Journal of Cardiology 68 (2016) 253–260

Contents lists available at ScienceDirect

Journal of Cardiology

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

Original article

Suppression of murine autoimmune myocarditis achieved with direct renin inhibition

Chisato Takamura (MD)[a], Jun-ichi Suzuki (MD, PhD, FJCC)[b,*], Masahito Ogawa (PhD)[a], Ryo Watanabe (BS)[a], Yuko Tada (MD)[c], Yasuhiro Maejima (MD, PhD)[a], Hiroshi Akazawa (MD, PhD, FJCC)[b], Issei Komuro (MD, PhD, FJCC)[c], Mitsuaki Isobe (MD, PhD, FJCC)[a]

[a] Department of Cardiovascular Medicine, Tokyo Medical and Dental University, Tokyo, Japan
[b] Department of Advanced Clinical Science and Therapeutics, The University of Tokyo, Tokyo, Japan
[c] Department of Cardiovascular Medicine, The University of Tokyo, Tokyo, Japan

ARTICLE INFO

Article history:
Received 27 May 2015
Received in revised form 21 August 2015
Accepted 9 September 2015
Available online 21 October 2015

Keywords:
Myocarditis
Renin
Inflammation
Cytokines

ABSTRACT

Background: The renin angiotensin system (RAS) plays an important role in the pathogenesis of cardiovascular diseases and inflammation. Myocarditis is an inflammatory disease of the heart, and the role of the RAS in its pathophysiology is unknown. Because the direct renin inhibitor, aliskiren, is thought to block RAS completely, we investigated the cardioprotective effect of aliskiren in mice with experimental autoimmune myocarditis (EAM).

Methods: A cardiac α-myosin heavy chain peptide was injected in mice on days 0 and 7. Aliskiren 25 mg/kg per day ($n = 10$) or vehicle ($n = 10$) was administered to EAM mice starting on day 0 and the animals were killed on day 21.

Results: Aliskiren significantly prevented the progression of left ventricular wall thickening in EAM hearts compared to the vehicle-treated group. Histologically, the inflammatory cell infiltration and fibrosis area ratios in the aliskiren-treated group were lower than that in the vehicle-treated group. Immunohistochemistry revealed that aliskiren suppressed CD4 positive cell infiltration in EAM hearts compared to vehicle. Moreover, aliskiren decreased mRNA levels of interleukin (IL)-2, interferon-γ, tumor necrosis factor-α, and collagen 1. In vitro study showed that aliskiren inhibited T cell proliferation and IL-2 production induced by myosin stimulation.

Conclusion: Our results suggest that aliskiren ameliorates EAM by suppressing T-cell activation and inflammatory cytokines, and has potential as a treatment for myocarditis.

Introduction

Myocarditis is an inflammatory heart disease that poses many risks to humans. It is progressive, can cause severe heart failure, arrhythmia, shock, and death [1]. Some cases of myocarditis are related to autoimmunity and lead to chronic immune-mediated inflammation, resulting in dilated cardiomyopathy [2–4]. Experimental autoimmune myocarditis (EAM) induced by inoculation with cardiac myosin is a model of post-infectious myocarditis and cardiomyopathy [5–7]. EAM is mediated by CD4+ T cells [5,8].

The renin angiotensin system (RAS) plays an important role in the pathogenesis of atherosclerosis, hypertension, left ventricular hypertrophy, myocardial infarction, and heart failure [9,10]. In addition to systemic RAS, RAS local to various end organs can produce angiotensin II (Ang II) locally and cause tissue injury and affect tissue regeneration [11]. For example, cardiac RAS regulates cardiac hypertrophy, remodeling, and fibrosis [12–14]. In some studies, these changes were independent of blood pressure and plasma Ang II [13,14]. Local RAS is also known to promote inflammation. Ang II can modulate adaptive immunity, acting directly on lymphocytes. In addition to the classic components required for T cell activation, Nataraj et al. reported that Ang II stimulates the proliferation of splenic lymphocytes, including B

* Corresponding author at: Department of Advanced Clinical Science and Therapeutics, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel.: +81 3 5800 9116; fax: +81 3 5800 9182.
E-mail address: junichisuzuki-circ@umin.ac.jp (J.-i. Suzuki).

http://dx.doi.org/10.1016/j.jjcc.2015.09.008
0914-5087/

cells and T cells [15]. In addition, T cells are equipped with all RAS components such as the angiotensin I converting enzyme (ACE) [16], renin, and angiotensinogen. They are also potentially able to produce and deliver Ang II [17,18]. The effects of Ang II on T cells have been suggested to be mediated by Ang II type 1 receptors (AT1R) or Ang II type 2 receptors (AT2R). These conclusions are based not only on pharmacological inhibition with AT1R or AT2R blockers, but also on studies of T cells from AT1R- and AT2R-deficient mice [18,19]. However, the anti-inflammatory effect due to inhibition of other RAS components on T cells has not been fully demonstrated.

The action of renin on angiotensinogen is the first and rate-limiting step in the RAS cascade. Aliskiren is the first available direct renin inhibitor. It inhibits the catalytic activity of renin by binding to the active site of renin and reduces synthesis of all subsequent components of the RAS cascade. Previous studies reported that aliskiren treatment resulted in amelioration of cardiac hypertrophy and vascular inflammation, and improved systolic function, all beyond its blood pressure lowering effects in animal models [20–23]. These effects of aliskiren suggest that it blocks not only systemic RAS but also local RAS. RAS blockade by ACE inhibitors (ACEIs) and Ang II receptor blockers (ARBs) induce the activation of feedback mechanisms that result in increased renin and its downstream RAS components. Therefore, aliskiren may provide a more complete block of local RAS than ACEIs and ARBs [24]. However, the effect of aliskiren on local RAS and inflammation has not been fully elucidated.

In this study, we demonstrate for the first time that aliskiren suppresses acute myocarditis in EAM models through the inhibition of local RAS.

Methods

Animals

Male BALB/c mice (7 weeks old, 23–26 g) were purchased from Japan CREA Corporation (Tokyo, Japan). They were given a standard diet and water and were maintained in compliance with the animal welfare guidelines of the Institute of Experimental Animals of Tokyo Medical and Dental University. All animal experiments were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University.

Inoculation (induction of experimental autoimmune myocarditis)

A specific peptide derived from murine cardiac α-myosin heavy chain [Myhc-$\alpha^{614-629}$ (Ac-SLKLMATLFSTYASAD-COOH)] (Japan Bio Services Corporation, Saitama, Japan) was used as an antigen as previously described [7,25]. The peptide was dissolved and emulsified with an equal volume of Freund's complete adjuvant (DIFCO Laboratories, Detroit, MI, USA). Each mouse was injected subcutaneously in the back with 150 μg/200 μl of peptide on days 0 and 7.

Aliskiren and administration

Aliskiren was provided by Novartis Pharma AG (Basel, Switzerland). Aliskiren was dissolved in 0.5% carboxymethylcellulose (CMC) vehicle immediately before use. EAM mice received daily subcutaneous injections of aliskiren (25 mg/kg) (ALS25, $n = 10$), aliskiren (50 mg/kg) (ALS50, $n = 6$) from day 0 to day 21. The day 0 is the day that the mice received their first inoculation. We selected the dose based on a previous study conducted also in mice [26]. Drug-free vehicle (0.5% CMC solution) was administered to another set of EAM mice as a positive control (Vehicle, $n = 10$). Non-immunized mice hearts were also used as a negative control (Control, $n = 5$).

Physiological examinations

Blood pressure was measured in the conscious state on days 0 and 21 in all three groups of mice using a tail-cuff system (BP-98A, Softron Co., Tokyo, Japan). Transthoracic echocardiography was performed on days 0 and 21 with ultrasound equipment (Nemio, Toshiba, Tokyo, Japan) on animals anesthetized by intraperitoneal administration of 3.6% chloral hydrate. Hearts were imaged in the two-dimensional mode in short-axis views at the level of papillary muscle. Ejection fraction (EF) was calculated by the Teichholz method on M-mode. Total wall thickness was calculated as the sum of septum and left ventricular posterior wall thickness.

Histological analysis

All mice were killed under anesthesia by intraperitoneal administration of 3.6% chloral hydrate on day 21. The hearts were harvested immediately and the upper one-third of the ventricles was fixed in 10% formalin, embedded in paraffin, and sectioned. We obtained transverse sections for histological examination. The heart sections were stained by hematoxylin and eosin (HE) or Mallory method. The extent of inflammatory cell infiltration was measured using HE staining. The degree of fibrosis was calculated using Mallory staining. The area of the heart and region affected by myocarditis (consisting of inflammatory cell infiltration and fibrosis) was determined by a computer-assisted analyzer (Scion Image beta 4.02, Scion Corporation, Frederick, MD, USA). The area ratio (affected/entire area expressed as a percentage) was calculated as described previously [27].

Immunohistochemistry

The middle one-third of ventricles was embedded in O.C.T. compound (Sakura Finetek, Tokyo, Japan) tissue medium, frozen in liquid nitrogen and stored at −80 °C. Tissue blocks were sectioned at 6 μm. Sections were then incubated with anti-CD4 (SouthernBiotech, Birmingham, AL, USA), anti-CD8 (PharMingen, San Diego, CA, USA) or anti-renin (no. 593) antibody (each at 1–10 μg/mL) at 4 °C. Histofine simple stain was used as a secondary antibody. Incubations with secondary antibodies were carried out at room temperature for 30 min. After incubation with avidin-biotin-horseradish peroxidase complexes, counterstaining was performed with hematoxylin. Immunostained type- and class-matched nonimmune phosphate-buffered saline was used as the negative control for each antibody [27,28]. Measurement of immunoreactivity of myocardial infiltrating cells for CD4 and CD8 was performed in 25 randomly selected fields in heart sections at 400-fold magnification of light microscopy.

Measurements of renin and angiotensin peptides

Blood samples were taken from the abdominal aorta under anesthesia on day 21. Plasma renin activity (PRA) and plasma angiotensin I (Ang I) levels were measured by standard radioimmunoassay methods (SRL Co., Tokyo, Japan).

Real-time polymerase chain reaction

Total RNA was isolated from the EAM hearts on day 21 after immunization; native hearts were used for control. cDNA was prepared with a real-time polymerase chain reaction (RT-PCR) kit (Stratagene Co., La Jolla, CA, USA). RT-PCR in a StepOne real-time PCR system (Applied Biosystems, CA, USA) was used to determine the mRNA expression of interleukin (IL)-2 (Mm00434256_m1), interferon (IFN)-γ (Mm01168134_m1), IL-4 (Mm00445259_m1),