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Original Research Article

CYP epoxygenase 2J2 prevents cardiac fibrosis by suppression of transmission of pro-inflammation from cardiomyocytes to macrophages

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ABSTRACT

Cytochrome P450 epoxygenase (CYP450)-derived epoxyeicosatrienoic acids (EETs) are important regulators of cardiac remodeling; but the underlying mechanism remains unclear. The present study aimed to elucidate how EETs regulated cardiac fibrosis in response to isoprenaline (Iso) or angiotensin (Ang) II. Cardiac-specific human CYP2J2 transgenic mice (Tr) and wild-type (WT) C57BL/6 littermates were infused with Iso- or Ang II. Two weeks after infusion, Tr mice showed more alleviative cardiac fibrosis and inflammation compared with WT mice. In vitro, we found Iso or Ang II induced nuclear transfer of NF-κB p65 and inflammatory cytokines expression in cardiomyocytes. Furthermore, inflammation response emerged in macrophages cultured in cardiomyocytes-conditioned medium. When pretreatment with 14,15-EET in cardiomyocytes, the inflammatory response was markedly suppressed and the transmission of inflammation from cardiomyocytes to macrophages was reduced. In conclusion, CYP2J2 and EETs prevent cardiac fibrosis and cardiac dysfunction by suppressing transmission of pro-inflammation from cardiomyocytes to macrophages in heart, suggesting that elevation of EETs level could be a potential strategy to prevent cardiac fibrosis.

1. Introduction

Cardiac fibrosis, an integral component of most cardiac pathologic conditions such as heart failure, is characterized by net accumulation of extracellular matrix (ECM) in the myocardium [1]. Cardiac fibrosis can be induced by various factors, such as the chronic activation of the sympathetic nervous system, myocardial hypoxia, ischemia, senescence, inflammation and hormones [2]. Consecutive administration with isoproterenol (Iso), a β-adrenergic receptors (β-AR) agonist, caused cardiac fibrosis in rats [3]. Emerging evidence suggests that activation of renin-angiotensin II (Ang II) system is the primary cause of cardiac fibrosis in hypertensive heart disease [4].

Recent studies demonstrated that inflammation plays a fundamental role in cardiovascular diseases, such as atherosclerosis, hypertension, and myocardial infarction [5]. Infiltration of inflammatory cells in the heart including macrophages is an early event. Fibrogenic growth factors secreted by macrophages induce cardiac remodeling. Accumulating evidences have suggested that myocardial infiltration of pro-inflammatory cells such as macrophages play pivotal roles in the initiation and development of cardiac fibrosis and dysfunction [6]. Upon activation, heart-infiltrated macrophages release various pro-inflammatory cytokines and chemokines, which interact with other cells, such as fibroblasts and cardiomyocytes, leading to cardiac remodeling [6–8]. Insults

Abbreviations: CYP2J2, cytochrome P450 2J2 epoxygenase; EET, epoxyeicosatrienoic acids; Iso, isoprenaline; Ang II, angiotensin II; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TGF-β1, transforming growth factor-beta 1; α-SMA, alpha smooth muscle actin; ECM, extracellular matrix; PPAR, nuclear receptors peroxisome-proliferator activated receptor; WT, wild type; Tr, transgene.

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result in cardiomyocyte death and induce an intense inflammatory response; subsequent activation of pro-fibrotic pathways is an important component of the reparative process, but also plays a role in the adverse remodeling [1]. However, whether the stressed cardiomyocytes could influence the activation of macrophages is incompletely understood. Exposed to different stress, the cardiomyocytes induce inflammatory response and promote cardiac fibrosis that attenuates cardiomyocyte loss. Therefore, we proposed in this study that inflammation in cardiomyocytes participates in the fibrotic process, by the direct crosstalk between cardiomyocytes and myofibroblasts or by the intermediate mechanisms; if this is the case, inhibition of inflammatory response in cardiomyocytes by transgene or drugs would prevent cardiac fibrosis.

Cytochrome P450 epoxygenase 2J2 (CYP2J2), which is of human origin and dominantly expressed in cardiovascular system, metabolizes arachidonic acid to epoxyeicosatrienoic acids (EETs) [9]. EETs possess diverse biological functions, and observations revealed that EETs exert beneficial effects on various cardiovascular diseases, including atherosclerosis, hypertension and heart failure [10–17]. Previous studies showed that 14,15-epoxyeicosatrienoic acid (14,15-EET) protected cardiomyocytes from various injuries [18,19]. It has been reported that administration of soluble epoxide hydrolase (s-EH) inhibitor, which prevents EET hydration, and overexpression of CYP2J2 could prevent angiotensin (Ang) II-induced cardiac hypertrophy and heart failure in mice [20,21]. However, the effect of CYP2J2 on cardiac fibrosis is incompletely understood. We hypothesized that inflammatory cardiomyocytes could aggravate cardiac fibrosis, and EETs in heart could inhibit cardiac inflammation and attenuate cardiac dysfunction.

In the current study, we investigated the effects of overexpression of CYP2J2 on Iso-or Ang II-induced cardiac fibrosis in mice, and determined the impact of CYP2J2 on inflammation signaling pathways involved in cardiac fibrosis.

2. Materials and methods

2.1. Reagents

Materials were obtained from the following suppliers: antibodies against NF-κB p65, LaminB and β-actin were from santa cruz biotechnology Inc. (Santa cruz, CA); antibody against MOMA-2 was from Cell Signaling Technology (Beverly, MA); antibodies against collagen I, collagen III and alpha smooth muscle actin (α-SMA) were from Boster Bio-Engineering Limited Company (Wuhan, China); EETs and 14,15-EEZE were from Cayman Chemical. All other chemicals and reagents were purchased from Sigma–Aldrich (Sigma–Aldrich China Inc., Shanghai, China).

2.2. Ethics statement

All animal studies were approved by Tongji Medical College Animal Care and Use Committee.

2.3. Animals

Cardiac-specific human CYP2J2 transgenic mice driven by αMHC promoter on a pure C57BL/6 genetic background were gifts from Dr. Darryl Zeldin's laboratory (NIEHS) and were identified as described previously [22]. All animals were housed at the animal care facility of Tongji Medical College at 25 °C with 12/12 h light/dark cycles and allowed free access to normal mice chow and water throughout the study period. All animal experimental protocols complied with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health.

Thirty male CYP2J2 transgenic mice and thirty C57BL/6 mice were used. Mini-osmotic pumps (Alzet model 1002; Durect, Cupertimo, California) were implanted as described previously [23]. The mice received a continuous subcutaneous infusion of Iso (dissolved in 0.002% ascorbic acid) [23,24] at a rate of 30 mg/kg/day for 14 days, while human Ang II was dissolved in 0.9% normal saline, and pumps were filled to deliver at the rate of 1.5 mg/kg/day over a period of 14 days. In control groups, vehicle (0.002% ascorbic acid or 0.9% normal saline) was used.

2.4. Analysis of cardiac function by echocardiography

Two weeks after infusion, echocardiography (Visualsonic Vevo 2100 System with a 40 MHz high resolution transducer) was used to detect the alterations of cardiac structure and functions. Mice were anaesthetized by isoflurane inhalation (1.5–2.5%). Measurements included IVS (interventricular septal wall thickness), LVID (left ventricular internal diameter), LVPW (left ventricular posterior wall), EF% and FS% under Long axis M-mode. All data and images were saved and analyzed by the Vevo 2100 Imaging System software version 1.0.0.

2.5. In vivo hemodynamics

In vivo LV function was assessed by Millar PV catheter as described previously [25]. Mice were anesthetized as described above and placed on heating pads with core temperature maintained at 37 °C. During the whole experiment, we monitored the vital signs (e.g. heart rate and blood pressure) under anesthesia. A microtip pressure–volume catheter (SPR-839; Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the left ventricle (LV) under pressure control. After stabilization for 20 min, the signals were continuously recorded at a sampling rate of 1000/s using an ARIA pressure-volume conductance system (Millar Instruments) coupled to a Powerlab/4SP analog-to-digital converter (AD Instruments, Mountain View, CA) and a personal computer. All pressure–volume loop data were analyzed using a cardiac pressure–volume analysis program (PVAN3.6; Millar Instruments), and HR, left ventricular end diastolic pressure (LVEDP), left ventricular end systolic pressure (LVESP), maximal slope of systolic pressure increment (dP/dtmax) and diastolic pressure decrement (dP/dtmin) were computed as described previously [26,27].

3. Cell culture

3.1. Primary cardiomyocytes and fibroblasts

All experiments involving animals were approved by Tongji Medical College Animal Care and Use Committee. 1–3 day old Sprague–Dawley rats were decapitated and the excised hearts placed in 1× PBS Buffer. The atria were carefully removed and the blood washed away. The ventricles were minced and incubated with 3 mL digestion buffer containing type II collagenase (0.025%) and trypsin (0.05%) at 37 °C while shaking at 80 RPM for 10 min. Then, stopped digestion with DMEM containing 20% newborn calf serum and stewed for 2 min. After that, discarded the supernatant and resuspended in 3 mL digestion buffer and incubated 37 °C with occasional agitation for 8 min. The steps for enzymatic digestion and isolation of myocytes were repeated 10–12 times to maximize yield. The myocytes were pooled and filtered in 200 mesh sieve and centrifuged at 1000 RPM for 8 min and resuspended in DMEM with 10% fetal bovine serum and penicillin–streptomycin (100 IU/ml) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. After culture for 1 h, transited the supernatant to another plates and

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