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Myeloid peroxisome proliferator-activated receptor gamma deficiency aggravates myocardial infarction in mice

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ABSTRACT

Background and aims: Agonists of peroxisome proliferator-activated receptor gamma (Pparγ) have been demonstrated to reduce the risk of myocardial infarction (MI) in clinical trials and animal experiments. However, the cellular and molecular mechanisms are not completely understood. We aimed to reveal the functions of myeloid Pparγ in MI and explore the potential mechanisms in this study.

Methods: Myeloid Pparγ knockout (*MPGKO*) mice (n = 12) and control mice (n = 8) underwent coronary artery ligation to induce MI. Another cohort of *MPGKO* mice and control mice underwent coronary artery ligation and were then treated with IgG or neutralizing antibodies against interleukin (IL)-1β. Infarct size was determined by TTC staining and cardiac function was measured using echocardiography. Conditioned media from GW9662- or vehicle-treated macrophages were used to treat *H9C2* cardiomyocyte cell line. Gene expression was analyzed using quantitative PCR. Reactive oxygen species were measured using flow cytometry.

Results: Myeloid Pparγ deficiency significantly increased myocardial infarct size. Cardiac hypertrophy was also exacerbated in *MPGKO* mice, with upregulation of β-myosin heavy chain (Mhc) and brain natriuretic peptide (Bnp) and downregulation of α-Mhc in the non-infarcted zone. Conditioned media from GW9662-treated macrophages increased expression of β-Mhc and Bnp in *H9C2* cells. Echocardiographic measurements showed that *MPGKO* mice had worsen cardiac dysfunction after MI. Myeloid Pparγ deficiency increased gene expression of NADPH oxidase subunits (Nox2 and Nox4) in the non-infarcted zone after MI. Conditioned media from GW9662-treated macrophages increased reactive oxygen species in *H9C2* cells. Expression of inflammatory genes such as IL-1β and IL-6 was upregulated in the non-infarcted zone of *MPGKO* mice after MI. With the injection of neutralizing antibodies against IL-1β, control mice and *MPGKO* mice had comparable cardiac function and expression of inflammatory genes after MI.

Conclusions: Myeloid Pparγ deficiency exacerbates MI, likely through increased oxidative stress and cardiac inflammation.

1. Introduction

Monocyte/macrophages have been demonstrated to play critical roles in cardiac remodeling after myocardial infarction (MI). Large amounts of monocytes migrate from the circulation and later from the spleen to the injury site following MI [1,2], not only in the early stage but throughout the whole course [3]. These monocytes show high plasticity and may differentiate into either pro-inflammatory

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or anti-inflammatory macrophages [3,4]. In addition, increased wall tension stimulates local macrophage proliferation after MI [5]. The recruitment of monocytes and local proliferation of macrophages both drive wound healing, inflammation, proteolysis and phagocytosis in the early phase and anti-inflammation, angiogenesis and fibrosis in the later stage post MI [1,6]. Resultantly, monocytes/macrophages are essential players in the process of myocardial salvage [7], left ventricular remodeling [8,9] and the recovery of left ventricular function [4]. Modulating the function of monocytes/macrophages has been proposed as a potential novel strategy for MI treatment [4].

Peroxisome proliferator-activated receptor-γ (Pparγ) plays important roles in cardiovascular diseases. Although it was controversial whether Pparγ agonist rosiglitazone was associated with increased risk of myocardial infarction, with initial analysis indicated such an association [10] and later data suggested otherwise [11,12]. Pparγ agonist pioglitazone reduces the risk of myocardial infarction in clinical trials [13–16]. Animal studies have demonstrated diverse functions of Pparγ in cardiovascular diseases. Pparγ has been shown to play an important role in ischemic liver injury in mice [17]. Genetic deletion of Pparγ leads to lower blood pressure and lipodystrophy in mice [18]. Pparγ deletion in vascular smooth muscle cells lowers blood pressure [19], exacerbates angiotensin (Ang) II-induced vascular remodeling [20], impairs intravascular thermoregulation [21], and exacerbates atherosclerosis [21–24] and vascular calcification [24] in mice. Pparγ deletion in endothelial cells and bone marrow cells results in dyslipidaemia, impairs vasoreactivity [25], and exacerbates atherosclerosis [26] and AngII-induced hypertension in mice [27]. Cardiomyocyte Pparγ deficiency promotes cardiac growth and embryonic gene expression, and induces cardiac hypertrophy [28,29]. Pioglitazone improves myocardial infarction independent of cardiomyocyte Pparγ [30], and attenuates fibrosis dependent of myeloid Pparγ but independent of cardiomyocyte Pparγ [29].

It has been reported that Pparγ regulates macrophage accumulation and inflammation. Pioglitazone inhibits AngII-induced cardiac macrophage accumulation through Pparγ [29]. Myeloid Pparγ deficiency increases macrophage accumulation and increases atherosclerosis in hypercholesterolemic mice [31]. Disruption of Pparγ in myeloid cells impairs alternative macrophage activation, increases inflammation in white adipose tissue, and aggravates diet-induced obesity, insulin resistance and glucose intolerance [32]. Moreover, myeloid Pparγ knockout mice have prolonged wound inflammation and delayed healing in type 2 diabetes [33]. However, the function of myeloid Pparγ in the process of MI has remained unclear.

In the current study, we aim to investigate the function of myeloid Pparγ in MI and explore whether myeloid Pparγ affects cardiac inflammation and oxidative stress. We first use myeloid Pparγ knockout (*MPGKO*) mice and floxed control (*FC*) mice, in combination with coronary artery ligation as a mouse model of myocardial infarction, to investigate whether myeloid Pparγ affects myocardial infarction. Then we further explore the possible mechanisms how myeloid Pparγ deficiency affects MI. Our results demonstrated that myeloid Pparγ deficiency exacerbated MI, suggesting that targeting Pparγ in myeloid cells specifically is a plausible novel strategy to treat MI.

2. Materials and methods

2.1. Animals and MI model

Myeloid Pparγ knockout (*MPGKO*: $Ppar\gamma^{fl/fl}$, LysM-Cre) and floxed control (*FC*: $Ppar\gamma^{fl/fl}$) mice were generated by crossing $Ppar\gamma^{fl/fl}$ mice [34] to LysM-Cre mice (from the Jackson Laboratory). Sex- and age-matched littermates were used for experiments. All animal protocols were approved by the Institutional Review and Ethics Board of Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine and the Institutional Animal Care and Use Committee of Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

After anesthesia with isoflurane, mice were put on a mechanical ventilator. Left-side thoracotomy was performed and the proximal portion of the left coronary artery was ligated to induce MI. Sham-operation was performed with the same surgical procedure except for the step of coronary artery ligation [35].

To neutralize IL-1β, mice were intraperitoneally injected with 10 mg/kg body weight of IL-1β-neutralizing antibody (BioXCell) 2 h after the induction of MI. The injection was repeated once at one week after MI. A mouse monoclonal IgG antibody was used as control.

2.2. Echocardiographic analysis

Transthoracic echocardiography was performed 14 or 28 days after coronary artery ligation as previously described [36]. The following parameters were recorded: left ventricle end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), LV end-diastolic internal diameter (LVIDd), and LV end-systolic inner diameter (LVIDs). LV ejection fraction (EF) was calculated as EF (%) = [(LVEDV-LVESV)/LVEDV] × 100. LV fraction shortening (FS) was calculated as FS (%) = [(LVIDd-LVIDs)/LVIDd] × 100.

2.3. Measurement of infarct size and cardiac hypertrophy

Mice were euthanized by anesthetic overdose and hearts were removed. Each heart was transversely sliced into sections. Each section was stained using 2,3,5-triphenyltetrazolium chloride (TTC). The non-infarcted zone stained red and the infarcted zone stained white. Infarcted size (%) was calculated as a percentage of infarct area to total LV area [37].

Hearts were removed and ventricles were dissected for weighing. Ratio of ventricular weight to body weight (VW/BW, mg/g) was calculated as an indicator of cardiac size [28].

2.4. Analysis of gene expression

Total RNA was isolated from ventricle samples and converted into cDNA using a PrimeScript Reverse Transcriptase reagent Kit (Takara). RT-QPCR was performed in a 7900 real time PCR machine (Life Technologies) using SYBER green master mixes and custom primers. Relative gene expression was determined using the ΔΔCt method, with *GAPDH* as a normalizing standard.

2.5. Cell culture

Thioglycollate elicited peritoneal macrophages were obtained as described before [38]. Peritoneal macrophages were treated with GW9662 (Sigma, 20 μM) containing 10% inactivated FBS or DMSO for 24 h and then conditioned media were collected for treatment in *H9C2* cells.

H9C2 cells were cultured in 6-well plates and treated with conditioned media in the presence of Angiotensin II (Sigma, 5 μM) or vehicle (ddH_2O) for 24 h. The cells were then collected for analysis of gene expression.

2.6. Measurement of intracellular reactive oxygen species (ROS) by flow cytometry

H9C2 cells were cultured in 12-well plates and treated with conditioned media in the presence of H_2O_2 (800 μM) or vehicle