Sphingosine 1-phosphate signaling contributes to cardiac inflammation, dysfunction, and remodeling following myocardial infarction

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1Department of Cardiology, Xijing Hospital, Fourth Military Medical University, Xi’an, Shannxi, China; 2Department of Orthopedics, Xijing Hospital, Fourth Military Medical University, Xi’an, Shannxi, China; and 3Center for Translational Medicine, Temple University, Philadelphia, Pennsylvania

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Zhang F, Xia Y, Yan W, Zhang H, Zhou F, Zhao S, Wang W, Zhu D, Xin C, Lee Y, Zhang L, He Y, Gao E, Tao L. Sphingosine 1-phosphate signaling contributes to cardiac inflammation, dysfunction, and remodeling following myocardial infarction. Am J Physiol Heart Circ Physiol 310: H250–H261, 2016. First published November 20, 2105; doi:10.1152/ajpheart.00372.2015—Sphingosine 1-phosphate (SIP) mediates multiple pathophysiological effects in the cardiovascular system. However, the role of SIP signaling in pathological cardiac remodeling following myocardial infarction (MI) remains controversial. In this study, we found that cardiac SIP greatly increased post-MI, accompanied with a significant upregulation of cardiac sphingosine kinase-1 (SphK1) and SIP receptor 1 (SIPR1) expression. In MI-operated mice, inhibition of SIP production by using PF543 (the SphK1 inhibitor) ameliorated cardiac remodeling and dysfunction. Conversely, interruption of SIP degradation by inhibiting SIP lyase augmented cardiac SIP accumulation and exacerbated cardiac remodeling and dysfunction. In the cardiomyocyte, SIP directly activated proinflammatory responses via a SIPR1-dependent manner. Furthermore, activation of SphK1/SIP/SIPR1 signaling attributed to β1-adrenergic receptor stimulation-induced proinflammatory responses in the cardiomyocyte. Administration of FTY720, a functional SIPR1 antagonist, obviously blocked cardiac SphK1/SIP/SIPR1 signaling, attenuated chronic cardiac inflammation, and then improved cardiac remodeling and dysfunction in vivo post-MI. In conclusion, our results demonstrate that cardiac SphK1/SIP/SIPR1 signaling plays an important role in the regulation of proinflammatory responses in the cardiomyocyte and targeting cardiac SIP signaling is a novel therapeutic strategy to improve post-MI cardiac remodeling and dysfunction.

Sphingosine 1-phosphate; myocardial infarction; inflammation; cardiac remodeling

NEW & NOTEWORTHY

The present study for the first time demonstrates that 1) cardiac SIP signaling is upregulated within 4 wk post-MI; 2) cardiac SIP signaling plays a critical role in the regulation of proinflammatory responses in the cardiomyocyte; and 3) interruption of cardiac SIP signaling ameliorates post-MI cardiac inflammation, dysfunction, and remodeling.

MYOCARDIAL INFARCTION (MI) is the most common cause of heart failure (HF) in the world (13a). Despite of significant advances achieved in HF therapies in recent decades, the incidence of HF remains extremely high. It has been reported that there are 5.1 million American adults suffering from HF in 2013 and the prevalence of HF will rapidly increase 25% by 2030 (13a). Therefore, there is an urgent need for identification of novel preventive and therapeutic strategies to improve life and survival quality of HF individuals.

Chronic inflammation is a hallmark of the failing heart. Abundant animal and human investigations have revealed that chronic inflammation contributes to post-MI left ventricular remodeling and HF progression (7). Targeting chronic cardiac inflammation has been well-recognized as a highly promising and effective strategy for HF treatment. However, the molecular and signaling mechanisms underlying chronic cardiac inflammation are not totally clear.

Sphingosine 1-phosphate (SIP) is a bioactive sphingolipid metabolite that is produced by two sphingosine kinases (SphKs), namely SphK1 and SphK2 (18a). Most of the biological effects of SIP are mediated through its ligation to five known G protein-coupled SIP receptors (SIPRs) called SIPR1 to SIPR5 (2, 18a). SIPR1 and SIPR3 are both expressed on the surface of the cardiomyocyte and cardiac SIP/SIPR1 signaling has been emerged as an important cardioprotective signaling against acute myocardial ischemia-reperfusion (MI/R) injury (15, 19). In the setting of MI/R injury, activation of cardiac SphK1/SIP/SIPR1 signaling reduces cardiomyocyte apoptosis and preserves cardiac function (33). Our previous work also demonstrated that SIP signaling mediates adiponectin cardioprotection against MI/R injury via improving calcium recycling in the cardiomyocyte (31). Although the role of cardiac SIP signaling in MI/R injury has been clarified, there is little direct evidence to verify the role of SIP signaling in chronic cardiac remodeling processes in response to long-term ischemic injuries, such as permanent MI. Due to rapidly increasing hospitalization and mortality caused by HF post-MI, it is of great significance to test whether cardiac SIP signaling contributes to chronic cardiac remodeling and HF progression or not. Therefore, we designed the present study to evaluate the role of SIP signaling in pathological cardiac remodeling using murine MI models, which are the most common animal models used to investigate myocardial remodeling and HF.

Previous studies suggest that activation of cardiac SphK1/SIP/SIPR1 signaling plays a deleterious role in chronic cardiac remodeling. The reasons are as the follows: first, SphK1/SIP/SIPR1 signaling is a major mediator of transforming growth factor-β (TGF-β)-stimulated myofibroblast activation and collagen deposition, which contributes to cardiac fibrosis and remodeling (13); second, activation of SphK1/SIP/SIPR1 signaling results in cardiac hypertrophic responses induced by several harmful stimuli, such as pressure overload (24, 26). The conclusion is also supported by the observation that...
upregulation of cardiac S1P production by SphK1 overexpression in rodent hearts causes progressive myocardial degeneration and interstitial fibrosis (29). Moreover, in post-MI HF animal models, increased vascular S1P signaling triggers peripheral vascular resistance and contributes to HF development (21). Taken together, these above investigations conclude that interruption of cardiac SphK1/S1P/S1PR1 signaling should be a promising therapeutic strategy for chronic cardiac remodeling and HF development. However, there are recent studies demonstrating that pharmacological or genetic activation of cardiac SphK1/S1P/S1PR1 signaling ameliorates post-MI cardiac remodeling and improves cardiac performance in mice after coronary artery ligation (9, 32). These results show that activation of S1PR1 signaling downregulates β1-AR expression and reduces β1-AR hyperactivation-induced cardiomyocyte apoptosis (8, 32).

Therefore, the aim of this study was to determine whether cardiac S1P signaling plays a protective or a detrimental role in pathological cardiac remodeling post-MI. In this study, we found that 1) pharmacological inhibition of cardiac S1P production ameliorated post-MI cardiac remodeling and dysfunction; 2) activation of cardiac SphK1/S1P/S1PR1 signaling was required for MI or β1-AR stimulation-induced proinflammatory responses in the cardiomyocyte; and 3) administration of FTY720, a functional S1PR1 antagonist, downregulated cardiac SphK1/S1P/S1PR1 signaling, blocked chronic cardiac inflammation, and ameliorated cardiac remodeling post-MI. Collectively, our data provide the evidence that targeting cardiac SphK1/S1P/S1PR1 signaling is a novel and promising therapeutic strategy to prevent chronic cardiac inflammation and pathological ventricular remodeling following MI.

MATERIALS AND METHODS

Mice and experimental protocol. All of the experimental protocols in this study were approved by the Animal Care and Use Committee of Fourth Military Medical University (FMMU). Male C57bl6 mice (aged 10–12 wk) were obtained from the Laboratory Animal Center of FMMU. To induce MI models, the mice were anesthetized by 1–2% isoflurane inhalation, the transthoracic two-dimensional mode echocardiography (VisualSonics) was performed. The LV end-systolic dimension (LVESD), end-diastolic dimension (LVEDD), and ejection fraction (LVEF) values were analyzed using the Vevo770 program software (VisualSonics).

Histological analysis. Ten sections (7- to 10-μM thick) per heart were prepared for the histological analysis. The Masson trichrome staining and the picrosirius red staining were used to evaluate interstitial fibrosis and structural changes of the heart. High-magnification light micrographs were obtained using a light microscopy. The size of interstitial fibrosis was measured using the software Image-Pro plus 6.0 (Media Cybernetics).

SIP measurement. LV tissues were sonicated in the ice-cold 50 mM Tris buffer (pH 7.4) containing 0.25 M sucrose, 25 mM KCl, 0.5 mM EDTA, and 1% phosphatase inhibitor cocktail (Beyotime). Then, LV homogenates or serum samples were centrifuged at 2,500 g for 10 min and SIP concentrations were quantified using a specific SIP enzyme-linked immunosorbent assay (ELISA) kit (Echelon Biosciences) as the manufacturer instructed.

SphK activity assay. SphK activity was determined as previously described (18). Briefly, the heart was rapidly excised from the anesthetized mouse and was clearly washed in the ice-cold PBS. Then the LV tissue was isolated and was homogenized in the ice-cold isolation buffer containing 0.13 M KCl, 20 mM HEPES, 1 mM EDTA, 1 μg/ml leupeptin, 0.25 μg/ml chymostatin, and 0.25 μg/ml pepstatin A. Each sample was assayed for SphK activity by incubation with d-erythro-sphingosine (Avanti Polar Lipids) and [γ-32P] ATP in the 96-well plate. Then, the product [γ-32P]sphingosine was quantified by the scintillation counting. The background values were determined by the negative controls, which were the reaction mixture without d-erythro-sphingosine.

Small interfering RNA transfection and neonatal rat ventricular cardiomyocytes culture. Primary ventricular cardiomyocytes were isolated from Sprague-Dawley rats (aged 1–2 days) as described previously in our work (31). Briefly, the isolated cardiomyocytes of the rats were seeded in six-well plates in the culture medium, of Dulbecco’s modified Eagle’s medium (DMEM), 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin. The specific rat S1PR1 small interfering RNA (siRNA) was purchased (GenePharma). The sequences of S1PR1 siRNA were 5'-GCUGCUUAUCAUCCUA-GATT-3' and 5'-UCUAGAUAUGAGACACGTT-3'. Cultured ventricular cardiomyocytes were transfected with scramble RNA or S1PR1 siRNA using the Lipofectamine2000 transfection reagent kit (Life Technologies) according to the manufacturer’s protocol. In brief, the cultured cardiomyocytes were serum-starved for 1 h and then were treated with 100 nM S1PR1 siRNA or scrambled RNA for 6 h in the FBS-free culture medium. Next, 10% FBS-containing culture medium was added to the cells for the following 72 h, and then the biochemical experiments were conducted.

Western blot. Total protein was extracted from the infarction boarder zone of frozen LV tissues or cultured cardiomyocytes. Then, the protein samples were separated by SDS-PAGE and were transferred to nitrocellulose membranes (Merck Millipore). After incubation overnight with primary antibodies, the membranes were washed using PBS with 0.1% Tween-20 and then were incubated with secondary antibodies conjugated to horseradish peroxidase (HRP) for 1 h at room temperature. The membranes were scanned using the Immobilon Western Chemiluminescent HRP Substrate (Merck Millipore) and the ChemiDOC XR system (Bio-Rad Laboratories). The blots were quantified by the Quantity-one analysis software (Bio-Rad Laboratories). The primary antibodies were anti-SphK1, anti-SphK2, anti-S1PR1, anti-S1PR3, and anti-β-actin (1:500; Santa Cruz Biotechnology) and anti-p-p65, anti-p65, anti-p-STAT3, and anti-STAT3 (1:1,000; Cell Signaling Technology).

Immunohistochemistry. Immunohistochemistry was performed by using an immunohistochemistry detection kit (Covin) according to the manufacturer’s instructions. Mouse CD45 primary antibody was purchased (Abcam) and images were visualized by a light microscopy.

Real-time PCR. Total RNA was extracted from frozen LV tissues or cultured cardiomyocytes using the RNA isolation kit (Tianz) the cDNA was synthesized from 2 μg RNA per sample using the PrimeScript cDNA synthesis kit (Takara Biotechnology). To examine the mRNA levels of SphK1, SphK2, S1PR1, S1PR3, TNF-α, IL-6,
atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), β-myosin heavy chain (β-MHC), transforming growth factor-β (TGF-β), connective tissue growth factor (CTGF), fibronectin, collagen I, and collagen III, real-time PCR (RT-PCR) was performed using the UltraSYBR Mixture (CoWin Biotechnology) and the CFX96 quantitative real-time PCR detection system (Bio-Rad Laboratories). All the primer used for RT-PCR were synthesized and validated by Takara biotechnology.

Statistical analyses. All the data are presented as means ± SE. Student’s t-test with a two-tail distribution was performed to test for statistical significance for two groups. For more than two groups, two-way ANOVA followed by a post hoc analysis was performed for analyzing two parameters. Statistical significance was analyzed by using the GraphPad Prism software, and P <0.05 was considered as statistically significant.

RESULTS

Cardiac SphK1/S1P/S1PR1 signaling is upregulated post-MI. The previous investigation demonstrated that cardiac SphK1/S1P/S1PR1 signaling was downregulated in advanced HF stage (9). However, the dynamic changes of circulating and cardiac S1P levels after MI have not been clearly illustrated before. Thus we detected serum and cardiac S1P levels of sham and MI mice at different time points after coronary artery ligation. Intriguingly, in our experimental settings, we found that cardiac S1P levels and cardiac SphK activities were significantly elevated at 24 h after MI, peaking at day 7 and remaining remarkably increased within 4 wk post-MI (Fig. 1, B and C), whereas there were no significant changes of serum S1P levels (Fig. 1A). As shown in Fig. 1D, cardiac SphK1 and S1PR1 expression was increased within 4 wk post-MI, while cardiac SphK2 and S1PR3 expression were unchanged (Fig. 1D). These results illustrate that cardiac SphK1/S1P/S1PR1 signaling is upregulated within 4 wk post-MI. Upregulated cardiac SphK1/S1P/S1PR1 signaling should play a role in the pathogenesis of post-MI cardiac remodeling and HF.

Pharmacological inhibition of SphK1 prevents cardiac dysfunction and remodeling induced by MI. To determine whether upregulated cardiac SphK1/S1P/S1PR1 signaling plays a protective role or a harmful role in post-MI cardiac remodeling, we evaluated the effects of PF543, a specific SphK1 inhibitor, on the mice subjected to predominant MI operation. We found that PF543 significantly inhibited cardiac SphK activity and reduced both serum and cardiac S1P levels after MI (Fig. 2, A–C). Administration of PF543 in sham-operated mice did not obviously affect cardiac function assessed by echocardiography (Fig. 2D) and had no obvious effects on cardiac structure evidenced by histological analysis (Fig. 2G). However, PF543 treatment significantly improved post-MI cardiac dysfunction, as assessed by left ventricular ejection fraction (LVEF; Fig. 2D). Furthermore, SphK1 inhibition reduced heart weight-to-body weight ratios (Fig. 2E) and lung weight-to-body weight ratios (Fig. 2F) and attenuated adverse cardiac structural changes.

Fig. 1. Cardiac sphingosine kinase-1 (SphK1)/sphingosine 1-phosphate (S1P)/S1P receptor 1 (S1PR1) signaling is upregulated post-myocardial infarction (MI). Animals were subjected to MI by left coronary artery ligation. Serum S1P (A), cardiac S1P (B), and cardiac SphK activity (C) were determined at different time points after the operation. The expression of SphK1, SphK2, S1PR1, and S1PR3 were determined by Western blot at different time points after MI (D). Data are presented as means ± SE; n = 8 per group. #P < 0.05 vs. sham.
changes (Fig. 2G) following MI. Next, we assessed transcriptional changes in post-MI hearts and found that PF543 markedly reduced remodeling genes expression, including ANP, BNP, and β-H9252-MHC (Fig. 2H). These results indicate that attenuation of cardiac SphK1 activity, via inhibiting cardiac SphK1 activity, plays a beneficial role in post-MI cardiac remodeling and dysfunction.

Amplification of cardiac S1P signaling by inhibiting S1P lyase activity exacerbates post-MI cardiac remodeling and dysfunction. In the mammalian tissues, S1P is irreversibly degraded by S1P lyase (SPL) (5). To determine whether inhibition of SPL activity, resulting in an elevation of cardiac S1P levels, aggravated pathological cardiac remodeling after MI or not, we examined the effects of THI, a specific SPL inhibitor, in both sham and MI-operated mice. The THI-fed mice exhibited higher S1P levels in the blood and myocardial tissues (Fig. 3, A and B). Our results showed that SPL inhibition aggravated MI-induced cardiac systolic dysfunction in terms of LVEF determined by echocardiography, compared with vehicle group (Fig. 3C). In addition, the THI-fed mice showed higher heart weight-to-body weight ratios (Fig. 3D), higher lung weight-to-body weight ratios (Fig. 3E), and severely adverse cardiac structural changes at day 28 after MI (Fig. 3F). SPL inhibition also markedly increased mRNA expression levels of remodeling genes, including ANP, BNP, and β-myosin heavy chain (β-MHC) (Fig. 3G). These data demonstrate that augment of cardiac S1P signaling exacerbates cardiac dysfunction and remodeling after MI. Taken together with the results obtained from the study of PF543 treatment group, these data suggest that upregulated cardiac SphK1/S1P signaling plays a harmful role in post-MI cardiac remodeling and HF progression.

S1P promotes the transcription of proinflammatory cytokines in the cardiomyocyte via its ligation to S1P1. Chronic induction of proinflammatory cytokines in the cardiomyocyte plays a harmful role in post-MI pathological cardiac remodeling and HF progression (10). To investigate the role of S1P in
the regulation of proinflammatory responses in the cardiomyocyte, we treated isolated neonatal rat ventricular myocytes (NRVMs) with different concentrations of S1P. Direct stimulation of S1P in the NRVMs for 24 h activated NF-κB (indicated by phosphorylated p65 subunit at ser311) and STAT3 (indicated by phosphorylated STAT3 at ser727) (Fig. 4A) and promoted proinflammatory cytokines (TNF-α and IL-6) expression in a dose-dependent manner (Fig. 4B). These data show that S1P directly regulates the transcription of proinflammatory cytokines in the cardiomyocyte. S1PR1 and S1PR3 are both expressed on the cardiomyocyte surface, while S1PR1 is the predominant S1P receptor subtype expressed in the cardiomyocyte (26). The former data demonstrated that S1PR1 protein expression were upregulated after MI but not S1PR3 (Fig. 1D). Given the observation that S1PR1 expression was upregulated in post-MI cardiac myocytes, we investigated whether the proinflammatory effects of S1P were mediated via its ligation to S1PR1 or not. We found that specific knockdown of S1PR1 expression by siRNA transfection in NRVMs obviously inhibited S1P-mediated activation of proinflammatory transcription factors (NF-κB and STAT3; Fig. 4D) and production of proinflammatory cytokines (TNF-α and IL-6; Fig. 4E). Similarly, blocking S1PR1 signaling by W146, a specific S1PR1 antagonist, also reduced S1P-induced NF-κB and STAT3 activation (Fig. 4G) and proinflammatory cytokines expression (Fig. 4I) in the cardiomyocyte. These in vitro results illustrate that S1P directly activates proinflammatory responses in the cardiomyocyte in a S1PR1-dependent manner.

Intriguingly, some S1PR1 agonists potently induce irreversible downregulation of S1PR1 expression by promoting its ubiquitinylation and proteasomal degradation (14). These S1PR1 agonists, referred as functional S1PR1 antagonists, block S1P/S1PR1 signaling and exert effects similar to those observed with other S1PR1 antagonists (14). FTY720, a clinical drug has been approved by US Food and Drug Administration for multiple sclerosis treatment, is one of the most effective functional S1PR1 antagonists due to its high binding affinity to S1PR1 (8). Here raises the following question. Can FTY720 block the proinflammatory effects of S1P in the cardiomyocyte? We found that FTY720 effectively decreased...
the expression of S1PR1 in the cardiomyocyte in a dose-dependent manner (Fig. 4F). Then, FTY720 significantly inhibited S1P-mediated proinflammatory transcription factor activation (Fig. 4H) and proinflammatory cytokines production (Fig. 4J), which mimicked the effects of specific S1PR1 antagonist W146. The above data support that FTY720 serves as an effective antagonist of S1PR1, instead of an agonist of S1PR1, in the cardiomyocyte.

Activation of SphK1/S1P/S1PR1 signaling contributes to β1-AR stimulation-induced proinflammatory responses in the cardiomyocyte. In the development of MI-induced cardiac remodeling, sustained β1-AR stimulation contributes to chronic production of proinflammatory cytokines, including TNF-α and IL-6 (23). According to the previous study, S1PR1 signaling downregulates β1-AR expression via upregulating G protein-coupled receptor 2 (GRK2) signaling in cultured cardiomyocyte (9). GRK2, the primary GRK isoform expressed in the cardiomyocyte, is a very important negative regulator of β1-AR signaling in the failing heart (34). However, whether S1PR1 antagonism affects cardiac β1-AR signaling in vivo or not has not been previously investigated. Here, we found that FTY720 obviously downregulated cardiac S1PR1 expression, suggesting that FTY720 is an effective agent to inhibit cardiac S1PR1 expression post-MI (Fig. 5A). Cardiac GRK2 expression was also not significantly changed by FTY720 administration (Fig. 5A).
Moreover, we found that cardiac cyclic adenosine monophosphate (cAMP), a critical second messenger of β1-AR signaling, was also not obviously affected by FTY720 administration (Fig. 5B). Collectively, these data demonstrate that S1PR1 signaling does not affect cardiac β1-AR signaling in vivo post-MI, which could be explained by the phenomenon that S1PR1 antagonism has extremely limited effects on the expression of GRK2 in postischemic cardiac myocytes.

Having demonstrated that S1PR1 inhibition failed to alter cardiac GRK2 expression and β1-AR signaling, we further determined whether S1PR1 signaling functions as a downstream molecule, mediating β1-AR activation-induced proinflammatory responses in the cardiomyocyte. After a 12-h exposure to β1-AR agonist isoprenaline (ISO), the expression of SphK1 and SphK2 was determined (C). Medium S1P levels were quantified by ELISA (D). ISO-stimulated cardiomyocytes were treated with vehicle, PF543 (10 μM), and W146 (10 μM) for 12 h. The mRNA expression levels of TNF-α and IL-6 in the cardiomyocyte were determined by RT-PCR (E and F). Data are presented as means ± SE; n = 6 per group. *P < 0.05 vs. control or sham group. &P < 0.05 vs. ISO group.

In vivo FTY720 administration interferes with cardiac SphK1/S1P/S1PR1 signaling and prevents chronic proinflammatory cytokines production following MI. The above results have indicated that S1PR1 plays a crucial role in the regulation

Fig. 5. Isoprenaline (ISO)-stimulated proinflammatory cytokines production involves the enlargement of SphK1/S1P/S1PR1 signaling in the cardiomyocyte. Cardiac β1-adrenergic receptor (β1-AR) and GRK2 expression levels were determined (A) and cardiac cAMP levels were assayed (B) in MI and MI + FTY720 group at 4 wk post-MI. Cardiomyocytes were isolated and cultured as methods described. Cells were treated with isoprenaline (ISO; 10 μM) for 12 h. The expression of SphK1 and SphK2 was determined (C). Medium S1P levels were quantified by ELISA (D). ISO-stimulated cardiomyocytes were treated with vehicle, PF543 (10 μM), and W146 (10 μM) for 12 h. The mRNA expression levels of TNF-α and IL-6 in the cardiomyocyte were determined by RT-PCR (E and F). Data are presented as means ± SE; n = 6 per group. *P < 0.05 vs. control or sham group. &P < 0.05 vs. ISO group.
of proinflammatory responses in the cardiomyocyte, a major mechanism underlying post-MI cardiac remodeling. We have doubted whether S1PR1 antagonism exerts beneficial effects on post-MI cardiac inflammation and remodeling in vivo or not. FTY720, also known as fingolimod, is one of the most effective functional S1PR1 antagonists and has been approved in the clinical use for other indications. Therefore, the drug has provided us an available and safe means to interrupt S1PR1 signaling in vivo. Here, we found that in vivo FTY720 administration significantly reduced S1PR1 expression in post-MI myocardial tissues (Fig. 6D). The previous study has demonstrated that FTY720 induces proteasomal degradation of SphK1 (16). Consistent with the previous observation, we also observed that FTY720 treatment obviously decreased cardiac SphK1 expression but not SphK2 (Fig. 6D). Accordingly, FTY720 treatment dramatically inhibited cardiac SphK activity and reversed cardiac S1P accumulation after MI (Fig. 6, B and C), whereas serum S1P levels were unchanged. These data demonstrate that FTY720 administration is an effective and an accessible approach to block cardiac SphK1/S1P/S1PR1 signaling in vivo.

Sustained activation of NF-κB and STAT3 in post-MI myocardium has been recognized as a dominant contributor to the chronic induction of proinflammatory cytokines in the cardiomyocyte, resulting in pathological cardiac remodeling and HF development (10). In the study, we examined the effects of FTY720 on chronic induction of proinflammatory cytokines in post-MI hearts. Compared with vehicle group, FTY720 administration significantly inhibited the activation of p65 NF-κB subunit (Fig. 6E) and decreased phosphorylated STAT3 levels (Fig. 6E) in post-MI cardiac myocytes. Moreover, FTY720 administration practically normalized proinflammatory cytokines levels in the heart (Fig. 6F). Proinflammatory cell infiltration is also an important indication of chronic cardiac inflammation after MI (10). It is well-known that S1P is a critical molecule in the regulation of immune cell migration (2). Thus we further tested leukocyte infiltration (CD45-positive cells) in the infarction border and remote zone. As shown in Fig. 6G, FTY720 treatment significantly reduced proinflammatory cell infiltration post-MI, which supports the conclusion that interruption of S1P/S1PR1 signaling by FTY720 treatment is an effective strategy to control chronic cardiac inflammation post-MI.

**FTY720 suppresses MI-induced cardiac remodeling and dysfunction.** In the following, we investigated whether inhibition of cardiac SphK1/S1P/S1PR1 signaling by using FTY720 ameliorated post-MI cardiac remodeling and dysfunction or not. We found that administration of FTY720 significantly preserved LVEF at day 28 after MI operation (Fig. 7, A and B), whereas the infarct size was not obviously influenced by FTY720 treatment (43.6 ± 4.1 vs. 40.7 ± 3.8%). Then, we examined the indexes of pathological cardiac remodeling evidenced by echocardiography and histological analysis. Compared with vehicle group, FTY720 administration obviously reduced heart weight-to-body weight ratios (Fig. 7G), attenuated lung edema (Fig. 7H), and lowered LVEDV (Fig. 7C) and LVEDD (Fig. 7D). Moreover, FTY720 treatment significantly ameliorated adverse cardiac structural changes (Fig. 7E), reduced interstitial fibrosis (Fig. 7F), and decreased TGF-β family genes (Fig. 7J) and collagen genes (Fig. 7K) expression. Expression levels of remodeling marker genes, including ANP, BNP, and β-MHC, were also evidently decreased in FTY720 treatment group (Fig. 7I). Taken together, these in vivo experiments suggest that interruption of cardiac SphK1/S1P/S1PR1 signaling by FTY720 treatment is a promising therapeutic approach to improve post-MI cardiac dysfunction and remodeling.

**DISCUSSION**

HF is a major public health burden in the modern world (13a). Numerous human and animal studies have provided the evidence that chronic cardiac inflammation, including the activation of proinflammatory transcription factors and the production of proinflammatory cytokines, contributes to pathological cardiac remodeling and HF development (7, 10, 28). However, the mechanisms underlying post-MI chronic cardiac inflammation are not well understood. S1P signaling has been linked with proinflammatory disorders. SphK1 and S1P are crucial mediators of TNF-α/NF-κB signaling (25). Moreover, S1P/S1PR1 signaling functions as the nodule point in the IL-6-STAT3 positive feedback loop and promotes tumorigenesis (17, 18). However, whether S1P signaling plays a role in the regulation of proinflammatory responses in the cardiomyocyte remains totally unknown. Here we first identify that upregulated cardiac SphK1/S1P/S1PR1 signaling promotes the activation of NF-κB and STAT3 in the cardiomyocyte, contributing to the overproduction of proinflammatory cytokines and pathological cardiac remodeling induced by MI. Chronic β1-AR stimulation is a well-recognized mechanism of chronic cardiac inflammation following MI. In this study, we confirm that β1-AR stimulation upregulates SphK1 expression and increases S1P production in the cardiomyocyte. Furthermore, our findings demonstrate that SphK1/S1P/S1PR1 signaling mediates β1-AR stimulation-induced activation of proinflammatory responses in the cardiomyocyte. Taken together, these findings for the first time provide the evidence that cardiac SphK1/S1P/S1PR1 signaling plays a crucial role in the regulation of proinflammatory responses in the cardiomyocyte and contributes to post-MI chronic cardiac inflammation.

In addition to regulating proinflammatory responses, S1P signaling has been reported to mediate multiple important biological functions in the cardiovascular system. For instance, it is notable that S1P/S1PR1 signaling acts as a critical signaling pathway in the regulation of vascular development, which is evidenced by the fact that deletion of endothelial S1PR1 leads to embryonic lethality due to vascular immature in mice (1). Numerous investigations have clearly demonstrated that activation of S1PR1 signaling exerts antiangiogenic effects in vitro and in vivo (4, 11, 20, 27). Given the fact that induction of cardiac angiogenesis protects against postischemic HF, interruption of cardiac S1P/S1PR1 signaling may be a novel approach to promote cardiac angiogenesis and to improve cardiac function following MI. Moreover, S1P signaling has also been identified as a critical regulator of immune cell migration. In this study, we observed that systemic inhibition of S1PR1 signaling by FTY720 treatment reduced cardiac leukocyte infiltration post-MI. These immunosuppressive effects may also contribute to S1PR1 antagonism-mediated cardioprotection against cardiac dysfunction and remodeling post-MI. Taken together, inhibition of SphK1/S1P/S1PR1 signaling improves post-MI cardiac perfor-
Fig. 6. In vivo administration of FTY720 blocks cardiac SphK1/S1P/S1PR1 signaling and inhibits activation of proinflammatory responses after MI. Mice were subjected to MI operation as methods described. Animals were randomized to receive vehicle or FTY720 for the following 4 wk post-MI. Serum S1P (A), cardiac S1P (B), and cardiac SphK activity (C) were determined. The SphK1, SphK2, and S1PR1 expression were determined by Western blot (D). The phosphorylation levels of p65 NF-κB and STAT3 were determined (E). Cardiac TNF-α and cardiac IL-6 were quantified by ELISA (F). Cardiac leukocyte infiltration was determined by CD45 immunohistochemistry staining (G). Data are presented as means ± SE; n = 12–15 per group. *P < 0.05 vs. sham. #P < 0.05 vs. MI.

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Fig. 6. In vivo administration of FTY720 blocks cardiac SphK1/S1P/S1PR1 signaling and inhibits activation of proinflammatory responses after MI. Mice were subjected to MI operation as methods described. Animals were randomized to receive vehicle or FTY720 for the following 4 wk post-MI. Serum S1P (A), cardiac S1P (B), and cardiac SphK activity (C) were determined. The SphK1, SphK2, and S1PR1 expression were determined by Western blot (D). The phosphorylation levels of p65 NF-κB and STAT3 were determined (E). Cardiac TNF-α and cardiac IL-6 were quantified by ELISA (F). Cardiac leukocyte infiltration was determined by CD45 immunohistochemistry staining (G). Data are presented as means ± SE; n = 12–15 per group. *P < 0.05 vs. sham. #P < 0.05 vs. MI.
Another notable point is that we observed that cardiac SphK1/S1P/S1PR1 signaling was upregulated within 4 wk after MI, opposite to the changes of cardiac S1P signaling in advanced HF stage (9). It is well-known that major remodeling events, including fibrotic and proinflammatory responses in the heart, peak within 1 wk after coronary artery ligation and early therapeutic approaches against remodeling events preserve cardiac function and improve the prognosis of individuals with MI (28, 30). Therefore, it is of great importance to clarify the dynamic changes of cardiac SphK1/S1P/S1PR1 signaling and to identify whether cardiac S1P signaling plays a protective or a detrimental role in a relatively early period of time post-MI. In the settings of our study, we observed that cardiac SphK1/S1P/S1PR1 signaling was upregulated within 4 wk after MI and interruption of cardiac S1P signaling benefited the infarcted heart. According to previous studies, SphK1-derived S1P promotes S1PR1 expression, forming a SphK1/S1P/S1PR1 amplification feedback loop (17). Here we found that, in postischemic myocardial tissues, the SphK1/S1P/S1PR1 positive feedback loop was activated within 4 wk post-MI, which caused cardiac S1P accumulation. FTY720 is a functional S1PR1 antagonist that is able to interrupt the SphK1/S1P/S1PR1 feedback loop (18). Our data showed that, by using FTY720, cardiac SphK1/S1P/S1PR1 feedback loop was interrupted and cardiac S1P accumulation was reversed, contributing to S1PR1 antagonism-mediated cardioprotection against post-MI cardiac dysfunction and remodeling. Taken together, these data demonstrate that interruption of cardiac SphK1/S1P/S1PR1 feedback loop is a novel therapeutic strategy for pathological cardiac remodeling and dysfunction during an early period of time post-MI. Our data also suggest that FTY720, a clinical drug for other indications, is a potential therapeutic candidate for post-MI HF treatment.

Fig. 7. FTY720 treatment improves MI-induced cardiac dysfunction and remodeling. Mice were subjected to MI via left coronary artery ligation. Mice were randomized to receive vehicle or FTY720 treatment for 4 wk post-MI. A: representative images of echocardiography. LVEF (B), left ventricular end-systolic diameter (LVESD; C), and left ventricular end-diastolic diameters (LVEDD; D) were determined by echocardiography at day 28 post-MI. E: representative images of masson trichrome-stained and picrosirius red-stained heart sections. F: size of interstitial fibrosis was calculated. Heart weight-to-body weight ratios (G) and lung weight-to-body weight ratios (H) were calculated. Relative expression levels of remodeling-related genes (I), TGF-β family genes (J), and collagen genes (K) were determined by RT-PCR. Data are presented as means ± SE; n = 12–15 per group. #P < 0.05 vs. sham. *P < 0.05 vs. MI group.
The limitation of the present study is that we did not observe the effects of SphK1 inhibition or S1PR1 antagonism on pathological cardiac remodeling and dysfunction during the period beyond 4 wk after MI. Thus the role of cardiac SphK1/S1P/S1PR1 signaling in pathological cardiac remodeling and HF development needs to be systemically investigated during a longer period of time post-MI in additional studies. Furthermore, we focused on S1PR1, which represents S1PR with the highest expression in the cardiomyocyte. However, S1PR3 is another S1PR subtype expressed in the cardiomyocyte and the role of S1PR3 in the pathogenesis of post-MI HF remains largely unknown. More studies are deeply needed to further investigate the relevant role of S1PR3 in post-MI cardiac dysfunction and remodeling.

In conclusion, the present study provides the evidence demonstrating that 1) cardiac SphK1/S1P/S1PR1 signaling increases within 4 wk after MI; 2) cardiac SphK1/S1P/S1PR1 signaling contributes to proinflammatory responses in the cardiomyocyte; and 3) inhibition of cardiac S1P signaling ameliorates chronic cardiac inflammation, remodeling, and dysfunction post-MI. Therefore, targeting cardiac SphK1/S1P/S1PR1 signaling is a promising and an effective therapeutic strategy for post-MI HF treatment.

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REFERENCES

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


