ESC HEART FAILURE
ESC Heart Failure 2023; **10**: 334–341
Published online 11 October 2022 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.14176

ORIGINAL ARTICLE

Sphingosine-1-phosphate improves outcome of no-reflow acute myocardial infarction via sphingosine-1-phosphate receptor 1

Amin Polzin[1,2]*, Lisa Dannenberg[1,2], Marcel Benkhoff[1], Maike Barcik[1], Petra Keul[3], Aysel Ayhan[1], Sarah Weske[3], Samantha Ahlbrecht[1], Kajetan Trojovsky[1], Carolin Helten[1,2], Sebastian Haberkorn[1,2], Ulrich Flögel[4], Tobias Zeus[1,2], Tina Müller[5], Markus H. Gräler[5], Malte Kelm[1,2] and Bodo Levkau[3]

[1]*Department of Cardiology, Pulmonary Diseases, and Vascular Medicine, Medical Faculty, CARID Cardiovascular Research Institute of Duesseldorf, Heinrich Heine University of Duesseldorf, Moorenstr. 5, 40225, Düsseldorf, Germany;* [2]*Division of Cardiology, Pulmonary Diseases and Vascular Medicine, University Hospital of Duesseldorf, Düsseldorf, Germany;* [3]*Institute of Molecular Medicine III, University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany;* [4]*Department of Molecular Cardiology, Heinrich Heine University, Düsseldorf, Germany; and* [5]*Department of Anesthesiology and Intensive Care, University Hospital Jena, Jena, Germany*

Abstract

Aims Therapeutic options targeting post-ischaemic cardiac remodelling are sparse. The bioactive sphingolipid sphingosine-1-phosphate (S1P) reduces ischaemia/reperfusion injury. However, its impact on post-ischaemic remodelling independently of its infarct size (IS)-reducing effect is yet unknown and was addressed in this study.

Methods and results Acute myocardial infarction (AMI) in mice was induced by permanent ligation of the left anterior descending artery (LAD). C57Bl6 were treated with the S1P lyase inhibitor 4-deoxypyridoxine (DOP) starting 7 days prior to AMI to increase endogenous S1P concentrations. Cardiac function and myocardial healing were assessed by cardiovascular magnetic resonance imaging (cMRI), murine echocardiography, histomorphology, and gene expression analysis. DOP effects were investigated in cardiomyocyte-specific S1P receptor 1 deficient (S1PR1 Cardio Cre+) and Cre− control mice and S1P concentrations measured by LC-MS/MS. IS and cardiac function did not differ between control and DOP-treated groups on day one after LAD-ligation despite fourfold increase in plasma S1P. In contrast, cardiac function was clearly improved and myocardial scar size reduced, respectively, on Day 21 in DOP-treated mice. The latter also exhibited smaller cardiomyocyte size and reduced embryonic gene expression. The benefit of DOP treatment was abolished in S1PR1 Cardio Cre+.

Conclusions S1P improves cardiac function and myocardial healing post AMI independently of initial infarct size and accomplishes this via the cardiomyocyte S1PR1. Hence, in addition to its beneficial effects on I/R injury, S1PR1 may be a promising target in post-infarction myocardial remodelling as adjunctive therapy to revascularization as well as in patients not eligible for standard interventional procedures.

Keywords Cardioprotection; LAD-ligation; Infarct size; Remodelling; Sphingosine-1-phosphate

Received: 17 May 2022; Revised: 3 September 2022; Accepted: 15 September 2022
**Correspondence to: Amin Polzin, Klinik für Kardiologie, Pneumologie und Angiologie, Universitätsklinikum Düsseldorf, Moorenstrasse 5, 40225 Düsseldorf, Germany. Email: amin.polzin@med.uni-duesseldorf.de*
Amin Polzin and Lisa Dannenberg both contributed equally.

Introduction

Revascularization of the infarct vessel is the golden standard in acute myocardial infarction (AMI).[1] However, clinical outcome varies inter-individually and even in Western countries, more than 10% of AMI patients are so-called 'latecomers'[2] in whom the benefit of revascularization is controversially discussed. Current guidelines even discourage revascularization of the infarcted artery if symptom onset has been longer than 48 h and the patient is asymptomatic.[3–5] However, these patients are at high risk for cardiovascular events and heart failure with reduced ejection fraction (HFrEF) with enormous socio-economic impact and high morbidity and mortality.[6,7] Nevertheless, druggable targets to improve cardiac remodelling independently of revascularization and initial infarct size (IS) are rare.

Plasma levels of the sphingosine-1-phosphate (S1P), an endogenous bioactive sphingolipid contained mainly in HDL, have been linked to the incidence and severity of coronary artery disease (CAD), AMI, and HFrEF.[8] Experimentally, administration of S1P prior to cardiac ischaemia/reperfusion diminishes IS and improves cardiac function in mice.[9–11] The same is true for mice with high endogenous S1P levels due to pharmacological or genetic inhibition of S1P degradation by the S1P lyase.[12] However, the effect of S1P on cardiac remodelling after AMI independently of initial IS has not been addressed yet. Hence, we have investigated the effect of pharmacological lyase inhibition on post-ischaemic myocardial healing in a murine model of permanent ligation of the left anterior descending artery (LAD).

Methods

Animals and myocardial ischaemia protocol

All experiments as stated by the European Convention of the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes were approved by the LANUV. Animals were treated according to the institutional guidelines (Council of Europe Treaty Series No. 123). C57BL/6 wild-type mice were purchased from Janvier Labs (Saint-Berthevin, France). S1PR1-deficient mice (S1P1 Cardio Cre+) were generated as previously described[13] and used for experiments at the age of 12 ± 2 weeks. DOP (30 mg/L via drinking water; purchased from Sigma-Aldrich, Taufkirchen, Germany) treatment started 7 days prior to AMI and was continued until mice were sacrificed. To accentuate DOP effects, vitamin B6 was omitted from the diet (Altromin GmbH & Co. KG, Lage, Germany). Isoflurane, ketamine, and xylazine were used for anaesthesia. Anaesthesia was maintained with 2 vol% isoflurane. Body temperature was controlled and held at physiological levels. Permanent LAD occlusion was conducted via open chest surgery. Successful ligation was controlled by the occurrence of characteristic ST-elevation in the electrocardiogram.

Myocardial infarct size and cardiac function measurement by CMR and murine echocardiography

Cardiac magnetic resonance (CMR) was conducted 24 h and 21 days after LAD-ligation with Bruker AVANCE III 9.4-T wide bore nuclear magnetic resonance spectrometer (Bruker, Rheinstetten, Germany) at 400.13 MHz (operation software: ParaVision 5.1). Bruker microimaging unit Micro 2.5 [25-mm birdcage resonator, actively shielded gradient sets (1.5 T/m)] was used for images. For CMR procedure, mice were anesthetized with isoflurane at 1.5%. Body temperature, ECG, and respiration were supervised and monitored by a M1025 system (SA Instruments, Stony Brook, NY). CMR data were synchronized to respiratory rate if necessary. For contrast agent, vasofix Safety IV Catheter (Braun Melsungen AG, Melsungen, Germany) was inserted intra-peritoneal. As previously described, cine movies were recorded in long-axis and short-axis orientation.[14] Then, gadolinium-diethylenetriamine pentaacetate (0.2 mmol/kg) was infused. Eight to ten short-axis slices were obtained for analysis of the left ventricle. In addition, murine echocardiography was conducted. For this, high-resolution 18–36-MHz ultrasound transducer (Vevo2100, VisualSonics Inc., Toronto, Canada) was used. Manufacturer's software was used for analysis. For structural analysis, left ventricular (LV) end-diastolic and end-systolic volume were assessed. For systolic function analysis, heart rate (HR), ejection fraction (EF), and stroke volume (SV) as well as the stroke volume index (SVI) as quotient of SV to HR and body surface area (BSA) and cardiac output (CO) were calculated.

Histological analysis, real time polymerase chain reaction and LC-MS/MS

Hearts were harvested after 21 days, embedded in paraffin, and sectioned as previously described.[15] Collagen content was determined by picrosirius red staining. Fibrosis was expressed as area in % from LV area. For cardiomyocyte (CM) size, periodic acid–Schiff (PAS) staining was performed, and CM width was measured at the site of longitudinally cut nuclei at 100-fold magnification. At least five representative CM diameters per section in five sections were assessed. AxioVision40 version 4.8.2.0 (ZEISS, Oberkochen, Germany) was used as software for quantification.

For gene expression analysis, RNA was extracted from the infarct area and the remote myocardium of the left ventricle, converted into cDNA with the Revert Aid First Strand cDNA Synthesis Kit (Qiagen) and real-time PCR performed on a Bio-Rad CFX96 system with iQ SYBR Green. The $2^{-\Delta\Delta CT}$ method normalized to GAPDH was used to calculate relative gene expression. Differences in expression levels by DOP treatment were calculated by ratio paired *t*-tests in fold of control without DOP treatment.

LC-MS/MS on plasma samples was performed as previously described.[8]

Statistical analysis

Statistical analyses were conducted using IBM SPSS© Software (NY, USA) and GraphPad Prism© 8.0 statistical software (GraphPad Software Inc., San Diego) using Student's *t*-test for normally distributed variables and the Mann–Whitney *U* test for non-normally distributed variables.

Two-way ANOVA analysis was conducted to compare DOP effects between S1PR1 Cardio Cre− and S1P1 Cardio Cre+. *P* values below 0.05 were defined as significant.

Results

S1P lyase inhibition improves post-ischaemic cardiac recovery

AMI in mice was induced by permanent ligation of the LAD in mice treated or not with the S1P lyase inhibitor 4-deoxypyridoxine (DOP) starting 7 days prior to AMI. As expected, DOP raised S1P plasma levels fourfold at the time of AMI from 0.97 ± 0.09 to 3.8 ± 0.09 μM (*n* = 6 each), $P < 0.0001$ (*Figure 1A*).

Scar size was measured by CMR after 24 h and did not differ between groups (control 26.3 ± 8.3% vs. DOP 31.6 ± 7.2%, *P* = 0.15; *Figure 1B*). Functional parameters such as ejection fraction [EF, (%) 34.8 ± 14.9 vs. 32.6 ± 8.7, *P* = 0.74], cardiac output [CO (μL/min), 11.5 ± 2.4 vs. 13.59 ± 2.2, *P* = 0.11] and stroke volume index [SVI (SV/heart rate/body surface area; μL/bpm/m^2) 0.51 ± 0.09 vs. 0.5 ± 0.14, *P* = 0.88] were similar. Neither did structural parameters differ [LV end-systolic volume (ESV; μL): 46.4 ± 19.3 vs. 49.6 ± 18.7, *P* = 0.75; LV end-diastolic volume (EDV; μL): 67.6 ± 18.7 vs. 72.2 ± 18.8, *P* = 0.64]. Myocardial mass was slightly increased in DOP-treated animals at this timepoint (55.11 ± 15.3 vs. 70.4 ± 10.46, *P* = 0.0443) (*Figure 1C*).

In contrast, there were clear differences between the control and DOP group at Day 21: Systolic function was much improved in the DOP group: CO [μL/min]: 14.73 ± 2.1 vs. 18.49 ± 3.9, *P* = 0.0454; EF [%]: 27.55 ± 8.7 vs. 38.22 ± 11.8, *P* = 0.0792; SVI [μl/bpm/m^2] 0.55 ± 0.07 vs. 0.80 ± 0.22, *P* = 0.0124 (*Figure 1E*) and representative cMRI images showing improved contraction (*Figure 1D*). End-systolic and end-diastolic volumes and myocardial mass were equal [ESV (μL): 78.54 ± 27.43 vs. 58.92 ± 25.34, *P* = 0.19; EDV (μL): 105.9 ± 26.72 vs. 92.23 ± 23.89, *P* = 0.33; myocardial mass: 69.06 ± 9.1 vs. 59.9 ± 9.7, *P* = 0.0843].

DOP treatment reduces cardiac scar size

Scar size was also reduced in hearts from DOP-treated mice compared with controls 21 days after AMI (19.30 ± 6.2% vs.13.44 ± 5.7%, *P* = 0.0494; *Figure 2A*). We then compared gene expression in the remote left ventricular myocardium and observed a reduction in brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP), and collagen1a2 (Coll1a2) in DOP treated mice compared with controls (BNP: 0.49 ± 0.24-fold of control, *P* = 0.0017; ANP: 0.58 ± 0.34-fold of control, *P* = 0.0688; Coll1a2: 0.45 ± 0.17-fold of control, $P < 0.0001$; *Figure 2B*). Finally, we observed that cardiomyocyte diameter in the remote myocardium was 21% smaller in DOP-treated mice (21.95 ± 1.59 μm vs. 17.35 ± 0.77 μm, *P* = 0.0020; *Figure 2C*).

DOP improves post-ischemic cardiac remodelling in a S1PR1-receptor-dependent manner

To test whether the myocardial S1R1 was involved in improved cardiac remodelling by DOP, we employed cardiomyocyte-specific S1PR1-deficient mice (S1P1 Cardio Cre+) that we have previously characterized.[13] As expected for the LAD-ligation model, EF and SVI were identical in Cardio Cre− and Cardio Cre+ with and without DOP treatment 24 h after LAD-ligation [EF (%): Cre− 27.2 ± 5.5 vs. 29.03 ± 6.9, *P* = 0.9724; Cre+ 28.1 ± 9.2 vs. 24.85 ± 6.5, *P* = 0.8795; SVI (μL/beat/m^2): Cre− 0.59 ± 0.18 vs. 0.75 ± 0.10, *P* = 0.6510; Cre+ 0.73 ± 0.29 vs. 0.87 ± 0.18, *P* = 0.7422; CO (μL/min): Cre−: 6.8 ± 1.7 vs. 9.5 ± 4.6, *P* = 0.5891; Cre+: 8.1 ± 3.3 vs. 8.6 ± 3.9, *P* = 0.9977; *Figure 3A*]. Structural parameters were equal in littermate controls with slight increase of ESV in S1PR1-deficient mice under DOP treatment (ESV: Cre− 46.6 ± 6.5 μL vs. 57.29 ± 10.26 μL, *P* = 0.1102, Cre+ 47.44 ± 6.8 μL vs. 63.91 ± 6.4 μL, *P* = 0.0081; EDV: Cre− 64.35 ± 9.9 μL vs. 81.7 ± 18.33 μL, *P* = 0.0858, Cre+ 68.46 ± 6.9 μL vs. 85.4 ± 9.4 μL, *P* = 0.1027,myocardial mass: Cre−: 105.6 ± 31.94 vs. 110.3 ± 24.77, *P* = 0.9860; Cre+: 79.90 ± 16.98 vs. 88.24 ± 14.69, *P* = 0.9214; *Figure 3A*).

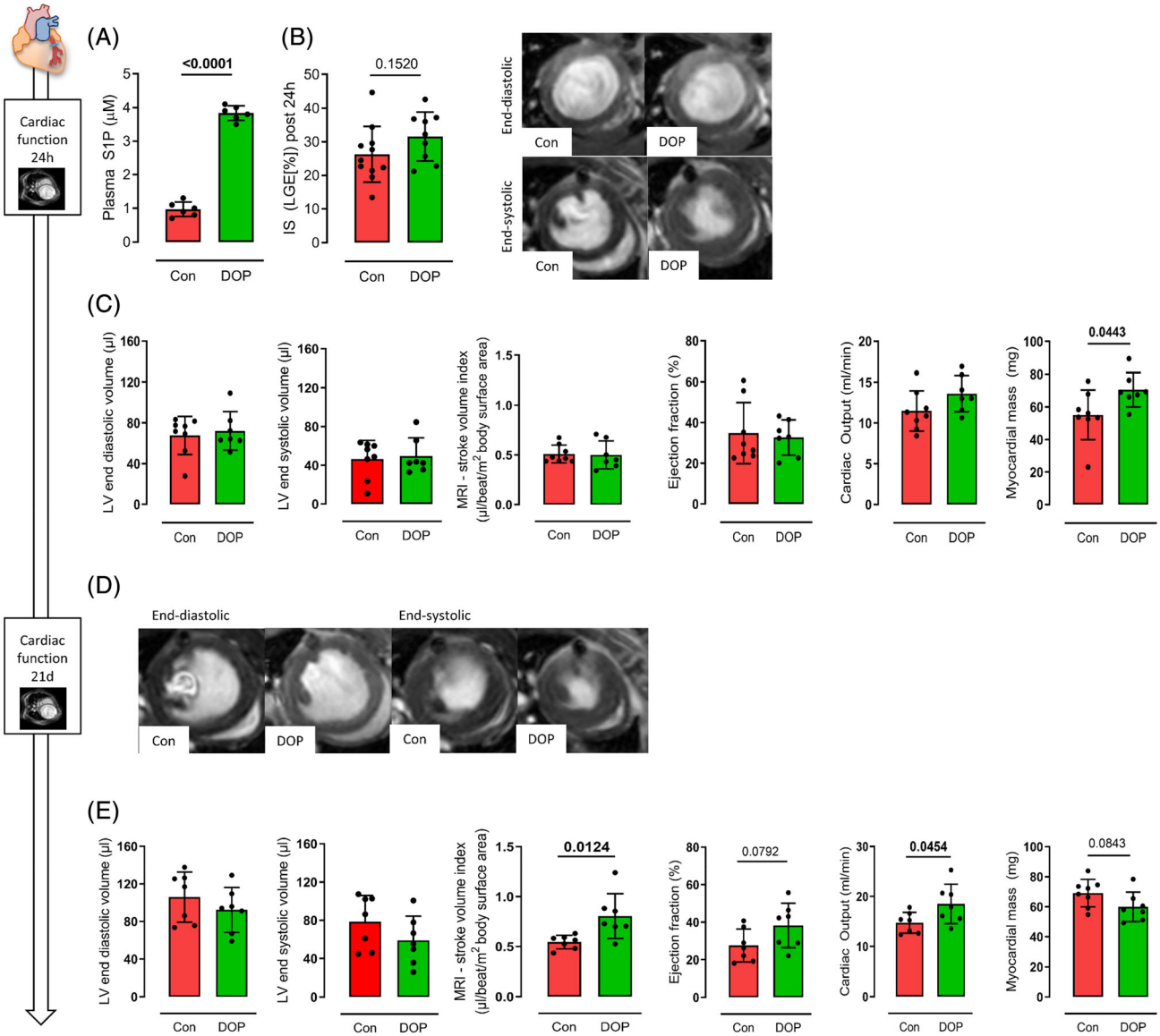
However, the situation was very different 21 days after LAD-ligation. Cre− mice treated with DOP showed an improved cardiac function: SVI was higher in the DOP group [CO (μL/min): 6.5 ± 2.2 vs. 10.2 ± 4.5, *P* = 0.2574; EF 19.5 ± 1.9% vs. 21.0 ± 5.5%, *P* = 0.9481; SVI (μL/bpm/BSA) 0.71 ± 0.13 vs. 1.159 ± 0.4, *P* = 0.0485; *Figure 3C*]. In clear contrast, DOP did not improve cardiac function in Cre+ mice (CO: 9.2 ± 2.6 vs. 9.98 ± 1.16, *P* = 0.9820; EF: 25.9 ± 5.7 vs. 18.4 ± 1.6, *P* = 0.0951; SVI 0.91 ± 0.23 μL/beat/m^2 vs. 0.97 ± 0.01 μL/beat/m^2, *P* = 0.9856). Similar to the C57Bl6 cohort, LV end-systolic and end-diastolic volumes and myocardial mass did not differ between Cre− and Cre+ with or without DOP (ESV: Cre− 79.5 ± 16.95 μL vs. 96.8 ± 24.01 μL, *P* = 0.71; Cre+ 78.13 ± 36.15 μL vs. 116.1 ± 14.01 μL, *P* = 0.1393; EDV: Cre− 98.1 ± 20.4 μL vs. 122.9 ± 30.8 μL, *P* = 0.5927; Cre+ 103.3 ± 40.8 μL vs. 143.0 ± 16.3, *P* = 0.2105; *Figure 3D*).

These data suggest that the beneficial effect of high S1P levels on post-ischaemic remodelling after coronary ligation is mediated by the myocardial S1PR1.

Discussion

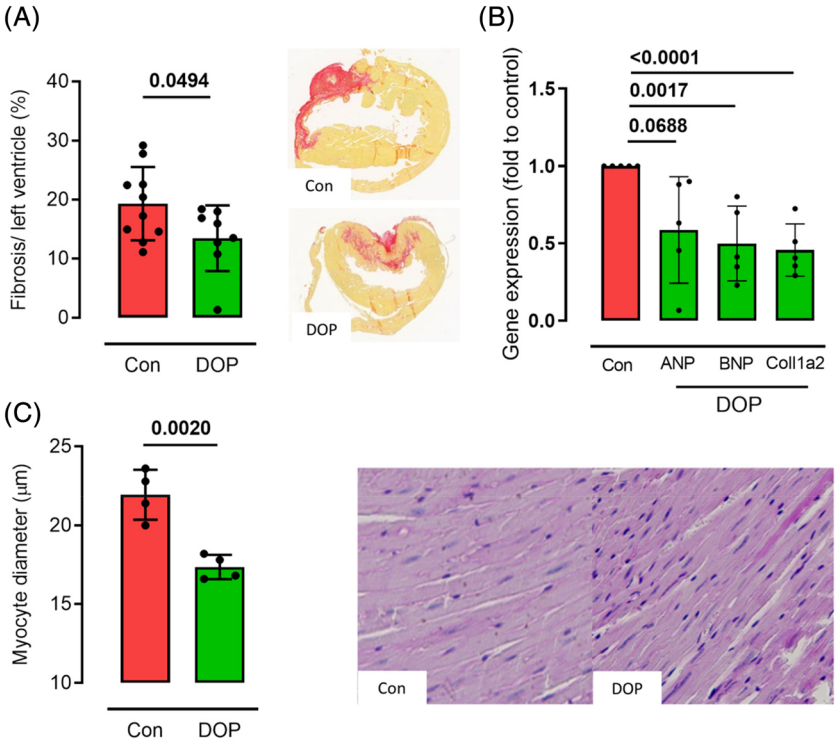
Approaches to additionally protect the heart after AMI beyond rapid coronary reperfusion remain challenging.[16]

Figure 1 (A) DOP raised S1P plasma levels fourfold at the time of AMI from 0.97 ± 0.09 to 3.8 ± 0.09 μM [n = 6 each], $P < 0.0001$. (B) Scar size after 24 h measured by CMR did not differ between groups (Con: 26.3 ± 8.3% vs. DOP: 31.6 ± 7.2%, P = 0.1520) with representative cMRI images after 24 h. (C) Functional parameters such as ejection fraction [EF, (%) 34.8 ± 14.9 vs. 32.6 ± 8.7, P = 0.74], cardiac output (CO, 11.5 ± 13.59 ± 2.2, P = 0.11), and stroke volume index [SVI (SV/heart rate/body surface area; μl/bpm/m^2) 0.51 ± 0.09vs. 0.5 ± 0.14, P = 0.88] were similar. Neither did structural parameters differ [LV end-systolic volume (ESV; μL): 46.4 ± 19.3 vs. 49.6 ± 18.7, P = 0.75; LV end-diastolic volume (EDV; μL): 67.6 ± 18.7 vs. 72.2 ± 18.8, P = 0.64]. Myocardial mass was slightly increased in DOP-treated animals (55.11 ± 15.3 vs. 70.4 ± 10.46, P = 0.0443). (D) Contractile function was improved 21 days after AMI; representative images 21 d after LAD-ligation. (E) Systolic function was improved by DOP treatment: CO [μL/min]: 14.73 ± 2.1 vs. 18.49 ± 3.9, P = 0.0454; EF [%]: 27.55 ± 8.7 vs. 38.22 ± 11.8, P = 0.0792; SVI [μl/bpm/m^2] 0.55 ± 0.07 vs. 0.80 ± 0.22, P = 0.0124. End-systolic and end-diastolic volumes and myocardial mass were numerically lower [ESV (μL): 78.54 ± 27.43 vs. 58.92 ± 25.34, P = 0.19; EDV (μL): 105.9 ± 26.72 vs. 92.23 ± 23.89, P = 0.33; myocardial mass: 69.06 ± 9.1 vs. 59.9 ± 9.7, P = 0.0843].



Whereas animal studies have often shown promising results, clinical studies seeking to implement their findings have failed to improve outcome after AMI.[16] The main reason for this is the already excellent clinical outcome in AMI patients who undergo early coronary revascularization.[1,3,17] However, a significant number of patients (>10%) with delayed or concealed symptoms leading to late clinical presentation do not benefit from coronary revascularization. In fact, coronary reperfusion is not recommended in patients with STEMI presenting later than 48 h after symptom onset.[3] These patients suffer from enhanced mortality and incidence of HFrEF.[7] Hence, pharmacological approaches

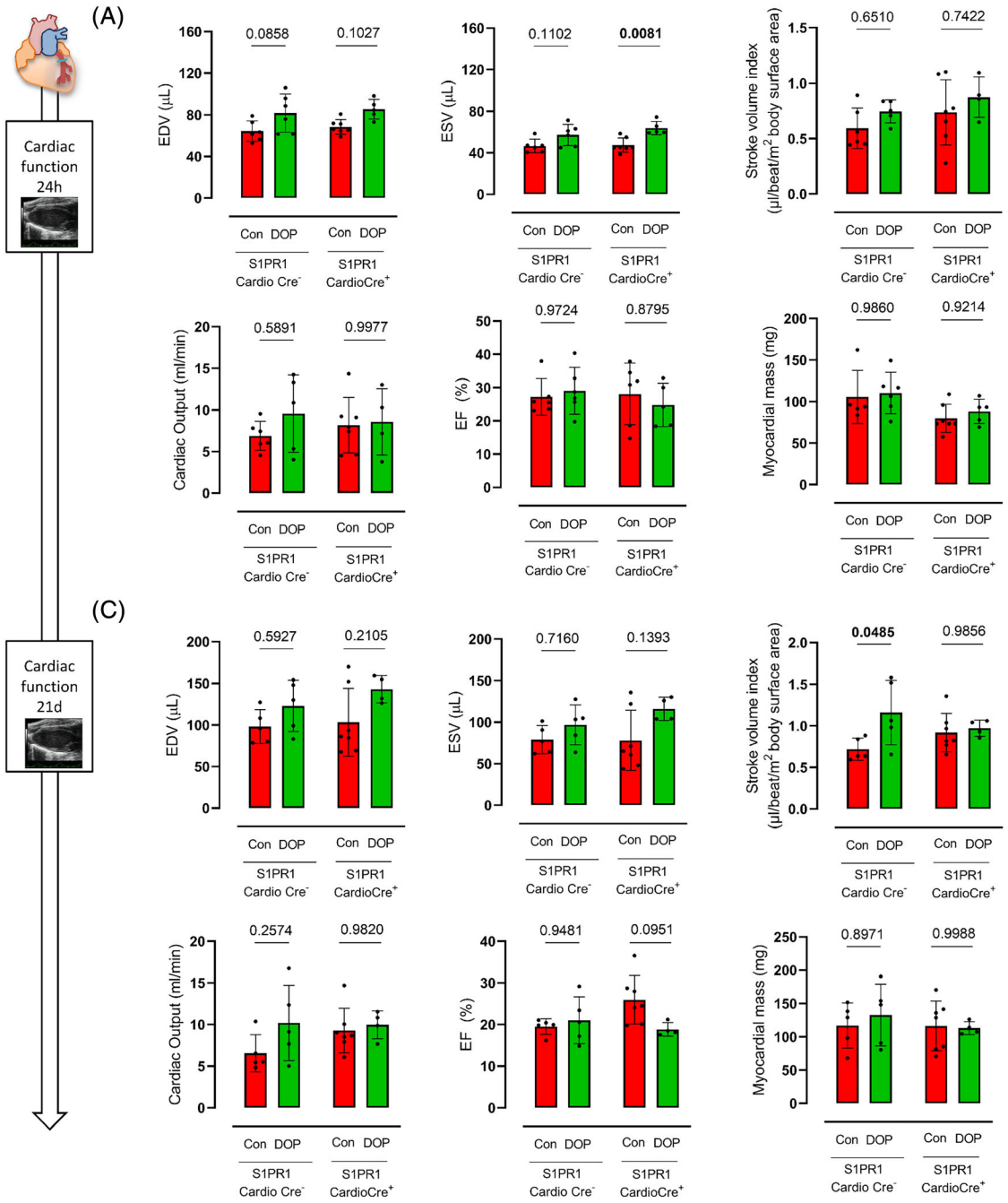
Figure 2 (A) Fibrosis was reduced in hearts from DOP-treated mice sacrificed 21 days after AMI (19.30 ± 6.2% vs.13.44 ± 5.7%, $P = 0.0494$). (B) Gene expression in the remote myocardium of the left ventricle was altered in DOP treated mice 21 days after ischemia (ANP: 0.58 ± 0.34-fold of control, $P = 0.0688$; BNP: 0.49 ± 0.24-fold of control, $P = 0.0017$; Coll1a2: 0.45 ± 0.17-fold of control, $P < 0.0001$). (C) Histomorphometry revealed reduced cardiomyocyte diameter in DOP treated mice (periodic acid–Schiff (PAS) staining, 100-fold magnification, measured in at least five cardiomyocytes in five sections). 21.95 ± 1.59 µm vs. 17.35 ± 0.77 µm, $P = 0.00$.



targeting cardiac remodelling independently of timely revascularization and initial infarct size are urgently needed.

Experimental studies that address cardiac remodelling independently of infarct size are rare but perhaps the most promising in this respect. Whereas high S1P levels due to S1P lyase inhibition and direct intravenous S1P administration, respectively, have been shown to reduce I/R injury ex vivo and in vivo,[10] their impact on post-ischaemic cardiac remodelling cannot be assessed independently of their IS-reducing effect. In contrast, we identify DOP as genuinely reperfusion-independent cardioprotective agent as exemplified by improved cardiac function and reduced fibrosis 21 days after LAD-ligation. We have identified the mechanism to be activation of the cardiomyocyte S1PR1 by high S1P as the benefit was lost in mice lacking S1PR1 in cardiomyocytes. Underlining the importance of this pathway, we have previously demonstrated that S1PR1 mediates ischaemic pre-conditioning.[13] Our study is also in line with reports on beneficial effects of exosomes from mesenchymal stem cells administered after AMI in the same LAD-ligation model that were mediated by S1PR1.[18,19] Joan Heller Brown's group was the first to show that S1PR1 activation in cardiomyocytes inhibits isoproterenol-stimulated cAMP accumulation through Gai (1). We have confirmed this in S1PR1 KO cardiomyocytes, where the ability of S1P to inhibit isoproterenol-stimulated contractility was abolished (2). We believe this to be a mechanism by which S1PR1 may confer cardioprotection in the present study in the wake of continuous engagement by elevated S1P levels. In fact, we have shown that S1PR1 KO mice fared worse under beta-adrenergic stimulation in vivo (2) without any S1PR1 signalling to dampen the cAMP response. This is a scenario that may certainly be also relevant in acute MI. Mechanistically, the cardiomyocyte S1PR1 is localized to caveolae where it is proficient to inhibit adenylyl cyclase via Gi but not to be able to activate Akt or ERK signalling (1). In contrast, cardiomyocyte S1PR2 and S1PR3 have no effect on cAMP but activate Akt (1) through beta/gamma subunits (3) after Gq and perhaps non-caveolar Gi activation, which is responsible for cardioprotection conferred by S1PR2 and S1PR3 in ischemic injury in vivo (4). Finally, cardiomyocyte S1PR1 may also have beneficial paracrine effects through inhibitory crosstalk with fibroblasts, where it may, for example, prevent adverse effects on remodelling by suppressing myofibroblast differentiation. Besides myocardial S1PR1, endothelial S1PR1 may also have a protective role in LV remodelling as has been addressed previously.[20] In LAD-ligation models, mortality is higher compared with I/R,[21] and aneurysmatic dilation of the infarcted

Figure 3 (A) EF and SVI were identical in Cardio Cre− and Cardio Cre+ with and without DOP treatment 24 h after LAD-ligation [EF (%): Cre− 27.2 ± 5.5 vs. 29.03 ± 6.9, *P* = 0.9724; Cre+ 28.1 ± 9.2 vs. 24.85 ± 6.5, *P* = 0.8795; SVI (μL/beat/m^2): Cre− 0.59 ± 0.18 vs. 0.75 ± 0.10, *P* = 0.6510; Cre+ 0.73 ± 0.29 vs. 0.87 ± 0.18, *P* = 0.7422; CO (μL/min): Cre−: 6.8 ± 1.7 vs. 9.5 ± 4.6, *P* = 0.5891; Cre+: 8.1 ± 3.3 vs. 8.6 ± 3.9, *P* = 0.9977; *A*]. Structural parameters were equal in littermate controls with slight increase of ESV in S1PR1-deficient mice under DOP treatment (ESV: Cre− 46.6 ± 6.5 μL vs. 57.29 ± 10.26 μL, *P* = 0.1102, Cre+ 47.44 ± 6.8 μL vs. 63.91 ± 6.4 μL, *P* = 0.0081; EDV: Cre− 64.35 ± 9.9 μL vs. 81.7 ± 18.33 μL, *P* = 0.0858, Cre+ 68.46 ± 6.9 μL vs. 85.4 ± 9.4 μL, *P* = 0.1027,myocardial mass: Cre−: 105.6 ± 31.94 vs. 110.3 ± 24.77, *P* = 0.9860; Cre+: 79.90 ± 16.98 vs. 88.24 ± 14.69, *P* = 0.9214). (B) Cre− mice treated with DOP showed an improved cardiac function: SVI was higher in the DOP group [CO (μL/min): 6.5 ± 2.2 vs. 10.2 ± 4.5, *P* = 0.2574; EF 19.5 ± 1.9% vs. 21.0 ± 5.5%, *P* = 0.9481; SVI (μL/bpm/BSA) 0.71 ± 0.13 vs. 1.159 ± 0.4, *P* = 0.0485; *C*]. DOP did not improve cardiac function in Cre+ mice (CO: 9.2 ± 2.6 vs. 9.98 ± 1.16, *P* = 0.9820; EF: 25.9 ± 5.7 vs. 18.4 ± 1.6, *P* = 0.0951; SVI 0.91 ± 0.23 μL/beat/m^2 vs. 0.97 ± 0.01 μL/beat/m^2, *P* = 0.9856). Similar to the C57Bl6 cohort, LV end-systolic and end-diastolic volumes and myocardial mass did not differ between Cre− and Cre+ with or without DOP (ESV: Cre− 79.5 ± 16.95 μL vs. 96.8 ± 24.01 μL, *P* = 0.71; Cre+ 78.13 ± 36.15 μL vs. 116.1 ± 14.01 μL, *P* = 0.1393; EDV: Cre− 98.1 ± 20.4 μL vs. 122.9 ± 30.8 μL, *P* = 0.5927; Cre+ 103.3 ± 40.8 μL vs. 143.0 ± 16.3, *P* = 0.2105).



area occurs at times,[22–24] prompting us to used three-dimensional MRI for precise functional quantification.

Remodelling after AMI is a complex process involving inflammation, scar formation, and myocardial adaptation, and S1P signalling may a play a role in all three processes. However, our data suggest that its role in the myocardium is most relevant in respect to post-ischaemic remodelling. In light of our finding that S1PR1 was instrumental in preventing reverse remodelling, S1PR1 agonists may appear attractive as adjunct therapeutics especially because several of them have been approved for multiple sclerosis. However, all of them persistently downregulate S1PR1, which, in our case, might be counterproductive. About DOP, we could show in further analysis that DOP does not affect S1P receptor down-regulation neither of S1PR1, S1PR2 nor S1PR3. Moreover, S1PR2 and S1PR3 expression was also equal in S1PR1-deficient mice. These data further underline the importance of S1PR1 in cardiac remodelling in the underlying study.

In addition, S1PR1 has major effects in cardiac fibroblasts, where its overexpression led to biventricular hypertrophy and diffuse interstitial cardiac fibrosis.[25] Understanding the biological pathways leading to improved cardiac recovery through S1PR1 are of utmost relevance for cardioprotection if S1PR-based drugs were to be considered as adjunct therapeutics.

Conclusions

Pharmacological S1P lyase inhibition improved myocardial remodelling after AMI independently of infarct size and through the cardiomyocyte S1PR1. Hence, targeting S1P signalling may be a promising adjunct therapy to enhance functional recovery after AMI.

Acknowledgement

The authors thank Stefanie Becher for experimental support. Open Access funding enabled and organized by Projekt DEAL.

Conflict of interest

No actual or potential conflict of interest to declare.

Funding

This work was supported by the Forschungskommission of the Medical Faculty, Heinrich-Heine-University (29-2019 to L.D., 18-2019 to A.P.) and by the German Research Foundation (LE 940/7-1 to B.L. and PO 2247/2-1 to A.P. and SFB1116 to A.P. and B.L.).

References

1. Neumann FJ, Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U, Byrne RA, Collet JP, Falk V, Head SJ, Juni P, Kastrati A, Koller A, Kristensen SD, Niebauer J, Richter DJ, Seferovic PM, Sibbing D, Stefanini GG, Windecker S, Yadav R, Zembala MO. 2018 ESC/EACTS guidelines on myocardial revascularization. *EuroIntervention*. 2019; **14**: 1435–1534.
2. Roberto M, Radovanovic D, de Benedetti E, Biasco L, Halasz G, Quagliana A, Erne P, Rickli H, Pedrazzini G, Moccetti M. Temporal trends in latecomer STEMI patients: Insights from the AMIS plus registry 1997-2017. *Rev Esp Cardiol (Engl Ed)*. 2020; **73**: 741–748.
3. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimsky P, Group ESCSD. 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The task force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J*. 2018; **39**: 119–177.
4. Konstam MA, Kramer DG, Patel AR, Maron MS, Udelson JE. Left ventricular remodeling in heart failure: Current concepts in clinical significance and assessment. *JACC Cardiovasc Imaging*. 2011; **4**: 98–108.
5. Heusch G, Libby P, Gersh B, Yellon D, Bohm M, Lopaschuk G, Opie L. Cardiovascular remodelling in coronary artery disease and heart failure. *Lancet*. 2014; **383**: 1933–1943.
6. Zheng W, Yu CM, Liu J, Xie WX, Wang M, Zhang YJ, Sun J, Nie SP, Zhao D. Patients with ST-segment elevation of myocardial infarction miss out on early reperfusion: When to undergo delayed revascularization. *J Geriatr Cardiol*. 2017; **14**: 524–531.
7. Montone RA, Niccoli G, Minelli S, Fracassi F, Vetrugno V, Aurigemma C, Burzotta F, Porto I, Trani C, Crea F. Clinical outcome and correlates of coronary microvascular obstruction in latecomers after acute myocardial infarction. *Int J Cardiol*. 2017; **236**: 30–35.
8. Polzin A, Piayda K, Keul P, Dannenberg L, Mohring A, Graler M, Zeus T, Kelm M, Levkau B. Plasma sphingosine-1-phosphate concentrations are associated with systolic heart failure in patients with ischemic heart disease. *J Mol Cell Cardiol*. 2017; **110**: 35–37.
9. Theilmeier G, Schmidt C, Herrmann J, Keul P, Schafers M, Herrgott I, Mersmann J, Larmann J, Hermann S, Stypmann J, Schober O, Hildebrand R, Schulz R, Heusch G, Haude M, von Wnuck LK, Herzog C, Schmitz M, Erbel R, Chun J, Levkau B. High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury in vivo via

the S1P3 lysophospholipid receptor. *Circulation*. 2006; **114**: 1403–1409.
10. Brulhart-Meynet MC, Braunersreuther V, Brinck J, Montecucco F, Prost JC, Thomas A, Galan K, Pelli G, Pedretti S, Vuilleumier N, Mach F, Lecour S, James RW, Frias MA. Improving reconstituted HDL composition for efficient post-ischemic reduction of ischemia reperfusion injury. *PLoS ONE*. 2015; **10**: e0119664.
11. 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*. 2016; **310**: H250–H261.
12. Bandhuvula P, Honbo N, Wang GY, Jin ZQ, Fyrst H, Zhang M, Borowsky AD, Dillard L, Karliner JS, Saba JD. S1P lyase: A novel therapeutic target for ischemia-reperfusion injury of the heart. *Am J Physiol Heart Circ Physiol*. 2011; **300**: H1753–H1761.
13. Keul P, van Borren MM, Ghanem A, Muller FU, Baartscheer A, Verkerk AO, Stumpel F, Schulte JS, Hamdani N, Linke WA, van Loenen P, Matus M, Schmitz W, Stypmann J, Tiemann K, Ravesloot JH, Alewijnse AE, Hermann S, Spijkers LJ, Hiller KH, Herr D, Heusch G, Schafers M, Peters SL, Chun J, Levkau B. Sphingosine-1-phosphate receptor 1 regulates cardiac function by modulating Ca2+ sensitivity and Na+/H+ exchange and mediates protection by ischemic preconditioning. *J Am Heart Assoc*. 2016; **5**: e003393.
14. Jacoby C, Molojavyi A, Flogel U, Merx MW, Ding Z, Schrader J. Direct comparison of magnetic resonance imaging and conductance microcatheter in the evaluation of left ventricular function in mice. *Basic Res Cardiol*. 2006; **101**: 87–95.
15. Keul P, Lucke S, von Wnuck Lipinski K, Bode C, Graler M, Heusch G, Levkau B. Sphingosine-1-phosphate receptor 3 promotes recruitment of monocyte/macrophages in inflammation and atherosclerosis. *Circ Res*. 2011; **108**: 314–323.
16. Heusch G. Critical issues for the translation of Cardioprotection. *Circ Res*. 2017; **120**: 1477–1486.
17. Wu WY, Biery DW, Singh A, Divakaran S, Berman AN, Ayuba G, DeFilippis EM, Nasir K, Januzzi JL, Di Carli MF, Bhatt DL, Blankstein R. Recovery of left ventricular systolic function and clinical outcomes in young adults with myocardial infarction. *J Am Coll Cardiol*. 2020; **75**: 2804–2815.
18. Deng S, Zhou X, Ge Z, Song Y, Wang H, Liu X, Zhang D. Exosomes from adipose-derived mesenchymal stem cells ameliorate cardiac damage after myocardial infarction by activating S1P/SK1/S1PR1 signaling and promoting macrophage M2 polarization. *Int J Biochem Cell Biol*. 2019; **114**: 105564.
19. Chen R, Cai X, Liu J, Bai B, Li X. Sphingosine 1-phosphate promotes mesenchymal stem cell-mediated cardioprotection against myocardial infarction via ERK1/2-MMP-9 and Akt signaling axis. *Life Sci*. 2018; **215**: 31–42.
20. Kuang Y, Li X, Liu X, Wei L, Chen X, Liu J, Zhuang T, Pi J, Wang Y, Zhu C, Gong X, Hu H, Yu Z, Li J, Yu P, Fan H, Zhang Y, Liu Z, Zhang L. Vascular endothelial S1pr1 ameliorates adverse cardiac remodelling via stimulating reparative macrophage proliferation after myocardial infarction. *Cardiovasc Res*. 2021; **117**: 585–599.
21. An W, Yu Y, Zhang Y, Zhang Z, Yu Y, Zhao X. Exogenous IL-19 attenuates acute ischaemic injury and improves survival in male mice with myocardial infarction. *Br J Pharmacol*. 2019; **176**: 699–710.
22. Kanamori H, Takemura G, Goto K, Maruyama R, Tsujimoto A, Ogino A, Takeyama T, Kawaguchi T, Watanabe T, Fujiwara T, Fujiwara H, Seishima M, Minatoguchi S. The role of autophagy emerging in postinfarction cardiac remodelling. *Cardiovasc Res*. 2011; **91**: 330–339.
23. Sciarretta S, Zhai P, Shao D, Maejima Y, Robbins J, Volpe M, Condorelli G, Sadoshima J. Rheb is a critical regulator of autophagy during myocardial ischemia: Pathophysiological implications in obesity and metabolic syndrome. *Circulation*. 2012; **125**: 1134–1146.
24. Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, Levine B, Sadoshima J. Distinct roles of autophagy in the heart during ischemia and reperfusion: Roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. *Circ Res*. 2007; **100**: 914–922.
25. Ohkura SI, Usui S, Takashima SI, Takuwa N, Yoshioka K, Okamoto Y, Inagaki Y, Sugimoto N, Kitano T, Takamura M, Wada T, Kaneko S, Takuwa Y. Augmented sphingosine 1 phosphate receptor-1 signaling in cardiac fibroblasts induces cardiac hypertrophy and fibrosis through angiotensin II and interleukin-6. *PLoS ONE*. 2017; **12**: e0182329.