Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Sphingomyelin phosphodiesterase 1 (SMPD1) mediates the attenuation of myocardial infarction-induced cardiac fibrosis by astaxanthin

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ARTICLE INFO

Article history:
Received 1 June 2018
Accepted 11 June 2018
Available online xxx

Keywords:
Cardiac fibrosis
Myocardial infarction
Astaxanthin
Lipidomics
SMPD1

ABSTRACT

Uncontrolled cardiac fibrosis following myocardial infarction (MI) is a critical pathological change leading to heart failure. Current pharmacotherapies are limited by unsatisfactory efficacy and undesired systemic side effects. Astaxanthin (ASX) is a natural carotenoid with strong antioxidant and anti-inflammatory activities. The effects of ASX on MI-induced cardiac fibrosis and the underlying mechanisms remain largely unknown. In this study, after the establishment of MI model, mice were administrated with ASX (200 mg/kg•d) for 4 weeks. We found that ASX treatment attenuated cardiac fibrosis and improved heart function following MI, as evidenced by reduced collagen I/III ratio, hydroxyproline content and left ventricular end diastolic pressure (LVEDP). Lipidomic analysis revealed the overaccumulation of myocardial ceramides in mice with cardiac fibrosis, which was normalized by ASX treatment. Molecular docking analysis showed that ASX produced a tight fit in the pocket of sphingomyelin phosphodiesterase 1 (SMPD1), a key enzyme in the production of ceramides. Western blot analysis confirmed the significant inhibition of SMPD1 expression by ASX. Furthermore, MI-induced overexpression of transforming growth factor β1 (TGF-β1) and phosphorylated SMAD2/3 were attenuated by ASX administration. SMPD1 knockout (KO) abrogated the beneficial effect of ASX. Taken together, our results suggest that the cardioprotective effects of ASX are mediated by SMPD1 through the indirection inhibition of TGF- β1/SMAD signaling cascade.

1. Introduction

Cardiac fibrosis refers to the excessive and disorganized deposition of myocardial collagens in response chronic insult to the myocardium such as myocardial infarction (MI) [1]. Uncontrolled fibrosis in the heart leads to fibrotic scar formation, wall stiffness, impairment of cardiac function and eventually to heart failure (HF) [2]. The process of fibrogenesis is primarily orchestrated by members of transforming growth factor (TGF)-β [3]. It was reported that overexpression of TGF- β isoform TGF-β1 in the mouse heart induces cardiac fibrosis [4].

Currently, there is lack of effective treatment for cardiac fibrosis. Interventions directly targeting TGF-β including TGF-β1 specific antibody and TGF-β1 kinase inhibitors either produce undesired systemic side effects or show no improvement due to the versatile physiological functions of TGF-β [5]. Inhibition of TGF-β downstream signaling pathways is proposed to be a better treatment strategy for fibrotic disorders [6]. TGF-β induces cardiac fibrosis via the activation of either canonical Smad signaling [7] or non-canonical signaling pathways such as PI3K/AKT and ERK/MAPK [8,9].

Ceramides, a class of sphingolipids, are not only the essential structural components of cell membranes but also function as second messenger molecules in cell signaling and inflammation [10]. Increasing evidence supports the direct role of ceramide signaling in cardiovascular diseases [11]. For instance, ceramides have been linked with processes involved in the atherosclerotic

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https://doi.org/10.1016/j.bbrc.2018.06.054
0006-291X/

development and cardiac remodeling and dysfunction [12]. Our previous also showed that increased plasma ceramides level might be used for the prediction of restenosis after percutaneous coronary intervention (PCI) [13]. Sphingomyelin phosphodiesterase 1 (SMPD1) is the key enzyme that coverts sphingomyelins (SMs) to the proinflammatory and proapoptotic second messenger ceramides [14]. SMPD1 is best known for its involvement in regulating immune cell functions and Niemann-Pick disease (NPD) [15]. However, the role of SMPD1 in the pathogenesis of cardiac fibrosis remained uncertain.

Astaxanthin (ASX) is a natural carotenoid and has been approved by US FDA as a food additive and dietary supplement [16]. In vitro and in vivo studies of ASX showed strong antioxidant and anti-inflammatory activities [17,18]. ASX has been shown to alleviate pulmonary, peritoneal and liver fibrosis through various tissue-specific mechanisms such as modulating autophagy and promoting apoptosis [19,20]. However, little information is available about its effect on cardiac fibrosis. The effect ASX on myocardial fibrosis following MI has not been reported yet. A recent study reported that ASX attenuated pressure overload-induced cardiac dysfunction and myocardial fibrosis partially by the activation of SIRT1 [21]. However, SIRT1 is a nuclear protein that mainly localized in the nucleus [22]. In contrast, ASX generally interacts with proteins on the plasma membrane due to its unique molecular structure [23]. The chance of the direct interaction between ASX and SIRT1 is fairly low. The mechanisms of action of ASX are still incompletely understood.

In this study, we investigated the effect of ASX on cardiac fibrosis after MI in mice. We aimed to elucidate the mechanisms of action of ASX using a comprehensive and unbiased lipidomic approach. The identified biological target of ASX was further validated by computational docking, western blot analysis and in vivo knockout tests. Our study highlighted the role of SMPD1 in the mediation of the attenuation of MI-induced cardiac fibrosis by ASX. The changes of TGF-β1 and the downstream signaling pathways in response to ASX treatment were also studied.

2. Experimental methods

2.1. Animals

Male C57BL/6 J mice (wild type, 8-week-old, 20–22 g) were obtained from the Experimental Animal Center of Shandong University. Smpd1 knockout mice (Smpd1$^{-/-}$, 8-week-old) were purchased from Nanjing Biomedical Research Institute of Nanjing University. All the animal protocols in this study were approved by Institutional Animal Care and Use Committee of Yantai Yuhuangding Hospital (YTYHD-2016-3218) and carried out in accordance with the National Institutes of Health (NIH) Guidelines for the use of animals in research.

2.2. Mice model of myocardial infarction (MI)

The mice model of MI was performed by the ligation of the left anterior descending artery (LAD) as described [24]. Briefly, mice were anesthetized with 2% isofluorane. The hearts were exposed and an 8-0 Prolene suture (Ethicon, Norderstedt, Germany) was used to ligate the LAD proximal with one single suture. The LAD position was at a site about 2–3 mm from LAD origin. The sham group underwent the same thoracotomy procedure except for LAD ligation.

2.3. Administration of astaxanthin (ASX)

ASX was purchased from Jingzhou Natural Astaxanthin Co. Ltd. (Jingzhou, China), dissolved in DMSO and diluted in 0.9% saline. The mice were randomly divided into three groups: (1) MI + ASX (Wild type with MI and treated with ASX), (2) MI + vehicle (Wild type, MI and 0.9% saline) (3) Sham + vehicle (Wild type with sham surgery and treated with 0.9% saline). Mice in ASX treated groups were administered with 200 mg/kg body weight of ASX daily for 4 weeks via oral gavage. Equal amount of 0.9% saline solution was given as vehicle treated groups. Eight mice were set up for each group.

2.4. Determination of hemodynamic parameters

Hemodynamic parameters including systolic blood pressure (SBP), diastolic blood pressure (DBP), left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), $+dp/dt_{max}$, $-dp/dt_{min}$ were measured after 4 weeks of treatment using a Millar pressure catheter. Mice were then sacrificed and the hearts were removed, weighed and stored at −80 °C for further analysis.

2.5. Quantification of myocardial hydroxyproline (Hyp)

The analysis of Hyp in mice heart tissue was conducted as described by a previous protocol [25]. Briefly, the samples were washed with saline and hydrolyzed with 6 mol/l HCl at 100 °C for overnight. The Hyp content was determined by the addition of *p*-dimethylaminobenzaldehyde and quantified on a Multiplate Spectrometer at 560 nm. The amount of Hyp in the issue was expressed as μg/g of fresh tissue. Measurements of each group were performed in triplicate, and the standard deviation was less than ±10%.

2.6. Immunohistochemical staining of collagen I and III

Immunohistochemistry was performed using DAKO EnVision^{+} detection kit (DakoCytomation, USA) according to the manufacturer's instructions. Primary antibodies against type I collagen (Abcam, USA) and type III collagen (Abcam, USA) were used. A positive reaction was observed as brown staining. The area of positive immunostaining was quantified using a computer image analysis system (Image Pro Plus 6.0). The ratio of type I/III collagen was calculated.

2.7. Lipidomic analysis of heart tissues

Lipidomic profiling was conducted as before with slight modifications [26]. Briefly, the lipids were extracted from 50 mg of frozen heart tissues using 1.5 mL of prechilled extraction buffer containing methanol-acetonitrile-acetone-water (30:30:30:10, v/v/v). The mixture was centrifuged at 16,000 g for 10 min and the supernatant was taken for lipidomic profiling using LC-MS/MS. Lipid analysis was conducted using a UHPLC 1260 system (Agilent, USA) coupled with a Qtrap 4500 mass spectrometer (SCIEX, USA) operated in multiple reaction monitoring (MRM) mode. The lipids were separated on an ACQUITY UPLC BEH C_{18} column (2.1 × 150 mm, 1.7 μm). Mobile phase A was 95% H_2O with 20 mM NH4HCOOH and 5% ACN (pH 4). Mobile phase. B was 100% acetonitrile. The flow rate was 300 μl/min. All major lipids classes in mice hearts were targeted.

2.8. Molecular docking analysis

Docking analysis between ASX and sphingomyelin phosphodiesterase 1 (SMPD1) protein was conducted using SwissDock (http://www.swissdock.ch/). The PDB code for SMPD1 was 5FIB and the zinc accession no. for ASX was 17653967. The data visualization and analysis were performed using Chimera 1.12.

Please cite this article in press as: Y. Shi, et al., Sphingomyelin phosphodiesterase 1 (SMPD1) mediates the attenuation of myocardial infarction-induced cardiac fibrosis by astaxanthin, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.06.054