Journal of Molecular and Cellular Cardiology 93 (2016) 69–72

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Short communication

Targeting acid sphingomyelinase reduces cardiac ceramide accumulation in the post-ischemic heart

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ARTICLE INFO

Article history:
Received 7 January 2016
Received in revised form 23 February 2016
Accepted 25 February 2016
Available online 28 February 2016

Keywords:
Ceramide
Bioactive lipids
Acid sphingomyelinase
Myocardial ischemia

ABSTRACT

Ceramide accumulation is known to accompany acute myocardial ischemia, but its role in the pathogenesis of ischemic heart disease is unclear. In this study, we aimed to determine how ceramides accumulate in the ischemic heart and to determine if cardiac function following ischemia can be improved by reducing ceramide accumulation.

To investigate the association between ceramide accumulation and heart function, we analyzed myocardial left ventricle biopsies from subjects with chronic ischemia and found that ceramide levels were higher in biopsies from subjects with reduced heart function. Ceramides are produced by either *de novo* synthesis or hydrolysis of sphingomyelin catalyzed by acid and/or neutral sphingomyelinase. We used cultured HL-1 cardiomyocytes to investigate these pathways and showed that acid sphingomyelinase activity rather than neutral sphingomyelinase activity or *de novo* sphingolipid synthesis was important for hypoxia-induced ceramide accumulation. We also used mice with a partial deficiency in acid sphingomyelinase (*Smpd1*$^{+/-}$ mice) to investigate if limiting ceramide accumulation under ischemic conditions would have a beneficial effect on heart function and survival. Although we showed that cardiac ceramide accumulation was reduced in *Smpd1*$^{+/-}$ mice 24 h after an induced myocardial infarction, this reduction was not accompanied by an improvement in heart function or survival.

Our findings show that accumulation of cardiac ceramides in the post-ischemic heart is mediated by acid sphingomyelinase. However, targeting ceramide accumulation in the ischemic heart may not be a beneficial treatment strategy.

1. Introduction

Ceramides are recognized not only as structural components of cellular membranes but also as bioactive lipids. These bioactive lipids may promote cellular stress responses such as inflammation, mitochondrial dysfunction, apoptosis and defects in insulin signaling [1]. Ceramides consist of a sphingosine base linked to a fatty acid and are produced either by *de novo* synthesis (from palmitate *via* the precursor dihydroceramides) or hydrolysis of sphingomyelin [2].

Abnormal ceramide accumulation has recently been implicated in acute myocardial ischemia [3,4]. Increasing evidence indicates that metabolic reprogramming in the ischemic heart contributes to pathological cardiac remodeling, leading to increased incidence of heart failure [5]. However, it is not known if myocardial ceramide accumulation plays a role in this process. Here, we investigated how ceramides accumulate and what role they play in the pathogenesis of myocardial ischemia.

2. Material and methods

Material and methods are described in the Supplemental material.

3. Results

To investigate the association between ceramide accumulation and heart function, we analyzed myocardial left ventricle biopsies from subjects with chronic ischemia and found that ceramide levels were higher in biopsies from subjects with reduced heart function (Fig. 1A). Furthermore, total triglycerides correlated with dihydroceramides (an upstream precursor to ceramides and a marker of *de novo* sphingolipid synthesis), indicating that increased accumulation of neutral lipids correlates with increased *de novo* sphingolipid synthesis. However, triglycerides did not correlate with ceramides (Fig. 1B), suggesting that myocardial ceramide accumulation in the post-ischemic human heart is disassociated from accumulation of neutral lipids and of the sphingolipid *de novo* synthesis pathway (see Fig. 1C for proposed pathway). Thus, our results suggest that cardiac ceramides accumulate

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http://dx.doi.org/10.1016/j.yjmcc.2016.02.019

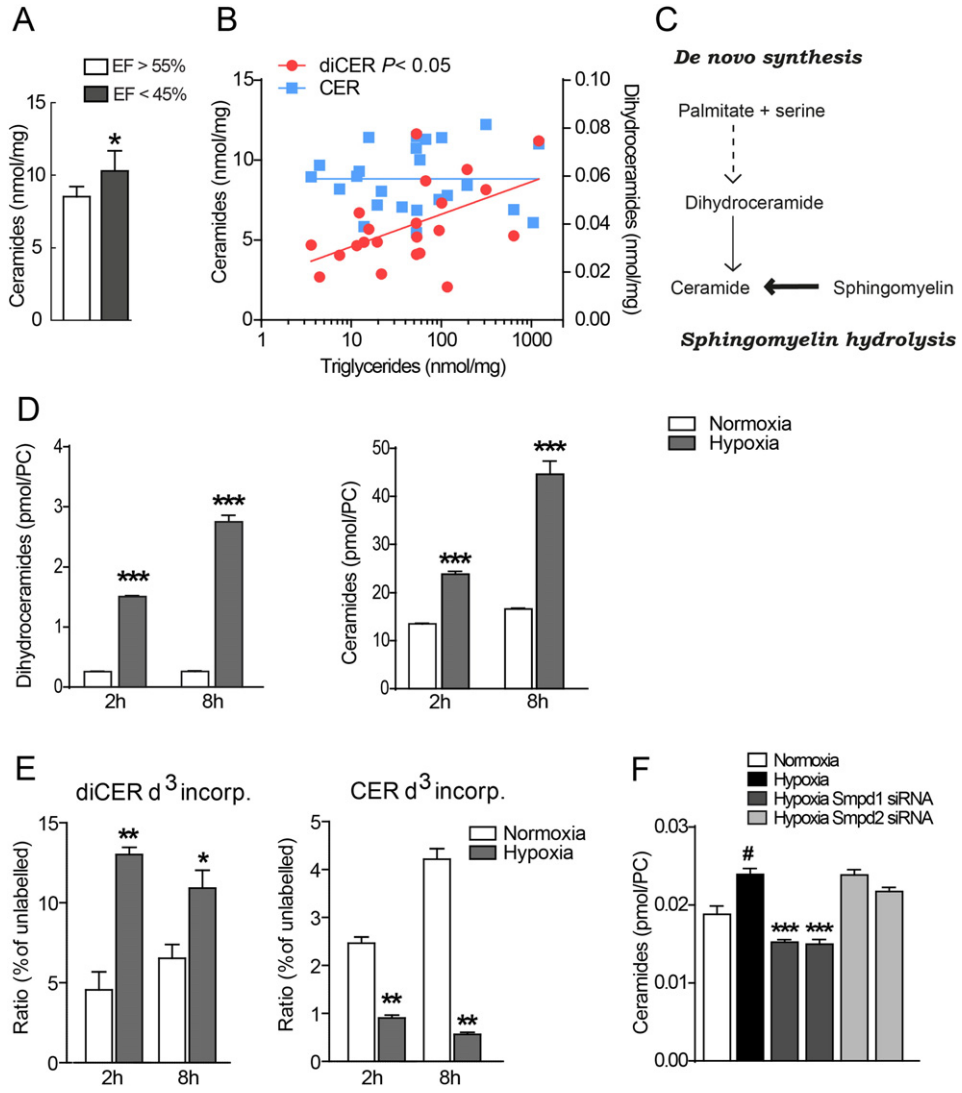


Fig. 1. Ceramides correlate with heart function and hypoxia-induced ceramide accumulation in cardiomyocytes is mediated by acid sphingomyelinase. (A) Ceramide concentrations in heart biopsies from patients with chronic cardiac ischemia and ejection fraction (EF) > 55% or <45% ($n = 30$). $^{*}P < 0.05$, *vs* EF > 55%. (B) Correlations between ceramides and dihydroceramides with triglycerides in heart biopsies from patients with chronic cardiac ischemia ($n = 30$). (C) Ceramide synthesis pathway. (D) Hypoxia-induced ceramide accumulation in HL-1 cardiomyocytes. HL-1 cells were incubated in normoxia or hypoxia for 2 h or 8 h and dihydroceramides and ceramides were measured with HPLC and mass spectrometry. ($n = 3$). Data are shown as mean ± SEM, $^{***}P < 0.001$ *vs* normoxia. (E) Incorporation of deuterium-labelled serine into dihydroceramides and ceramides in HL-1 cardiomyocytes following incubation in normoxia or hypoxia for 2 h or 8 h ($n = 3$) $^{***}P < 0.001$ *vs* normoxia. (F) Ceramide levels in HL-1 cardiomyocytes transfected with control siRNA, *Smpd1* siRNA (ID #1 and #2) and *Smpd2* siRNA (ID #1 and #2), incubated in normoxia or hypoxia for 24 h ($n = 3$–6). Data are mean ± SEM.

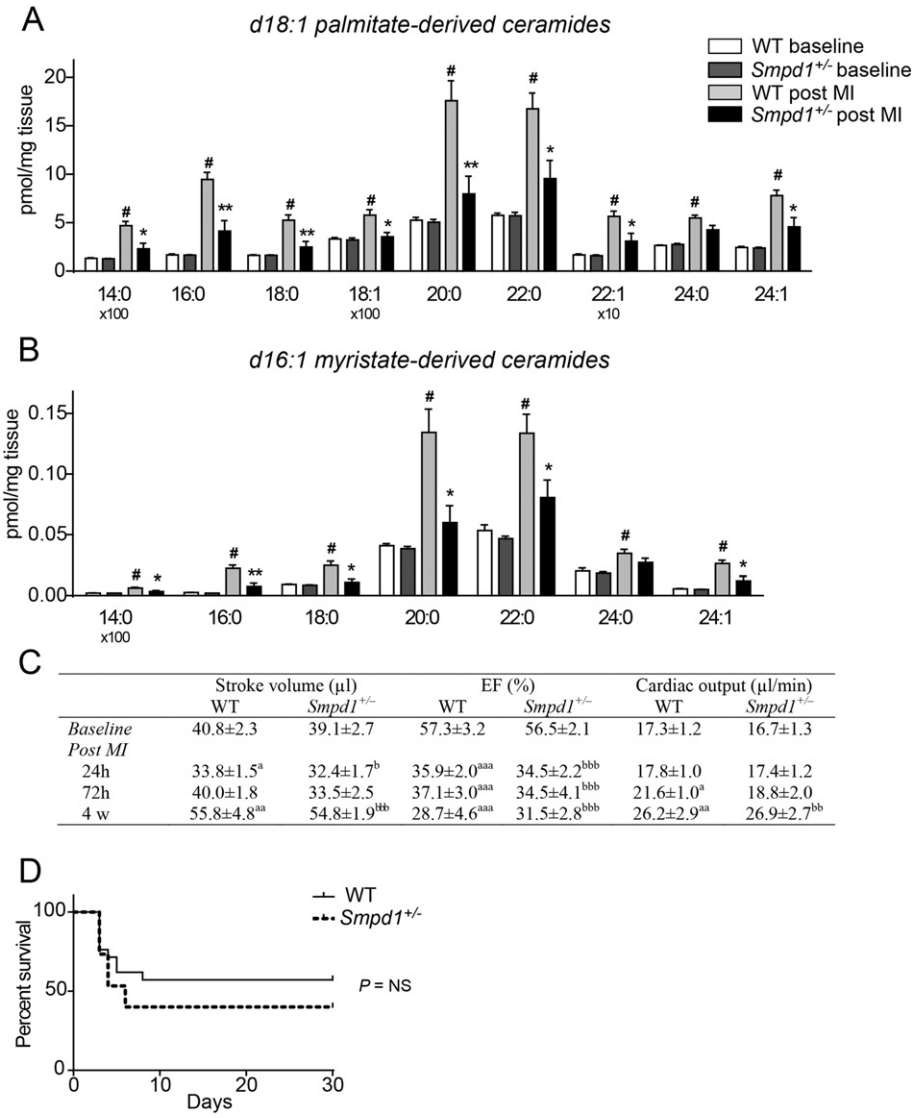
independently of the *do novo* sphingolipid synthesis pathway and that they associate with heart function in the post-ischemic heart.

We further investigated how hypoxia induces ceramide accumulation using cultured HL-1 cardiomyocytes. Levels of ceramides and dihydroceramides were upregulated by hypoxia in HL-1 cardiomyocytes (Fig. 1D). We next incubated the cells with deuterium-labeled serine (D3-serine) and found that hypoxia promoted a significant increase in the D3-serine pool in dihydroceramides but a decrease in the D3-serine pool in ceramides (Fig. 1E), thus confirming that the hypoxia-induced increase in ceramides does not originate from *de novo* synthesis.

To test whether hypoxia-induced ceramide accumulation instead resulted from hydrolysis of sphingomyelin, we used siRNA to inhibit acid sphingomyelinase (*Smpd1*) and neutral sphingomyelinase (*Smpd2*) in HL-1 cardiomyocytes. Importantly, we showed that inhibiting *Smpd1* completely blocked ceramide accumulation during hypoxia, whereas blocking *Smpd2* did not have any effect (Fig. 1F). Thus, our results suggest that acid sphingomyelinase mediates the ceramide accumulation induced by hypoxia in cardiomyocytes.

Next, we used mice with a heterozygous mutation in *Smpd1* to test if partial inhibition of acid sphingomyelinase would reduce ceramide accumulation in the post-ischemic heart. *Smpd1*$^{+/-}$ mice had normal levels of cardiac glycerolipids and sphingolipids under baseline conditions (Fig. S1). By comparing ceramide species in hearts from WT and *Smpd1*$^{+/-}$ mice before and 24 h after an induced myocardial infarction, we showed that the ischemia-induced accumulation of all ceramide species in WT hearts was significantly reduced in *Smpd1*$^{+/-}$ hearts, with the exception of the palmitate- and myristate-derived 24:0 species (Fig. 2A and B). Thus, ischemia-induced cardiac ceramide accumulation can be reduced by targeting acid sphingomyelinase.

We also assessed the cardiac function of WT and *Smpd1*$^{+/-}$ mice before and after an induced myocardial infarction. There were no differences in stroke volume, ejection fraction or cardiac output between the groups under baseline conditions nor, surprisingly, 24 h, 72 h and 4 weeks after an induced myocardial infarction (Fig. 2C). In addition, we did not observe any difference in survival of these mice for up to 30 days after an induced myocardial infarction (Fig. 2D). Thus, in our



C

	Stroke volume (µl)		EF (%)		Cardiac output (µl/min)	
	WT	*Smpd1*$^{+/-}$	WT	*Smpd1*$^{+/-}$	WT	*Smpd1*$^{+/-}$
Baseline	40.8±2.3	39.1±2.7	57.3±3.2	56.5±2.1	17.3±1.2	16.7±1.3
Post MI						
24h	33.8±1.5^{a}	32.4±1.7^{b}	35.9±2.0^{aaa}	34.5±2.2^{bbb}	17.8±1.0	17.4±1.2
72h	40.0±1.8	33.5±2.5	37.1±3.0^{aaa}	34.5±4.1^{bbb}	21.6±1.0^{a}	18.8±2.0
4 w	55.8±4.8^{aa}	54.8±1.9^{bbb}	28.7±4.6^{aaa}	31.5±2.8^{bbb}	26.2±2.9^{aa}	26.9±2.7^{bb}

Fig. 2. Inactivation of acid sphingomyelinase (*Smpd1*) reduces ischemia-induced cardiac ceramide accumulation but does not affect heart function. (A–B) Concentrations of d18:1 palmitate-derived (A) and d16:1 myristate-derived (B) ceramide species in WT and *Smpd1*$^{+/-}$ mice at baseline and 24 h after an induced myocardial infarction (MI) ($n = 5$–6). Data are mean ± SEM. $^{\#}P < 0.01$ *vs* WT baseline $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* WT post MI. (C) Echocardiographic analysis of WT and *Smpd1*$^{+/-}$ mice at baseline and after an induced MI ($n = 9$ at baseline; $n = 15$–20 at 24 h; $n = 7$–9 at 72 h; $n = 6$–7 at 4 weeks). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* WT baseline; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* *Smpd1*$^{+/-}$ baseline. (D) Kaplan–Meier curve showing survival of WT and *Smpd1*$^{+/-}$ mice after an induced MI ($n = 15$–21). Data are analyzed using the log-rank test and $P = 0.33$.

mouse model, reducing ischemia-induced cardiac ceramide accumulation does not result in improved heart function and survival.

4. Discussion

In this study, we showed that ceramide accumulation associates with heart function in the human post-ischemic heart and, importantly, that the increase in ceramides appears to be mediated by acid sphingomyelinase and not by *de novo* sphingolipid synthesis. However, we found that inhibiting acid sphingomyelinase in mice does not result in improved heart function or survival after an induced myocardial infarction despite reducing ischemia-induced ceramide accumulation.

Targeting cardiac ceramide accumulation by inhibition of *de novo* sphingolipid synthesis has emerged as a potential strategy to improve heart function in obesity and diabetes. Pharmacological inhibition of serine palmitoyltransferase, for example, has been shown to prevent cardiac toxicity caused by increased lipid uptake and excess ceramides in a mouse model of dilated cardiomyopathy [6]. However, although *de novo* sphingolipid synthesis may be enhanced in the post-ischemic heart, our results from heart biopsies and cardiomyocytes indicate that ischemia/hypoxia-induced cardiac ceramide accumulation is independent of the sphingolipid *de novo* synthesis pathway but instead results from hydrolysis of sphingomyelin catalyzed by acid sphingomyelinase. Indeed, it is known that the enzyme catalyzing ceramide synthesis from dihydroceramides is oxygen dependent [7], and thus it is unlikely that *de novo* synthesis of ceramides would occur in ischemic tissue [8]. Targeting acid sphingomyelinase in the heart would represent a novel approach to reduce cardiac ceramide accumulation.

We used mice with a partial deficiency in acid sphingomyelinase to test the hypothesis that limiting ceramide accumulation after a myocardial infarction would improve cardiac function and outcome. However, contrary to our expectations, cardiac function and outcome following a myocardial infarction in *Smpd1*$^{+/-}$ mice were not improved despite reduced ceramide accumulation compared with WT mice. We used *Smpd1*$^{+/-}$ mice as they have normal cardiac ceramide levels under baseline conditions. However, because they have 50% of normal acid sphingomyelinase activity [9], ischemia-induced ceramide accumulation is not entirely abolished in these mice. It is plausible that the reduction of ceramides was not robust enough to counteract the effects of this multifactorial pathological process, and we cannot rule out the

possibility that elevated post-ischemic ceramide levels remain a factor in impaired cardiac function. Furthermore, although long-chained ceramides were efficiently reduced in the *Smpd1*$^{+/-}$ mice, very long-chained ceramides were reduced to a lesser extent. Very long-chained ceramides may be more harmful than long and medium-chained ceramides [4,10], and we cannot exclude the possibility that these species preferentially mediate the harmful effects on heart function in our mouse model. An additional limitation to the study is that the *Smpd1*$^{+/-}$ mouse model is a global partial deficiency model, and any differences in heart function or outcome after a myocardial infarction could potentially be counteracted or enhanced by systemic effects.

In conclusion, our study reveals that ceramide accumulation in the post-ischemic heart is mediated by acid sphingomyelinase. However, inhibiting acid sphingomyelinase in mice does not result in improved heart function or survival after an induced myocardial infarction despite reducing ischemia-induced ceramide accumulation. Further investigations are therefore needed to determine the potential of targeting ceramide accumulation as a treatment for ischemic heart disease.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yjmcc.2016.02.019.

Sources of funding

This work was supported by the Swedish Research Council (C0166301), the Swedish Heart–Lung Foundation (20120399), Novo Nordisk (10799) Foundation, Swedish Diabetes Foundation (DIA2012-095), Diabetes Research Wellness Foundation (6648/2013SW), the Sahlgrenska University Hospital ALF (ALFGBG-380231) research grants.

Disclosures

None.

Acknowledgments

We thank Azra Miljanovic for technical assistance, and Rosie Perkins for scientific editing of the manuscript.

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