



Bioactive Sphingolipids as Major Regulators of Coronary Artery Disease

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Abstract

Atherosclerosis is the deposition of plaque in the main arteries. It is an inflammatory condition involving the accumulation of macrophages and various lipids (low-density lipoprotein [LDL] cholesterol, ceramide, S1P). Moreover, endothelial cells, macrophages, leukocytes, and smooth muscle cells are the major players in the atherogenic process. Sphingolipids are now emerging as important regulators in various pathophysiological processes, including the atherogenic process. Various sphingolipids exist, such as the ceramides, ceramide-1-phosphate, sphingosine, sphinganine, sphingosine-1-phosphate (S1P), sphingomyelin, and hundreds of glycosphingolipids. Among these, ceramides, glycosphingolipids, and S1P play important roles in the atherogenic processes. The atherosclerotic plaque consists of higher amounts of ceramide, glycosphingolipids, and sphingomyelin. The inhibition of the *de novo* ceramide biosynthesis reduces the development of atherosclerosis. S1P regulates atherogenesis via binding to the S1P receptor (S1PR). Among the five S1PRs (S1PR1-5), S1PR1 and S1PR3 mainly exert anti-atherosclerotic properties. This review mainly focuses on the effects of ceramide and S1P via the S1PR in the development of atherosclerosis. Moreover, it discusses the recent findings and potential therapeutic implications in atherosclerosis.

Key Words: Sphingolipid, Ceramide, Sphingosine 1-phosphate, Sphingosine-1-phosphate receptor, Atherosclerosis

INTRODUCTION

Atherosclerosis is the most common cause of cardiovascular disease and one of the leading causes of death worldwide (Murray and Lopez, 1997). Atherosclerotic plaques are formed in large and medium-sized arteries and are characterized by inflammation, lipid and macrophage accumulation, cell death, and fibrosis. Clinical complications occur when advanced lesions limit the blood flow or these plaques rupture, leading to unstable angina, myocardial infarction, stroke, or sudden death. The major lipids found in human atherosclerotic lesions are cholesterol, glycerophospholipids, and sphingolipids.

Recent findings show that atherosclerotic lesions contain a high concentration of sphingolipids. More than 50 years of study on human aortas obtained at autopsy revealed that the concentrations of sphingomyelin and cholesterol are high in advanced atherosclerosis; sphingomyelin can account for up to 70-80 % of the phospholipids in the necrotic lesions (Smith,

1960). Similar results were obtained in a monkey model that showed higher sphingomyelin in aortic lesions and plasma of atherosclerotic monkeys compared to the control monkeys (Portman and Alexander, 1970). Such findings were essentially confirmed in a more recent study that visualized increased concentrations of cholesterol, cholesteryl esters, glycerophosphocholines, and sphingomyelins in human plaques using desorption electrospray ionization mass spectrometry scanning (Manicke *et al.*, 2009).

The origin of atherosclerotic sphingolipids is not quite clear. In the majority of studies, it is attributed to an enhanced uptake of sphingolipid-rich lipoproteins. However, increased local sphingolipid synthesis in the atherosclerotic plaque has also been observed and is considered a contributing mechanism (Zilversmit *et al.*, 1961; Portman and Alexander, 1970).

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MODULATION OF CERAMIDES IN ATHEROSCLEROSIS AND RELEVANCE AS BIOMARKERS

Ceramides are accumulated in atherosclerotic lesions and in obesity (Schissel *et al.*, 1996). They are involved in insulin resistance (Hla and Kolesnick, 2014), lipoprotein uptake and aggregation (Walters and Wrenn, 2008), vascular tone (Sasset *et al.*, 2016), inflammation, oxidative stress, and apoptosis. Moreover, circulating ceramides are strongly correlated with diabetes, and some specific species have served as predictive biomarkers of future adverse cardiovascular events (Summers, 2018).

Since 2009, several small cross-sectional studies have shown that diabetic patients had elevated plasma ceramide levels (Haus *et al.*, 2009; Boon *et al.*, 2013; Lopez *et al.*, 2013; Bergman *et al.*, 2015). Lately, prospective studies based on a large population further revealed that higher concentrations of several ceramide species (e.g., C16:0, C18:0, and dihydroceramides) were associated with fasting insulin levels (Lemaitre *et al.*, 2018; Neeland *et al.*, 2018) and an increased future risk of diabetes in non-diabetic individuals (Wigger *et al.*, 2017). However, after adjustment for age, sex, and race, none of the ceramide species was independently associated with the incidence of type 2 diabetes (Neeland *et al.*, 2018).

Coronary artery disease (CAD) continues to be a leading cause of death in developed countries and starts affecting people at young ages (Lozano *et al.*, 2012). Because of the advanced stages of atherosclerotic plaques in the blood vessels, oxygen supply to the heart is reduced. This may lead to angina pectoris, myocardial infarction, as well as sudden cardiac death. Sphingolipid metabolism has already been implicated in CAD pathogenesis, including the elevation of plasma

sphingomyelin levels in CAD patients compared to the control groups (Jiang *et al.*, 2000).

In studies on patients with CAD, the concentration of ceramide species (C16:0, C18:0, C22:0, C24:0, and C24:1) was quantified, and their ratios were used to perform risk estimation for plaque instability (Cheng *et al.*, 2015b), adverse CAD incidents (Pan *et al.*, 2014; Laaksonen *et al.*, 2016; Meeusen *et al.*, 2018), and future mortality (Tarasov *et al.*, 2014; Yu *et al.*, 2015; Peterson *et al.*, 2018).

In studies on healthy individuals, serum ceramides were strongly associated with lower aerobic capacity (Fabbri *et al.*, 2016) and could help predict adverse cardiovascular outcomes (Havulinna *et al.*, 2016; Wang *et al.*, 2017). Research findings from different groups were not very consistent, and the cardioprotective role of C24:0-ceramides remained controversial (Sigrüener *et al.*, 2014). The inconsistency in the results of these groups might be because of differences in the patient selection methodology and/or different quantification methods.

A recent study investigated the importance of plasma ceramides as biomarkers for cardiovascular death in three independent CAD cohorts. It was found that the adjusted ceramide ratios of C16:0/C24:0, C18:0/C24:0, and C24:1/C24:0 were associated with an increased risk of cardiac death in patients with CAD (Laaksonen *et al.*, 2016). These findings were confirmed by Meeusen *et al.* (2018) who found that elevation of plasma ceramide C16:0, C18:0, and C24:1 was indicative of negative cardiovascular outcomes and increased hazard ratios of 1.42-1.5 from four years (C16:0, C18:0, and C24:1) up to 18 years post-enrollment (C16:0 and C24:1). The ceramide levels were shown to be more precise than the currently used lipid prediction markers and offered a promising means of

Table 1. Clinical studies investigating the role of diverse ceramides as cardiometabolic biomarkers

Year	Population	Correlating lipid (↑, higher; ↓, lower)	Clinical endpoint	Reference
2009	CAD patients (n=33)	↑ Total ceramides	CAD	de Mello <i>et al.</i> , 2009
2014	CAD patients (n=304)	↑ Total ceramides	Acute coronary syndrome	Pan <i>et al.</i> , 2014
		↑ Sphingomyelin		
2014	CAD patients (n=258)	↑ C16:0, C18:0	Cardiovascular death	Tarasov <i>et al.</i> , 2014
2015	CAD patients (n=581)	↑ C16:0, C24:0, and C16:0/C24:0 ratio	MACE	Cheng <i>et al.</i> , 2015a
2015	CHF patients (n=423)	↑ Total ceramides	Mortality	Yu <i>et al.</i> , 2015
2016	Healthy volunteers (n=433)	↑ C18:0, C20:0, and C24:1	Lower aerobic capacity	Fabbri <i>et al.</i> , 2016
		↑ Dihydroceramides		
2016	Three CAD cohorts (n=80, 51, and 81)	↑ C16:0, C18:0, C24:1, and C16:0/C24:0 ratio	Cardiovascular death	Laaksonen <i>et al.</i> , 2016
2016	Healthy individuals (n=8,101)	↑ C16:0, C18:0, C24:1, and ratios with C24:0	Cardiovascular death	Havulinna <i>et al.</i> , 2016
2017	PREDIMED trial (n=980)	↑ C16:0, C22:0, C24:, and C24:1	Non-fatal acute myocardial infarction, non-fatal stroke, cardiovascular death	Wang <i>et al.</i> , 2017
2018	Participants before non-urgent coronary angiography (n=265)	↑ C16:0, C18:0, C24:1, and ratios with C24:0	MACE	Meeusen <i>et al.</i> , 2018
2018	Two cohorts (n=2,462 and n=3134)	↓ C24:0/C16:0, C22:0/C16:0 ratios	Incident CAD and total mortality	Peterson <i>et al.</i> , 2018

CAD, coronary artery disease; CHF, chronic heart failure; MACE, major adverse cardiac events; PREDIMED, the Prevencion con Dieta Mediterranea.

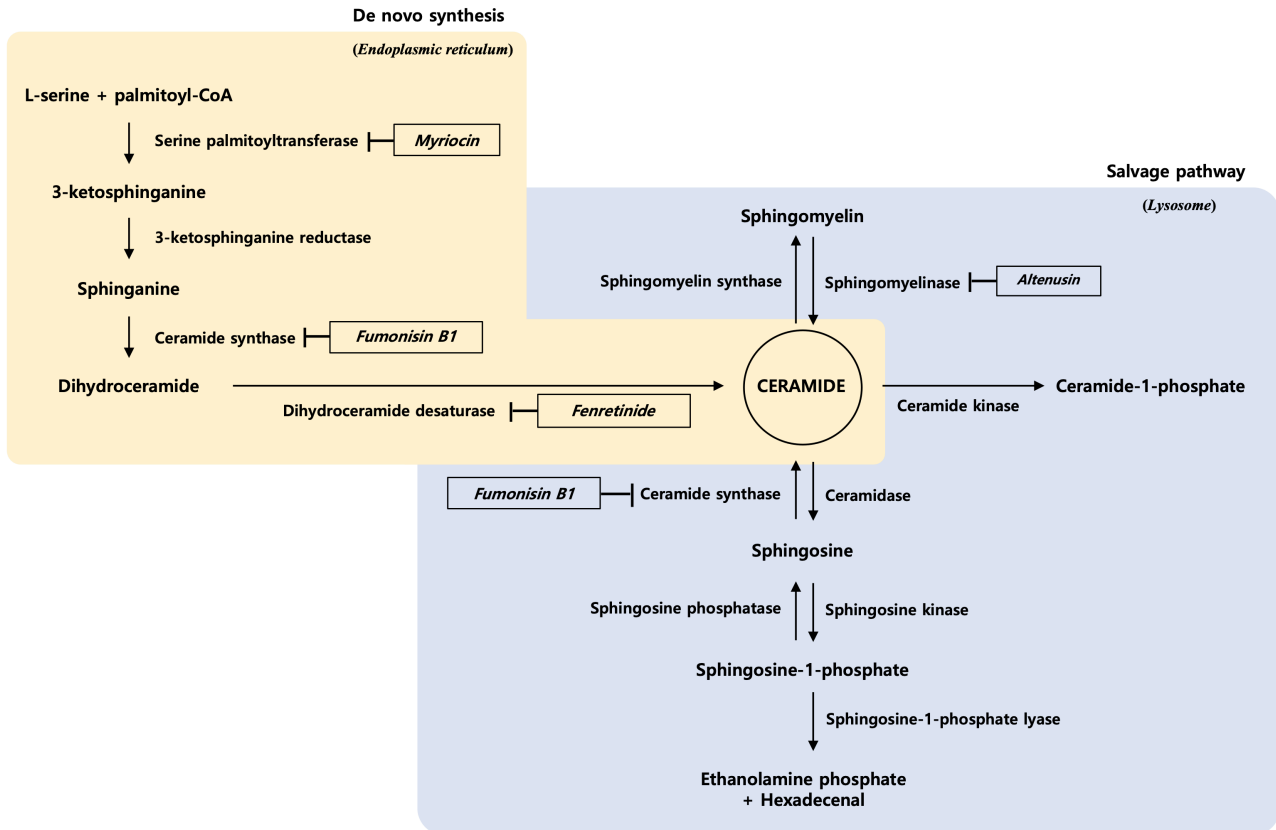


Fig. 1. Biosynthetic and salvage pathways of ceramide and sphingolipid metabolites.

identification of patients in need of more aggressive treatment. This is being tested in clinical trials in the US (Table 1). Subsequently, a ceramide plasma test for evaluation of cardiac risk was commercially released in 2016 by the Mayo Medical Laboratories (Nicholls, 2017). In general, this supportive evidence of plasma ceramides driving cardiometabolic dysfunction provided the basis for developing interventions for reduction in ceramide levels.

CERAMIDE BIOSYNTHESIS PATHWAY

Sphingolipids are based on a ceramide parent structure that is composed of a hydrophobic sphingoid backbone and a fatty acyl chain linked via an amide bond (Maula *et al.*, 2015). Three metabolic pathways are involved in ceramide production. Biosynthetic and salvage pathways of ceramide are shown in Fig. 1.

(i) *De novo* ceramide synthesis begins in the cytosolic layer of the endoplasmic reticulum. It entails the condensation of the amino acid serine and palmitoyl-coenzyme A by serine palmitoyltransferase (SPT) and generates 3-ketosphinganine. 3-Ketosphinganine is then reduced to sphinganine, an 18-carbon backbone, via 3-ketosphinganine reductase (KSR). Finally, the sphinganine gets N-acylated by the ceramide synthases (CerS), generating dihydroceramide, which is subsequently converted into ceramide by dihydroceramide desaturase (DES) (Castro *et al.*, 2014). The *de novo* synthetic pathway is

the major source of ceramide in cells. All eukaryotic cells have the capacity to produce sphingolipids in this manner.

(ii) A catabolic pathway occurs in the lysosomes that involves the hydrolysis of sphingomyelin by sphingomyelinase (SMase) and catabolism of glycosphingolipids by glycosidases that hydrolyze glycosidic bonds (Maceyka and Spiegel, 2014).

(iii) A salvage pathway generates ceramides by recycling sphingosine via CerS. Ceramidase (CDase) catalyzes the hydrolysis of ceramide to generate sphingosine (Galadari *et al.*, 2015). At least half of the sphingosine enters this reutilization pathway and plays an important role in sphingolipid homeostasis (Maceyka and Spiegel, 2014).

THE ROLE OF CERAMIDES IN ATHEROSCLEROSIS

Serine palmitoyltransferase (SPT)

SPT catalyzes the first reaction that condenses serine with palmitoyl-coenzyme A to produce 3-ketosphinganine (Hanada, 2003). Two physically associated gene products (SPTLC1 and SPTLC2) are necessary for the enzyme activity. A putative third subunit has recently been identified in both yeast (Gable *et al.*, 2000) and mammals (Hornemann *et al.*, 2006). In all organisms, the enzyme is highly selective for saturated fatty acyl-CoA molecules containing 16 carbons. The rate of this reaction is influenced largely by the availability of the free fatty acid (FFA) substrate (Merrill, 2002). This explains

the mechanism by which only saturated fats (and not the unsaturated ones) drive the synthesis of sphingolipids (Chavez *et al.*, 2003; Holland *et al.*, 2007). Inhibitors of SPT include the highly specific sphingofungins, lipoxamycin, and myriocin and the relatively less specific β -chloroalanine and cycloserine (Delgado *et al.*, 2006).

Myriocin, a commonly used SPT inhibitor (also known as thermozymocidin) inhibits the first step in the *de novo* synthesis pathway. It is derived from a traditional Chinese medicine called *Isaria sinclairii*, which is classified as a fungal species. Park *et al.* first investigated the beneficial effects of myriocin against the development of atherosclerosis in ApoE^{-/-} mice (Park *et al.*, 2004). Myriocin administration could dramatically prevent the progression of atherosclerotic lesions and even regress the pre-existing plaques and lower the plasma levels of total cholesterol, triglycerides, ceramides, sphingomyelins, S1P, sphingosine, and glycosphingolipids (Hojjati *et al.*, 2005; Park *et al.*, 2006; Glaros *et al.*, 2007; Park *et al.*, 2008). Besides, myriocin was found to improve insulin sensitivity (Dekker *et al.*, 2013; Campana *et al.*, 2018; Zabielski *et al.*, 2019), ameliorate hepatic lipid accumulation, and further reverse non-alcoholic fatty liver disease (NAFLD) (Kurek *et al.*, 2014; Kasumov *et al.*, 2015). Because myriocin inhibits the initial step in the synthesis of a number of sphingolipids, the identity of the critical species involved is unknown. Nevertheless, experimental results supported the hypothesis that SPT inhibition could be a novel therapeutic target in atherosclerosis and metabolic diseases.

Ceramide synthases (CerS)

CerS acylates sphinganine to produce dihydroceramide (Pewzner-Jung *et al.*, 2006). Recent studies indicate that a large family of CerS isoforms exists, each demonstrating selectivity for particular fatty acyl-CoA substrates (Pewzner-Jung *et al.*, 2006). Because of the existence of multiple specific enzymes catalyzing this reaction, it is tempting to speculate that individual ceramide subspecies may have distinct biological functions. However, this assumption has not yet been confirmed through experimentation. A number of fungal metabolites have been shown to inhibit this step; fumonisin B1 is the most widely used reagent among these (Delgado *et al.*, 2006).

Six fatty acyl-selective CerSs (CerS1–6) exist in mammals and are distributed in distinct tissues (Park *et al.*, 2014). The regulation of CerSs is elaborate at multiple levels, and the enzyme activity may not be consistent with mRNA or protein expression levels. Mutations in the CerSs genes or deregulation of the contents and enzyme activities of CerSs are all correlated with human disease. Over the past few years, each CerS knockout was established in mice. It showed that specific CerS deactivation may cause serious impacts and may be lethal, such as the CerS1-null mice exhibiting Purkinje cell death (Zhao *et al.*, 2011), CerS2-null mice generating myelin sheath defects and hepatocellular carcinomas (Imgrund *et al.*, 2009), CerS3-null mice dying shortly after birth (Jennemann *et al.*, 2012), and CerS4-null mice developing alopecia (Ebel *et al.*, 2014). CerS5/6-null mice showed a relatively milder phenotype that presented some behavioral abnormalities (Ebel *et al.*, 2013).

Turpin *et al.* (2014) identified that only CerS6 expression was positively correlated with obesity. Further, they generated conventional CerS6-deficient mice as well as specific brown adipose tissue and liver CerS6 deletion mice. Thus, they dem-

onstrated that CerS6 ablation could up-regulate β -oxidation and increase lipid utilization (Turpin *et al.*, 2014). Conversely, overexpression of CerS6 increases the levels of a specific species, known as C16:0 ceramides, in the mitochondrial membranes, where they interact with the mitochondrial fission factor (Mff) and thereby promote mitochondrial fragmentation (Hammerschmidt *et al.*, 2019). However, silencing of this Mff in mice with simultaneous overexpression of CerS6 prevented mitochondrial fragmentation and glucose intolerance, despite increased levels of the C16:0 ceramides (Hammerschmidt *et al.*, 2019).

However, conclusive data showing that targeting specifically unique CerS could benefit atherosclerotic degeneration are not available. Moreover, pharmacological inhibitors with high degree of selectivity for any particular CerS are not yet available. Given that CerS6 inhibition is beneficial for obesity and diabetes, it will probably restrain atherosclerosis development. Nevertheless, further novel studies are required to provide favorable evidence about this assumption.

Dihydroceramide desaturases (DES)

DES oxidizes inactive dihydroceramide into active ceramide. Two isoforms have been identified. Dihydroceramide desaturase 1 (DES1) inserts a key double bond in most peripheral tissues (Omae *et al.*, 2004), whereas the DES2 isoform preferentially produces phytosphingolipids and is largely restricted to the gut and the kidneys (Omae *et al.*, 2004). The anticancer and antidiabetic agent fenretinide (Zheng *et al.*, 2006; Kravaka *et al.*, 2007), a cyclopropene-containing sphingolipid (termed GT11), and a rationally designed compound (termed XM642) are inhibitors of this enzyme (Delgado *et al.*, 2006; Munoz-Olaya *et al.*, 2008).

In the last few years, multiple publications have shown that dihydroceramides are implicated in a far wider spectrum of biological functions than previously thought (Rodriguez-Cuenca *et al.*, 2015). Heterozygous deletion of *Des1* in mice was also demonstrated to prevent diet-induced vascular dysfunction and hypertension in mice (Zhang *et al.*, 2012). Importantly, pharmacological inhibition of DES1 protected humans from obesity and insulin resistance. The most notable inhibitory compound is fenretinide, which has been tested in several clinical trials (Mody and McIlroy, 2014). Fenretinide treatment could positively balance the metabolic profile by improving insulin sensitivity in overweight premenopausal women (Johansson *et al.*, 2008). Moreover, long-term therapy with fenretinide could alleviate diet-induced adiposity and dyslipidemia and prevent hepatic steatosis in mice (Preitner *et al.*, 2009; Bikman *et al.*, 2012; Koh *et al.*, 2012).

A recent study by Chaurasia *et al.* (2019) established the importance of ceramides through the genetic manipulation of *Des1* in mice. An inducible whole-body deletion of *Des1* in both lean and obese (leptin-deficient) mice altered the energy balance. Finally, an adeno-associated vector containing a small hairpin RNA, targeting the *Des1* mRNA for degradation, improved the metabolic measures in mice that either were already obese or were of normal weight but subsequently administered a high-fat diet. An added benefit of preventing obesity was observed in the latter group of mice. In summary, the deletion of *Des1* improved insulin signaling and mitochondrial function and decreased lipogenesis and lipid uptake. Altogether, although there was no direct evidence of inhibition of *Des1* preventing atherosclerosis, it is reasonable to hypothesize

DES1 as an effective target for normalizing vascular homeostasis by controlling ceramide production.

Ceramidases (CDase)

CDase deacylates ceramide to produce sphingosine that can, in turn, be phosphorylated by sphingosine kinase to produce sphingosine 1-phosphate (S1P). S1P often opposes the ceramide action, leading researchers to propose the existence of a ceramide:S1P rheostat that controls cellular responses (Hait *et al.*, 2006). Ceramidases, which can sometimes catalyze the reverse reaction to convert sphingosine back into ceramide, can be distinguished by their pH optima (Kolesnick, 2002). Collectively, ceramidases are ubiquitously distributed throughout the cellular membranes and also secreted into the extracellular milieu.

Inhibitors targeting the process of ceramide biosynthesis are potential means for the treatment of metabolic syndrome. Hence, we can presume that promoting ceramide degradation may provide similar benefits. Deacylation of ceramide species is initiated by a family of enzymes called the CDase. Chavez *et al.* (2005) demonstrated that overexpression of CDase negated the inhibitory effects of exogenous free fatty acids on muscle insulin sensitivity by blocking ceramide accumulation *in vitro*. Holland *et al.* (2011) found that adiponectin, a protein hormone with antidiabetic and cardioprotective properties, could stimulate CDase activity and further lower the concentration of cellular ceramides. CDase was found to have some homology with the adiponectin receptors, AdipoR1 and AdipoR2. From *in vivo* studies, it was found that targeted induction of ceramide degradation in adipose tissue or liver by overexpressing transgenic CDase was sufficient to recapitulate most adiponectin actions (Xia *et al.*, 2015). Moreover, overexpression of AdipoR1 or AdipoR2 in either the adipocyte or hepatocyte resulted in enhanced CDase activation, improved hyperglycemia and glucose intolerance, and inhibition of hepatic steatosis (Holland *et al.*, 2017). Together, adiponectin exerted its metabolic improvement functions through CDase signaling (Reibe-Pal and Febbraio, 2017). These findings support the strategy of CDase replacement as a potential treatment for atherosclerosis.

Sphingomyelinase (SMase)

SMases are also known to be implicated in atherogenesis. Mice deficient in the acid sphingomyelinase (ASMase) displayed 40-50% reduction in early foam cell formation and 87% reduction in lipoprotein trapping in ApoE^{-/-} mice (Devlin *et al.*, 2008). ASMase can be secreted and further activated after binding to the matrix (collagen, fibronectin, laminin, and heparin), (Marathe *et al.*, 1999). Furthermore, ASMase not only regulated inflammation and apoptosis in the macrophages (Jin *et al.*, 2013; Zhao *et al.*, 2016), but also played an important role in cholesterol trafficking and efflux (Jin *et al.*, 2013). Inhibition and deficiency of neutral sphingomyelinase (NSMase) (by GW4869) also decreased the inflammatory response by oxidized LDL and had significantly less ICAM1, VCAM1, MCP-1 expressions, which are chemo-attractants of macrophage/monocytes, leading to reduction in atherosclerosis development (Lallemand *et al.*, 2018). The deficiency of sphingomyelin synthases 1 and 2 (SMS1, SMS2) prevented atherogenesis (Liu *et al.*, 2009; Li *et al.*, 2012). Mice deficient in SMS1 had attenuated toll-like receptor 4 (TLR4)-mediated NF- κ B activation. SMS2-deficient mice showed reduced cho-

lesterol efflux and reduction in the levels of interleukin-6 and tumor necrosis factor α . Further, SMS2-deficient mice had significantly less necrotic core area, less macrophage infiltration, and more collagen content in the atherosclerotic lesions. SMS2 deficiency attenuated NF- κ B activation, suggesting that SMS2 plays a critical role in inflammation (Hailemariam *et al.*, 2008). Conversely, SMS2 overexpression by AAV (adenovirus-associated virus) increased the atherosclerotic lesions and inflammation (Wang *et al.*, 2011; Zhao *et al.*, 2012). SMS1 and SMS2 overexpression by AAV increased non-HDL cholesterol and non-HDL-SM, serum ApoB, and hepatic SR-B1 expression, suggesting increased atherogenic potential (Dong *et al.*, 2006). Taken together, even though the protective mechanisms in atherogenesis are not understood, SMase and SMS could be therapeutic strategies against atherosclerosis generation.

THE ROLE OF S1P IN ATHEROSCLEROSIS

S1P

S1P is a bioactive lipid, primarily carried by apolipoprotein M (apoM) on HDL and signals its G-protein-coupled receptors, named S1PR1-5 (Hla and Dannenberg, 2012). S1P is degraded by two pathways: dephosphorylation by S1P phosphatases (SPP1/2) and irreversible cleavage by S1P lyase (SPL). The roles of S1P in the fields of atherosclerosis have been demonstrated in many elegant reports. In general, S1P possesses protective roles in atherosclerosis, but it can also exert harmful effects during the pathogenesis of atherosclerosis.

S1P receptors

Various reports have investigated the physiological roles of S1P and S1P receptors in the pathogenesis of atherosclerosis using animal experiments. For example, S1PR3 knockout mice exhibited resistance to the protective properties of HDL or S1P in a model of coronary infarction (Theilmeier *et al.*, 2006), while increased recruitment of monocyte/macrophage and neointima formation was caused by carotid artery ligation in S1PR3 knockout mice (Keul *et al.*, 2011). S1PR2 knockout mice developed atherosclerotic lesions to a lesser degree when they had a background of apoE deficiency and reduced macrophage recruitment (Skoura *et al.*, 2011). Although S1PR1 knockout mice are embryonically lethal (Liu *et al.*, 2000), pharmacological experiments showed that an S1PR1 agonist protected the LDL receptor knockout mice from atherosclerosis (Poti *et al.*, 2013). S1P preserves the endothelium via S1PR1/3 (Kurano and Yatomi, 2018), inhibits the migration of smooth muscle cells via S1PR2, and shows anti-inflammatory properties via S1PR4 (Fettel *et al.*, 2019). However, S1P also promotes inflammatory monocyte/macrophage recruitment through S1PR2/3 (Michaud *et al.*, 2010; Keul *et al.*, 2011). Together, these reports suggest that S1PR1 and S1PR3 mainly exert anti-atherosclerotic properties, while S1PR2 exerts pro-atherosclerotic properties. Further studies are required to investigate the involvement of S1P receptors in the pathogenesis of atherosclerosis.

The S1P receptor modulator FTY720 was approved by the FDA, USA and is now used for treating multiple sclerosis (Keul *et al.*, 2007; Wang *et al.*, 2014). It was successful in reducing atherosclerotic lesions, macrophage infiltration, and collagen content (Keul *et al.*, 2007). FTY720 treatment also showed

a reduction in the infarct size in cardiac ischemia-reperfusion injury (Wang *et al.*, 2014). It increased the levels of Niemann-Pick C1 protein and ATP-binding cassette transporter A1 protein, further increasing the efflux of endosomal cholesterol to apolipoprotein A1. Moreover, it decreased the cholesterol toxicity in macrophages by reducing the delivery of cholesterol to the ER (Blom *et al.*, 2010).

Sphingosine kinases

Regarding the effects of S1P levels, a sphingosine kinase (SphK) inhibitor has been demonstrated to suppress atherosclerotic lesions in LDL receptor knockout mice that were fed a high cholesterol diet, but not in mice that were fed normal chow (Poti *et al.*, 2015). Contrary to the possible anti-atherosclerotic properties of lowering S1P using an SphK inhibitor, overexpression of apoM in the LDL receptor-knockout mice and apoE-knockout mice protected the mice against atherosclerosis, although the association with S1P was not mentioned (Wolfrum *et al.*, 2005). These results suggest that S1P bound to apoM might possess distinct properties from S1P bound to albumin in the pathogenesis of atherosclerosis.

Clinical correlation of plasma S1P and CAD

Several clinical data reported that plasma S1P concentrations are negatively associated with the prevalence and severity of CAD and myocardial infarction. At present, only four studies have shown an association between plasma S1P levels and atherosclerotic diseases (Knapp *et al.*, 2009; Sattler *et al.*, 2010; Knapp *et al.*, 2013; Sattler *et al.*, 2014) (Table 2). Among them, Sattler *et al.* (2010) reported that S1P uncoupled from HDL was more prevalent than the bound form in subjects with myocardial infarction and stable angina. This supports the idea that S1P bound to apolipoprotein M (apoM) possesses anti-atherosclerotic properties while S1P bound to albumin might somehow be involved in the harmful effects of S1P. Other clinical studies have also demonstrated that plasma S1P levels were lower in patients with myocardial infarction (Knapp *et al.*, 2009, 2013) and that S1P bound to HDL might predict the severity of coronary heart disease (Sattler *et al.*, 2014). Although an association between the total apoM level and atherosclerosis is not shown (Takahashi *et al.*, 2017), apoM polymorphism is reported to be associated with atherosclerosis (Jiao *et al.*, 2007; Xu *et al.*, 2008). Further

studies are required to elucidate the involvement of apoM in human atherosclerosis.

THE ROLE OF OTHER SPHINGOLIPIDS IN ATHEROSCLEROSIS

Glycosphingolipids and atherosclerosis

Glycosphingolipids are extremely diverse. They are composed of hydrophobic ceramide scaffolds and hydrophilic sugar chains. Their glycosyl groups are different, such as D-glucose, D-galactose, D-acetylglucosamine, D-acetylgalactosamine, L-fucose, D-mannose, and sialic acid. Sphingoglycolipids are generally divided into cerebrosides, sulfatides, globosides, and gangliosides. According to the number of glycosides, they are divided into monohexosylceramide (MHC), dihexosylceramide (DHC), trihexosylceramide (THC), and tetrahexosylceramide.

The following observations led to the association of glycosphingolipids with atherosclerosis: (a) gangliosides (Breckenridge *et al.*, 1975), especially ganglioside GM3, glucosylceramide (GluCer), and lactosylceramide (LacCer) accumulate in the atherosclerotic plaques (Mukhin *et al.*, 1995; Bobryshev *et al.*, 1997; Chatterjee *et al.*, 1997; Gracheva *et al.*, 2009). (b) GluCer and LacCer not only stimulate the proliferation of aortic smooth muscle (Bhunja *et al.*, 1997), but also suppress apoE production in macrophages and cholesterol-loaded foam cells (Garner *et al.*, 2002). (c) LacCer stimulates the recruitment of monocytes to the endothelium (Gong *et al.*, 2004). (d) GM3 stimulates monocyte differentiation into macrophages and increases the uptake of LDL in macrophages (Prokazova *et al.*, 1991; Gracheva *et al.*, 2009). Recently, specific plasma glycosphingolipids such as DHC, GluCer, and LacCer were identified as discriminatory risk-associated lipids for unstable CAD and CAD mortality (Meikle *et al.*, 2011; Tarasov *et al.*, 2014; Cheng *et al.*, 2015a). Li *et al.* (2014) successfully separated the isomer pair of galactosylceramide (GalCer) and GluCer. They showed that plasma GalCer levels, and not GluCer levels, had increased in atherosclerotic patients. In animal studies, inhibition of glycosphingolipid synthesis inhibited atherosclerosis development (Bietrix *et al.*, 2010; Chatterjee *et al.*, 2014). Numerous distinct glycosphingolipid species exist, making it difficult to determine the one that is critical for the

Table 2. Clinical studies investigating the diverse roles of S1P as a cardiometabolic biomarker

Year	Population	Correlating lipid (↑, higher; ↓, lower)	Clinical end point	Reference
2003	CAD patients (n=318)	↑ S1P	CAD	Deutschman <i>et al.</i> , 2003
2009	MI patients (n=22)	↓ S1P	MI	Knapp <i>et al.</i> , 2009
2010	CAD patients (n=178)	↓ HDL-bound S1P ↑ non HDL-bound S1P	CAD and MI	Sattler <i>et al.</i> , 2010
2011	CCHS (n=95)	↓ HDL-bound S1P ↓ Dihydro-S1P and ceramide (C24:1)	CAD	Argraves <i>et al.</i> , 2011
2013	MI patients (n=32)	↓ S1P	MI	Knapp <i>et al.</i> , 2013
2014	CAD patients (n=59)	↓ HDL-bound S1P	CAD	Sattler <i>et al.</i> , 2014
2017	Patients with ischemic heart disease	↓ S1P and sphingomyelins	Reduced left ventricular ejection fraction	Polzin <i>et al.</i> , 2017

CAD, coronary artery disease; CCHS, Copenhagen City Heart Study; MI, myocardial infarction.

onset of atherosclerosis. Therefore, inhibition of glycosphingolipid synthesis may be an effective approach for the treatment of atherosclerosis.

CONCLUSION

Sphingolipids are a large class of lipids with varied structural and signaling roles. Their levels are increased in atherosclerotic lesions (Smith, 1960; Zilversmit *et al.*, 1961; Portman and Alexander, 1970; Manicke *et al.*, 2009). Researchers have significantly improved knowledge of the diverse mediators of obesity-related diseases. Several enzymes involved in the different pathways of ceramide metabolism, such as SPT, CerS family, or the DES, can be potential targets to manipulate ceramide generation. Nowadays, genetic inhibition of enzymes that control the global ceramide synthesis is more often considered an attractive therapeutic target for the treatment of metabolic diseases and obesity (Adams *et al.*, 2004; Gault *et al.*, 2010; Chavez *et al.*, 2012; Chaurasia and Summers, 2015). It is worth noting that ceramides participate in various cellular processes, so there exists a risk of negative effects of the inhibition of systemic sphingolipid synthesis. It seems to be extremely important to determine the enzymes in the ceramide pathway that participate in metabolic disease progression.

New data suggest a pro-atherogenic role for sphingolipids that might be specifically significant in the scenario of the metabolic syndrome. Sphingolipid metabolism can be regulated at the level of LDL absorption, lipoprotein transport, *de novo* synthesis, and turnover. Increased levels of the sphingolipids in the plasma and the vascular wall are strongly associated with atherosclerosis in the patients as well as experimental models. Intracellular ceramides may exert their pro-atherogenic activities via the stimulation of apoptosis, stress signaling, and cholesterol synthesis. The ceramide derivative S1P may, in part, counterbalance the ceramide activities via antiapoptotic and pro-proliferative signaling. S1P itself exists in the extracellular form (primarily in association with HDL) and as an intracellular messenger that may exert both pro- and anti-atherogenic signaling. Various targets along the sphingolipid pathways provide numerous opportunities for drug discovery. In future, those efforts may yield compounds that can be used as a combination therapy with lipid-lowering agents and/or as a stand-alone therapy to prevent the progression of metabolic syndrome towards atherosclerosis.

CONFLICT OF INTEREST

The authors declare that they have no conflicting interest.

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