Targeting Sphingolipid Metabolism in Atherosclerosis

Literature Review

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Contents

Abstract

Cardiovascular diseases (CVDs) are by far the leading cause of death due to non-communicable diseases in the world. The development of CVDs in many cases is caused by atherosclerosis. In atherosclerosis, plaques in blood vessels are formed by the excessive accumulation of lipids in the endothelial lining. Besides triglycerides and cholesterol, these atherosclerotic plaques also consist of bioactive lipids which have signaling roles in addition to structural and energetic functions. Among these bioactive lipids, sphingolipids are especially damaging to the blood vessels. A large number of studies suggest that sphingolipids contribute to the development of atherosclerosis by disrupting arterial function and inducing pro-inflammatory pathways. Discoveries in the metabolism of sphingolipids have elucidated several potential therapeutic targets in treating atherosclerosis. These targets focus on lowering sphingolipid levels through several strategies in order to alleviate atherosclerosis. In this review, we present a brief background on the metabolism of sphingolipids and the most recent findings on their contribution to atherosclerosis. Moreover, we give an overview of therapeutic targets in sphingolipid metabolism to treat atherosclerosis and discuss their feasibility.

Introduction

Cardiovascular disease (CVD) is the leading cause of death due to non-communicable disease according to the latest report of the World Health Organization (WHO) (Arnold et al., 2022). A main underlying issue in CVD development is atherosclerosis. Excessive lipid deposition in non-adipose organs, such as blood vessels and the heart, results in the formation of atherosclerotic plaques (Cornier et al., 2011). These plaques restrict blood flow in the cardiovascular system and can result in plaque rupture, ultimately leading to heart ischemia and death.

Besides triglycerides and cholesterol, lipid aggregates also consist of bioactive lipids (Chiurchiù et al., 2018), which have signaling roles in addition to structural and energetic functions. (Nishizuka, 1992; Smith et al., 2000). Among bioactive lipids, sphingolipids are particularly damaging to blood vessels and heart tissue when accumulated (Bielawska et al., 1997; H. Li et al., 2002).

Sphingolipids represent one of the major classes of lipids in eukaryotic cells and consist of a wide range of lipids with highly diverse functions (Hla, 2004; Obeid et al., 1993; Venable et al., 1995). Among these lipids, ceramides play a central role as they are precursors of the most complex sphingolipids. Plasma levels of complex sphingolipids and ceramides are positively correlated with CVDs and disruption of sphingolipid metabolism has revealed new therapeutic targets (Yu et al., 2019). Besides apoptosis of cardiovascular tissue, ceramides and complex sphingolipids also contribute to arterial dysfunction in CVDs (Zhang et al., 2003; Zheng et al., 2000).

In the field of cardiovascular drug development, the focus has been mainly put on lipid-lowering and revascularization treatments, overlooking mechanism-based therapies (MacRae et al., 2016). Mechanism-based therapies target more underlying components of CVDs such as lipid metabolism and inflammation in contrast to result-oriented therapies which focus on symptoms of CVD. The role of sphingolipids in CVD development makes them an interesting target for these necessary novel therapeutic avenues.

In this review, we first present a brief background on the metabolism of sphingolipids and put their contribution to atherosclerosis development into perspective. Building upon these findings, we then give an overview of the therapeutic targeting of sphingolipid metabolism in relation to atherosclerosis.

Sphingolipid metabolism

Sphingolipid metabolism

The sphingolipid class consists of lipids with vastly diverse functions. All of these sphingolipids are metabolically connected by a set of enzymes that catabolize, recycle and interconvert sphingolipids into one another, through different intermediates(Bandhuvula & Saba, 2007; Ichikawa & Hirabayashi, 1998; Wijesinghe et al., 2005). In this sub-section, we discuss the *de novo* synthesis and interconversion of sphingolipids.

De novo synthesis

De novo sphingolipid synthesis starts with the condensation of serine and the lipid palmitate into sphingoid backbones, catalyzed by a serine palmitoyl transferase (SPT) enzyme complex (Fig. 1). After this initial reaction, a multitude of enzymes converts the backbones into dihydroceramide. Dihydroceramides are ceramide pre-cursors in *de novo* synthesis formed by ceramide synthases (CERS1-CERS6). The different synthases vary in the tissue of residence and substrate specificity, leading to differences in sphingolipid profiles across cells and tissues. Lastly in *de novo* synthesis, dihydroceramide is converted into ceramide by dihydroceramide desaturases (DES1-2). Similar to CERS, DES1 and DES2 are expressed differently across tissues, with DES1 being expressed in most tissues (Causeret et al., 2000).

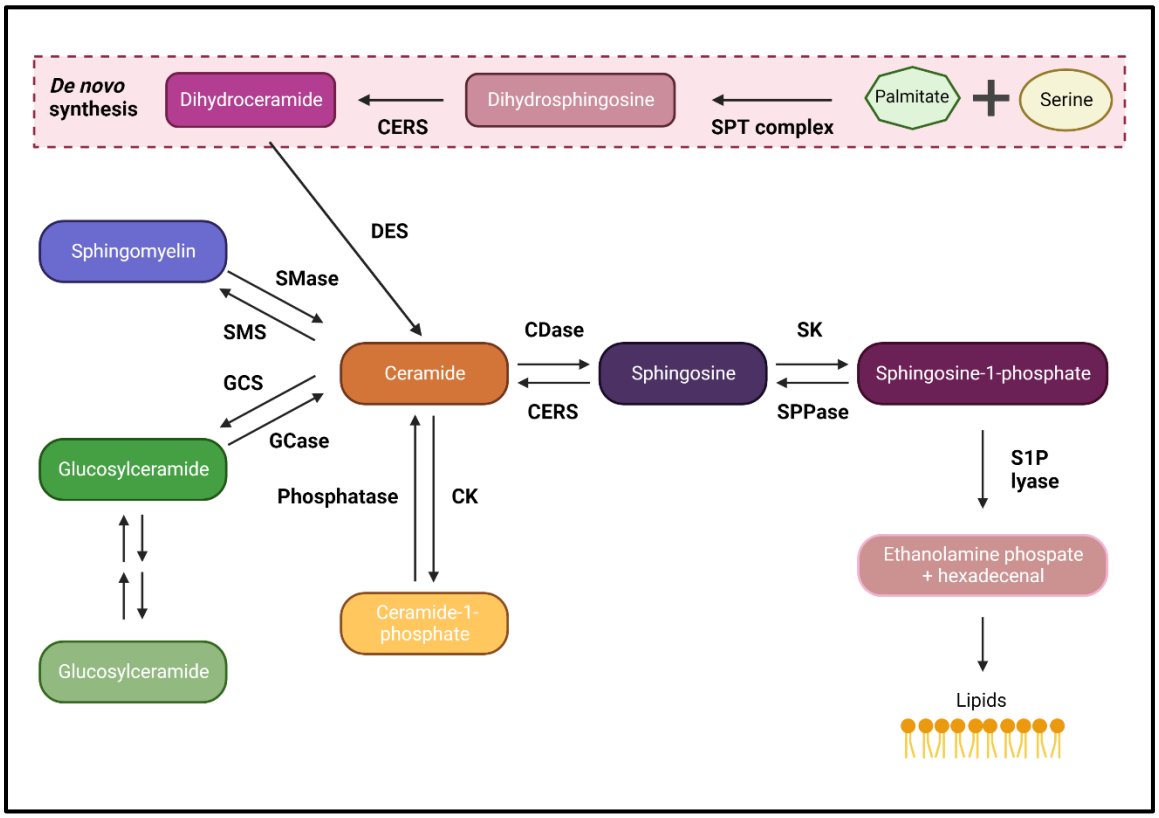

Figure 1. Sphingolipid metabolism and their interconnection. Ceramides are at the center of sphingolipid metabolism and are initially synthesized *de novo* through the formation of sphingoid backbones. These backbones are consequently converted by ceramide synthases (CerS) and desaturases (DES) to form ceramides. Ceramides are the central intermediate sphingolipid in the interconversion of complex sphingolipids by a multitude of enzymes.

Interconversion of complex sphingolipids

Within sphingolipid metabolism, ceramides act as the central intermediate sphingolipid, generating several complex sphingolipids. These sphingolipids have many diverse functions, including roles in cell growth, death, migration, and adhesion. The complex sphingolipids are interconnected by a set of enzymes that convert them into one another (Fig. 1). The interconversion of sphingolipid levels allows cells to coordinate cellular responses according to their needs. For example, while ceramide promotes apoptosis, S1P activates cell survival pathways (Cuvillier et al., 1996; Juchnicka et al., 2021). Therefore, the ratio of ceramide/S1P fluctuates to dynamically regulate apoptosis versus growth through (in)activation of interconverting enzymes (Newton et al., 2015; Pchejetski et al., 2007).

Studies of enzymes and pathways in sphingolipid metabolism have revealed the roles of sphingolipids in disease development and potential therapeutic avenues. Specifically in cardiovascular diseases, ceramides and other sphingolipids have been linked to their development.

Sphingolipids as drivers of atherosclerosis

While sphingolipids play a beneficial role in a healthy cardiovascular system, they can play a harmful role in the pathogenesis of atherosclerosis. In the following paragraphs, we discuss the pathogenesis of atherosclerosis and the role of sphingolipids in this process.

Atherosclerosis

Atherosclerosis development initially involves lipoprotein retention in the inner layer of arteries that are subjected to low shear stress and disturbed blood flow (Davies, 1995) (Figure 2). These mechanical stimuli and retained lipoproteins, such as low-density lipoproteins (LDL), activate signaling pathways, leading to a pro-apoptotic and pro-inflammatory endothelium lining (Gimbrone & García-Cardeña, 2013; Zeng et al., 2009). The activated endothelial cells (ECs) recruit several immune cells, among which monocytes that infiltrate the vascular intima (Galkina & Ley, 2007; Gerszten et al., 1999). These infiltrated monocytes differentiate into macrophages and internalize lipoproteins (Brown & Goldstein, 1983). Subsequently, they become macrophage foam cells and activate inflammatory signaling pathways, leading to more leukocyte recruitment and LDL uptake(Colin et al., 2014; Peled & Fisher, 2014). The infiltration of all these activated leukocytes results in the progression of atherosclerotic lesions, becoming more advanced when untreated. These lesions become more advanced as vascular smooth muscle cells (VSMCs) also start to infiltrate and proliferate in the vascular intima, due to chemoattractants and growth factors secreted by the activated macrophages (Johnson, 2007; Raines, 2004).

As a defense mechanism, accumulated VSMCs in the vascular intima generate extracellular matrix to form a fibrous cap covering macrophage foam cells, protecting the blood from prothrombotic factors, and limiting thrombosis. Although the barrier may protect the blood from clotting, the inflammatory environment of the atherosclerotic lesion remains unresolved. Foam cells start to become susceptible to apoptosis and enhance the secretion of inflammatory components, resulting in a necrotic core. A necrotic core is a space within the lesion composed of leaked oxidative and inflammatory components that thin the fibrous cap and make it prone to rupture. Plaque rupture can lead to thrombus formation and ultimately infarction.

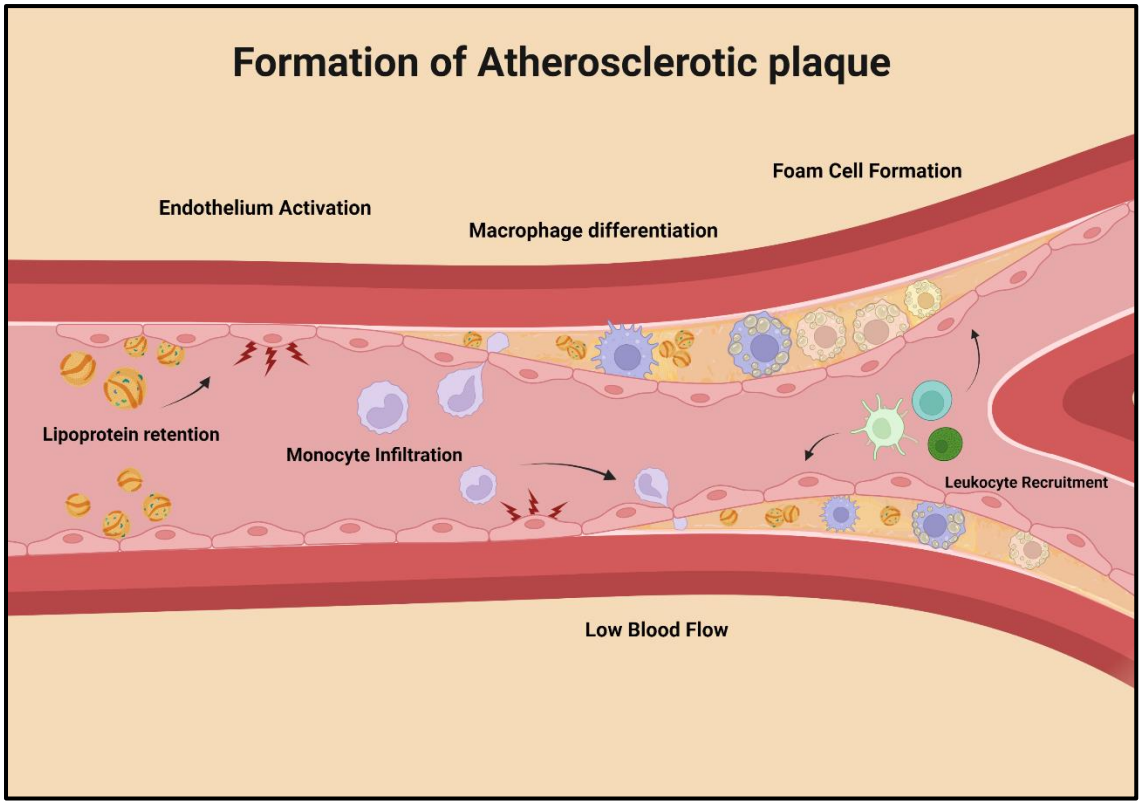

Figure 2. Development of atherosclerotic plaques. Atherosclerosis progression begins with dysfunctional endothelial cells (ECs) and the retention of lipoproteins, which consequently activate ECs. These activated ECs recruit monocytes that infiltrate the endothelial layer and differentiate into macrophages which internalize the retained lipoproteins. In turn, these macrophages become foam cells and activate inflammatory signaling pathways, resulting in more leukocyte recruitment and lipoprotein uptake.

Sphingolipids in relation to atherosclerosis development

Among sphingolipids, ceramides appear to be the most correlated with atherosclerosis development. Several studies have shown that the previously mentioned ceramide/S1P dynamic regulation is also apparent in vascular ECs (Wende et al., 2012). In blood vessels, ceramides decrease the generation of nitric oxide (NO) (Bharath et al., 2015). NO promotes EC proliferation, which along with its effects on other cells (VSMCs and platelets), inhibits intimal hyperplasia (Lei et al., 2013). Limited NO bioavailability, as a consequence of high ceramide levels, leads to increased EC dysfunction and apoptosis, which in turn, will result in vascular dysfunction and an increased risk of atherosclerosis (Matthys & Bult, 1997; Symons & Abel, 2013). These studies indicate that ceramides contribute to mechanical stimuli, low shear stress and disturbed blood flow, which lead to dysfunctional endothelium and in turn to atherosclerotic lesions. Additionally, ceramides accumulate in atherosclerotic plaques and are associated with and possibly induce plaque inflammation (Edsfeldt et al., 2016). In contrast to ceramides, S1P is a regulatory sphingolipid with several atheroprotective properties, such as inhibiting EC inflammation and promoting vasorelaxation (Igarashi & Michel, 2009; Jozefczuk et al., 2020; Kennedy et al., 2009). Feuerborn et al. showed that the elevation of S1P alleviates atherosclerosis in mice through these properties.

Glycosphingolipids, specifically glucosylceramide and lactosylceramide, also appear to be associated with atherosclerosis development. Similar to ceramides, these glycosphingolipids accumulate in plaques (S. B. Chatterjee et al., 1997) and are strongly correlated with levels of proinflammatory cytokines (Edsfeldt et al., 2016). Lactosylceramide also stimulates the recruitment of monocytes to the endothelium (Gong et al., 2004), adding to the argument that glycosphingolipids contribute to the pro-inflammatory environment of atherosclerotic lesions.

In summary, ceramides and glycosphingolipids contribute to the mechanical stimuli and pro-inflammatory environment, respectively, which leads to the development of atherosclerotic lesions.

Approaches to modulate Sphingolipid metabolism

The discoveries made in regard to sphingolipid metabolism and its role in atherosclerosis resulted in the elucidation of potential therapeutic targets. Ceramides and glycosphingolipids in particular appear to contribute to atherosclerosis development. In this sub-section, we give an overview of approaches to modulate sphingolipid metabolism by targeting ceramides and glycosphingolipids (Fig. 3).

Disruption of *de novo* ceramide synthesis

As previously mentioned, *de novo* ceramide synthesis starts with the condensation of serine and palmitate into sphingoid backbones, catalyzed by an SPT enzyme complex. These backbones are converted by CERS and DES, respectively, into ceramides. Several studies show that inhibiting *de novo* ceramide synthesis by targeting its involved enzymes alleviates atherogenic processes (Park et al., 2004; Chaurasia et al., 2019) .

Park et al. first presented that myriocin, an SPT inhibitor, lowers plasma lipid levels and significantly improved the anti-atherogenic activity in an atherosclerosis mouse model ($ApoE^{-/-}$). Myriocin specifically reduced levels of ceramides and sphingomyelin, leading to cholesterol and triglyceride lowering, resulting in the inhibition of early atherosclerosis development. Further studies also showed that myriocin also reduced plasma glycosphingolipid levels (Glaros et al., 2007).

Other targets in *de novo* ceramide synthesis are ceramide synthases (CERS), which convert sphingoid backbones into dihydroceramide (Fig. 3). In particular, CERS6 appears to be an interesting target since its expression is positively correlated with obesity, a risk factor for atherosclerosis (Turpin et al., 2014). In the same study, the researchers showed that CERS6 inhibition protected mice from high-fat-induced obesity, highlighting it as an approach to treating obesity. However, the effects of this intervention on atherosclerosis are still unknown. CERS6 is also involved in the conversion of sphingosine to ceramide within the complex sphingolipids (Fig. 1), thus inhibition might also have an effect on the metabolism of complex sphingolipids.

Another approach of disrupting *de novo* synthesis is to target dihydroceramide desaturases (DES), which convert dihydroceramide into ceramide (Fig. 3). As previously mentioned, DES1 is the major desaturase in most tissues, including the heart and vessels. Inhibition of DES1 in mice with a chemical inhibitor (fenretinide) or through genetic modification prevents the elevation of lipid levels and vascular dysfunction, respectively (Mody & Mcilroy, 2014; Q.-J. Zhang et al., 2012). Unfortunately, fenretinide treatment worsens atherosclerosis in ApoE$^{-/-}$ mice. While lipid levels were reduced, inflammation was enhanced resulting in aggravated atherosclerosis. Although this effect is driven by the retinoid actions of the inhibitor and thus independent of DES1 inhibition. (Busnelli et al., 2020). Genetically inhibiting DES1 did not show these adverse reactions and appears to be a more promising approach to alleviate the risk of atherosclerosis (Chaurasia et al., 2019).

Taken together, disruption of *de novo* synthesis can be a potential therapeutic avenue in treating atherosclerosis development, however, it needs to be balanced delicately in order to realize it. Sphingolipids serve essential cellular functions and lowering their overall levels through this therapeutic approach might have a detrimental effect on the patient's health. For example, myriocin, along with other SPT inhibitors, is reported to be toxic to the gut and liver (Genin et al., 2016; Salaun et al., 2016). Enzymes downstream in *de novo* synthesis, CERS, and DES, appear to be promising targets, however, additional studies are needed to determine whether their inhibition affects atherosclerosis development.

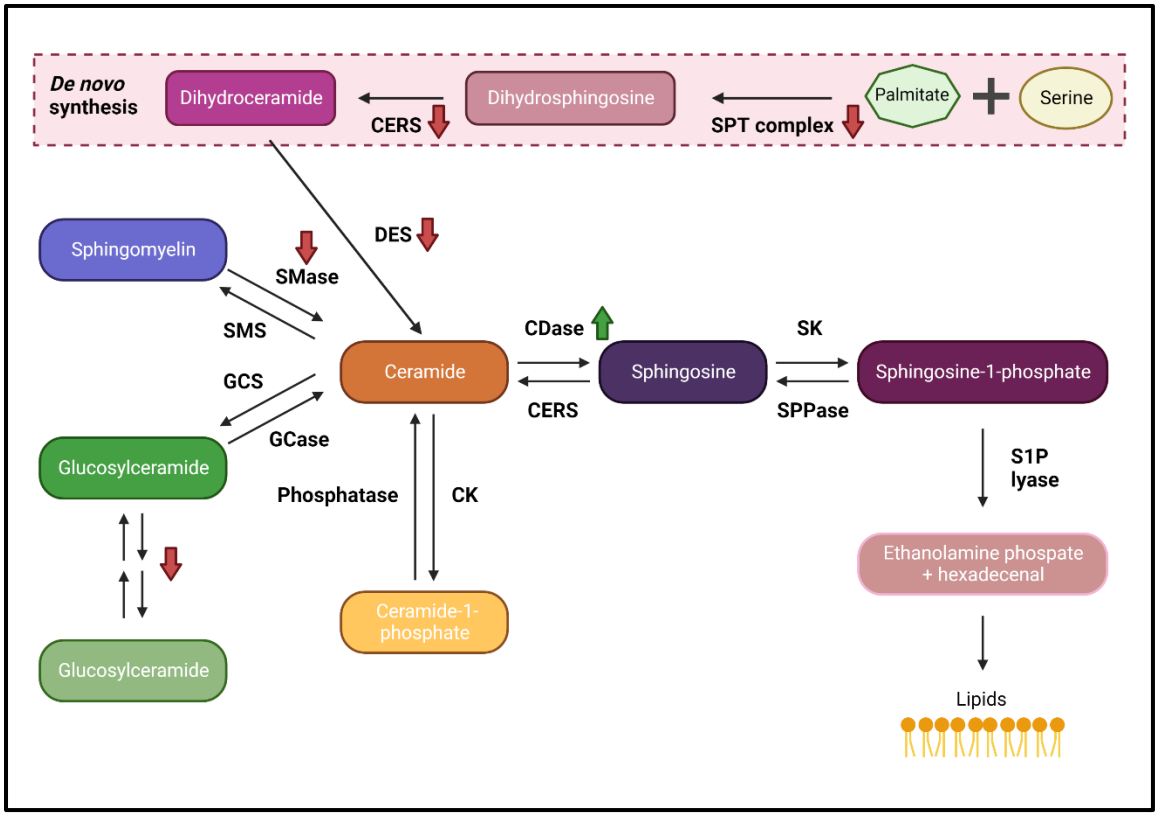

Figure 3. Overview of modulations within sphingolipid metabolism. The activity of several enzymes within sphingolipid metabolism can be regulated to disrupt *de novo* synthesis and interconversion. Inhibition of enzymes involved *de novo* synthesis (SPT, CERS and DES) leads to decreased overall sphingolipid levels. Furthermore, complex sphingolipid levels can be shifted by regulating enzymes that interconvert these sphingolipids.

Inhibition of (complex) sphingolipid metabolism

In contrast to the disruption of *de novo* synthesis, the inhibition of complex sphingolipid metabolism affects sphingolipid levels in a more controlled manner. The sphingolipid metabolism can be affected by altering the activity of enzymes that interconvert sphingolipids and their intermediates. This results in a shift in sphingolipid balances rather than a decrease in the overall sphingolipid level.

Ceramides contribute to atherosclerosis development by disturbing endothelial cell function, resulting in vascular dysfunction. In healthy patients, ceramide levels are maintained within normal range through the dynamically regulated ceramide/S1P balance. Within this regulation, ceramidases (CDase) and sphingosine kinase (SK) are key enzymes in converting ceramide into S1P (Fig. 3). Targeting this pathway within sphingolipid metabolism is specifically interesting for treating atherosclerosis as it leads to both reduced ceramide levels and generation of S1P. Multiple studies presented that CDase, which produces the precursor of S1P (Fig. 3), can be stimulated through adiponectin (Holland et al., 2011; Reibe-Pal & Febbraio, 2017). These findings support the generation of S1P as a promising therapeutic strategy for treating atherosclerosis.

Besides S1P generation, ceramide levels can also be lowered by targeting the conversion of sphingomyelin to ceramides. Within sphingolipid metabolism, sphingomyelinase (SMase) converts sphingomyelin to ceramides in order to generate a supply of ceramide (Fig. 3). Several studies have shown that inhibition of sphingomyelinase through the use of chemical inhibitors or genetic modification reduces atherosclerosis in atherosclerosis-prone mice (Fan et al., 2010; Lallemand et al., 2018; Lu et al., 2020). These inhibitions suppress atherosclerosis by alleviating the associated mechanical stimuli and its pro-inflammatory features. Although chemical inhibitors and genetic modifications of sphingomyelinase in these studies are potent against atherosclerosis, adverse effects, such as cerebral dysfunction, were observed throughout. The adverse effects occurred due to the systemic inhibition of sphingomyelinase. These difficulties could be overcome by utilizing a targeted approach to inhibit sphingomyelinase in endothelial cells and monocytes.

In sphingolipid metabolism, ceramides can be glycosylated by enzymes, such as glucosylceramide, to form glycosphingolipids. As previously mentioned, glycosphingolipids contribute to the development of atherosclerosis, specifically to the pro-inflammatory environment. Among glycosphingolipids, glucosylceramide and lactosylceramide are key contributors to atherosclerosis development. Their generation can be inhibited by D-PDMP, an inhibitor of their corresponding synthases, and can reduce atherosclerosis in ApoE-knockout mice (S. Chatterjee et al., 2014). D-PDMP treatment also alleviated arterial stiffness within these mice.

In summary, the elucidation of sphingolipid metabolism and their interconnection has revealed many potential therapeutic targets in treating atherosclerosis. However, further research is necessary to realize these strategies in the field of pharmacology. The aforementioned therapeutic strategies either lacked further research or exhibited adverse effects. These adverse effects occurred due to an uncontrolled loss of essential sphingolipid functions. Difficulties in uncontrolled therapeutic effects can be overcome by the use of a targeted approach, such as targeted drug delivery.

Use of (targeted) drug delivery to modulate sphingolipid metabolism

Targeted drug delivery can be applied in several ways to administer therapeutics in a controlled manner. One of which is nanomedicine, an application of targeted drug delivery that allows for the controlled and targeted release of therapeutics via nanoparticles (NPs). NPs can be used as efficient drug carriers to deliver therapeutics to specific tissues and cells with the use of a targeting moiety. Within the context of atherosclerosis, these targets can be cells that play a significant role in the disease's development, such as endothelial cells and macrophages. These two cell types are major drivers of atherosclerosis as they are the main cause of the pro-inflammatory environment which can ultimately lead to plaque rupture and infarction.

Several active targeting strategies for atherosclerosis have been studied over the past decade as nanomedicine has emerged in the field of pharmaceutics. These strategies target the key players in atherosclerosis.

Endothelial cells (ECs) in atherosclerotic plaques over-express different adhesion molecules (e.g. intracellular adhesion molecule-1 (ICAM-1) and selectins (Khodabandehlou et al., 2017), an important surface molecule in inflammation. Serrano et al. present an approach to actively target ECs by using an antibody against ICAM-1. In this study, anti-ICAM1 was conjugated to polystyrene particles and successfully internalized by ECs through cell adhesion molecule-mediated endocytosis. Furthermore, particles targeting E-selectins through a thioaptamer molecule can also be taken up ECs (Ma et al., 2016).

Besides endothelial cells, macrophages also play an important role in the formation and progression of atherosclerosis. For this reason, macrophages have been considered extensively as a target in treating atherosclerosis. One of many approaches for actively targeting macrophages is binding to osteopontin (OPN), a cytokine expressed by foamy macrophages to recruit leukocytes (Scatena et al., 2007). An antibody for this cytokine has been used for imaging vulnerable atherosclerotic plaques and could facilitate targeted drug delivery to foam macrophage cells (Qiao et al., 2017). An additional molecule for targeting apoptotic macrophages is annexin v, a protein that can target phosphatidylserine present on the membrane of apoptotic macrophages in plaques (X. Li et al., 2016).

The aforementioned moieties may facilitate drug delivery to endothelial cells and macrophages heavily involved in atherosclerosis development. However, these cells have a short lifespan relative to the chronic inflammation present in atherosclerosis. The therapeutic effect would be short-lived and better results might be achieved when targeting longer-lived cells. In contrast to macrophages, hematopoietic stem and progenitor cells (HSPCs) have a significantly longer lifespan. HSPCs are progenitors of all myeloid cells, including macrophages, and reside within the bone marrow. van Leent et al. present a high-density lipoprotein (HDL) NP with high bone marrow uptake and affinity for myeloid cells and their progenitors. This nanoparticle targets myeloid cells and could induce long-lived therapeutic effects, efficiently treating the chronic inflammation associated with atherosclerosis.

The use of nanomedicine allows for controlled drug release and has the potential to efficiently treat atherosclerosis. With this application, promising therapeutic targets in sphingolipid metabolism can be realized in the field of pharmacology circumventing the adverse effects caused by their uncontrolled actions. In addition to controlled drug release, nanomedicine also provides the opportunity to actively target cells that play a key role in atherosclerosis development.

Conclusion

Cardiovascular diseases are a major cause of death worldwide and atherosclerosis is the main underlying mechanism of these diseases. Atherosclerotic plaques consist of various lipid species among which sphingolipids elicit the pro-inflammatory environment and vascular defects in atherosclerosis. Out of the sphingolipid class, increased ceramide levels are found to contribute to the development of atherosclerosis in several ways. Discoveries made in regard to sphingolipid metabolism have resulted in findings of potential therapeutic targets for atherosclerosis treatment. Focusing on these therapeutic targets allows for the lowering of sphingolipid levels, a strategy that has been proven to alleviate the disease's development. Sphingolipid level lowering is possible through the inhibition of *de novo* synthesis and shifting sphingolipid metabolism. Although these are promising strategies for treating atherosclerosis, sphingolipid metabolism needs to be targeted in atherosclerosis-related cells to maintain essential systemic sphingolipid roles and avoid adverse effects. Sphingolipid metabolism can be modulated in a controlled manner by using nanomedicine. Nanomedicine allows for the targeting of key players in atherosclerosis development and long-lived therapeutic effects, which is necessary to combat chronic inflammation in atherosclerosis. Further studies are needed in the promising strategy of lowering sphingolipid levels to treat atherosclerosis. The precise mechanisms of controlling sphingolipid metabolism have to be understood in order to treat atherosclerosis in an effective and safe manner.

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