Sphingolipids metabolism alteration in the central nervous system: Amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases

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ABSTRACT

Sphingolipids are complex lipids. They play a structural role in neurons, but are also involved in regulating cellular communication, and neuronal differentiation and maturation. There is increasing evidence to suggest that dysregulated metabolism of sphingolipids is linked to neurodegenerative processes in amyotrophic lateral sclerosis (ALS), Parkinson’s disease and Gaucher’s disease. In this review, we provide an overview of the role of sphingolipids in the development and maintenance of the nervous system. We describe the implications of altered metabolism of sphingolipids in the pathophysiology of certain neurodegenerative diseases, with a primary focus on ALS. Finally, we provide an update of potential treatments that could be used to target the metabolism of sphingolipids in neurodegenerative diseases.

1. Introduction

Lipids represent around 60% of the constituents of nervous tissue and are essential in the propagation of electrical and chemical signals [1]. The sphingolipids represent almost 20% of the lipids of the nervous system [2]. They belong to a major class of complex lipids that are involved in various physiological and developmental processes, thus ensuring the proper functioning of the nervous system. In this review, we describe the generalities of sphingolipids and their physiological roles in the nervous system, as well as the importance of sphingolipids in neurodegenerative diseases. Finally, we discuss therapeutic approaches associated with sphingolipids in animal models and clinical trials.

2. Biosynthesis of sphingolipids in the nervous system

The biosynthesis of sphingolipids begins at the cytosolic layer of the endoplasmic reticulum (ER) and progresses through several subcellular structures. The general structure of sphingolipids is defined by a sphingosine skeleton. Ceramide is the simplest sphingolipid. Made up of a sphingosine molecule and one fatty acid, it is the key precursor for the synthesis of many sphingolipids. Ceramide is obtained by the transformation of sphinganine into dihydroceramide by the ceramide synthase enzymes, which represent a large family of ER enzymes. Dihydroceramide is subsequently converted to ceramide by ceramidase [3]. During synthesis, the subclass of sphingolipids is determined through the addition of different chemical groups.
groups (radical group “R”) to the ceramide (Fig. 1). Among these subclasses are the glycosphingolipids which are characterized by the addition of one or more monosaccharides. Once formed in the ER, ceramide can be directly transformed into galactosylceramide through the addition of one galactose by the enzyme ceramide galactosyltransferase (UGT8), or transported to the Golgi apparatus to give rise to several glycosphingolipids and gangliosides (Fig. 2). For example, when ceramide is transported to the cis-Golgi network, the addition of glucose leads to the formation of glucosylceramide. At this stage, glucosylceramide is transported to the trans-Golgi apparatus via two mechanisms: through vesicular transport or through the transport protein four-phosphate adaptator protein 2 (FAPP2) (4,5). At the trans-Golgi network, glucosylceramide is transformed into different glycosphingolipids (e.g. lactosylceramide, gangliosides, etc.). Using the ceramide transferase protein, ceramide can be transported directly to the trans-Golgi network, where the addition of a phosphocholine produces sphingomyelin. At the trans-Golgi network, several glycosphingolipids are formed. These glycosphingolipids are transported to the plasma membrane where they are integrated into lipid rafts. Finally, the addition of glucose and galactose to the ceramide allows for the formation of lactosylceramide. Addition of sialic acid to lactosylceramide leads to the production of the first ganglioside, GM3. Various derivatives can then be generated through the addition of N-acetylgalactosamine and galactose, which gives rise to GM2, GM1, and so on (Fig. 1).

The degradation of glycosphingolipids occurs in the endosomes and lysosomes. Endosomal glycosphingolipids are recycled to subcellular compartments (ER and Golgi apparatus) while the glycosphingolipids that are redirected to the lysosomes are hydrolyzed to ceramide and then to sphingosine.

3. Sphingolipids have major roles in the nervous system

Several studies show that sphingolipids are widely distributed across the central nervous system (CNS). The CNS is mainly composed of glycosphingolipids and gangliosides [6,7]. Gangliosides are mainly located in neurons, whereas galactosylceramide is mainly localized in the oligodendrocytes [8]. The distribution of sphingolipids in the CNS highlights their importance in the formation of different cell types, and in neurodevelopment.

3.1. Sphingolipids: driving the maturation of the nervous system

The gangliosides are the major class of sphingolipids that are found at high concentrations in the CNS. As such, they are indispensable for the development of the nervous system, axonal growth, and neuronal differentiation [9,10]. During embryonic development, ganglioside levels vary depending on the stage of maturation (Fig. 3). During the early stages of development, GM3 and GD3 are synthesized in large quantities during the formation of the neural tube, and they also participate in the proliferation and differentiation of neural stem cells [7,10,11]. Next, several derivatives of GM3 (like GD1a, GM1, GD1b and GT1b) participate in neuronal differentiation, synaptogenesis, and myelination until adulthood [12]. Sphingomyelin, galactosylceramide and sulfatide also participate in axonal arborization during development. Not surprisingly, an absence of GD3 synthase drastically delays axonal growth and myelination in mice [13]. By contrast, administration of GD3 improves the regeneration process after sciatic nerve injury in GD3 synthase knockout (KO) mice [14]. This age- and development-dependent expression of gangliosides highlights their crucial role in neurogenesis.

3.2. Implication of glycosphingolipids in neuronal differentiation and axonal growth

The other actors involved in the development of the nervous system are the glycosphingolipids. As gangliosides, glycosphingolipids are involved in establishing neuronal proliferation and maturation, as well as axonal growth [7,15,16]. In addition, many glycosphingolipid enzymes such as the synthetic enzymes of ceramide and glucosylceramide, ceramide synthase and the UDP-glucose ceramide glycosyltransferase (CERT) (3) where it is transformed into sphingomyelin (SM). (4) Ceramide can also be transported from the ER to the cis-Golgi network to be transformed into glucosylceramide (GlcCer). (5) GlcCer can be transported to the trans-Golgi apparatus through vesicular transport. At the trans-Golgi network, GlcCer is transformed into different glycosphingolipids (GSLs). (6) Four-phosphate adaptator protein 2 (FAPP2) can transport GlcCer back to the ER where it can be degraded to Cer by GBA2. (7) GlcCer is transported to the membrane by the glycolipid transfer protein (GLTP) where it can integrate into lipid rafts. (8) GSLs are transported to the plasma membrane through vesicular transport, (9) where they can integrate into lipid rafts. (10) GSLs are recycled at the endosome (11) to the ER or Golgi apparatus (12) or degraded by lysosomal pathway. (13) Degradation of GlcCer at the lysosome level occurs by GBA1 (inspired by [6]).
Fig. 2. Schematic representation of sphingolipid metabolism. Sphingolipids are characterized by the addition of a radical group to determine the subclass of sphingolipids; in this diagram the different classes of ceramide/dihydroceramide are not shown (e.g., hydrogen atom for ceramide is not shown). Sphingomyelin degradation by sphingomyelinase (SMase) generates ceramide (Cer). From the Cer, sphingosine and galactosylceramide (GalCer) are then synthesized by acid ceramidase (ASAHI) and ceramide galactosyltransferase (GALC) respectively. Glucosylceramide (GlcCer) is a glycosphingolipid which is obtained from Cer by glucocerebrosidase (GBA1/GBA2). The addition of galactose to GlcCer by galactosyltransferase (B4GLNT6) leads to the generation of lactosylceramide (LacCer). Then, the addition of sialic acid to LacCer forms GM3, a precursor of gangliosides. Ganglioside synthesis is continued through the successive addition of N-acetylgalactosamine (GalNac) and galactose residues by GalNac transferase 1 (GM2 synthase/ B4GLNT1 and B3GALNT4) respectively for GM2 and GM1.

3.3. Location and function in cell membranes of the nervous system

Cell membranes are composed of micro-domains, called lipid rafts, which are supplemented with phospholipids, cholesterol and sphingolipids. These lipid rafts play a structural role in cells but are also critical for signal transduction. Cholesterol is critical for the formation and maintenance of lipid rafts where they play a structural and anchoring role for membrane proteins involved in endocytosis, signal transduction, cell adhesion and rearrangement of the cytoskeleton [21–24]. Sphingolipids are an integral part of cell membranes where they act as modulators of signaling pathways. Ceramides, by their degree of saturation or chain length, can induce membrane gel domains, with far-reaching effects on signal transduction [25,26].

Several studies also suggest that lipid rafts, including sphingolipids, are directly responsible for the spatial organization of signaling molecules. These rafts group G proteins and neurotransmitter receptors to promote signal transduction [21]. Indeed, several sphingolipids (e.g., GM1, GM2, etc.) interact with G protein coupled receptors [27–29]. Moreover, the glycosylated region of gangliosides, such as GM1, are positioned in the extracellular environment in a way that allows interaction with BDNF and NGF receptors [30–32]. Of note, GM1 is also found in the nuclear envelope of cells, where it regulates gene expression through its association with a sodium-calcium exchanger as during neuronal development [33–35]. To ensure the integrity of tissue in the nervous system, the gangliosides stabilize the myelin-associated glycoproteins (MAGs), thereby allowing strong architecture between the axon and the myelin sheath [7,36,37]. In 2005, Yamashita and collaborators reported disruptions in axon-myelin interactions in mice lacking GM3 and GM2 syntheses, two enzymes that synthesize gangliosides [38]. Finally, sphingolipids are involved in cellular communication through the vesicles of endosomes and exosomes. Indeed, sphingomyelinase (SMase) for example, participates in the formation and secretion of exosome vesicles, and sphingosine kinase is involved in the regulation of endosomes [28]. All these data underline the importance of sphingolipids in cell integrity but also in the establishment of cellular communication.

3. The expression of gangliosides in the nervous system during development.

Ganglioside composition changes during development. The gangliosides GD3 and GM3 are produced during the formation of the neural tube, and during neural stem cell proliferation. During neuronal differentiation, the expression of gangliosides such as GD1a, GM1, GD1b and GT1b continue to increase. Galactosylceramide (GalCer), sphingomyelin (SM) and sulfatide are synthesized during synaptogenesis. Their synthesis is maintained until adulthood (inspired by [8,11]).

(UGCG), have been shown to be essential for axonal and dendritic growth. In this regard, inhibition of ceramide synthase activity in murine neurons in vitro leads to a considerable decrease in levels of glycosphingolipid, thereby impairing neuronal growth [17,18]. Similarly, deletion of UGCG in the neural cells of mice causes axonal degeneration and demyelination of peripheral nerves, leading to the death [19].

In 2009, it was also shown that inhibition of UGCG with a shRNA led to a decrease in the expression of glycosphingolipids in mouse embryonic stem cells [20]. In sum, sphingolipids and the degradation of glucosylceramide into ceramide is crucial for the development of the nervous system.
It is widely accepted that GM1 interacts with neurotrophins, which themselves are essential for the growth and survival of neurons. GM1 is anchored to the cell membrane via its ceramide group, and 5 sugars (radical R) are exposed to the extracellular environment. The extracellular portion of GM1 can interact with growth factor receptors. Pioneering studies demonstrated that GM1 promoted the neurite growth in murine neuroblastomas [39–41]. Subsequently, a direct interaction between GM1 and the TrkA receptor, as well as the ability of GM1 to increase the activation of TrkA induced by NGF, was shown [30,42]. In the early 2000s, the enhanced effect of NGF on GM1-potentiated TrkA receptor activity was demonstrated in vivo in rats [32]. More recently, it has been proposed that this effect is due to the binding of NGF to the TrkA receptor, and activation of the Ras/Raf/MEK/Erk pathway [7]. Interestingly, cells deficient in GM1 do not express the TrkA receptor [43]. In line with these data, it has been shown that endogenous GM1 directly modulates the activity of the TrkA and TrkB receptors, as well as their associated signaling cascades [31,44,45]. GM1 also activates the various Trk receptors (A, B and C) by phosphorylation [32] and participates in the autophosphorylation of TrkC [46]. Critically, a 2002 study by Bachis and colleagues noted that GM1 appeared to prevent glutamate-related excitotoxicity by mimicking the action of BDNF on TrkB [47]. Collectively, these studies consolidate the close link between GM1 and Trk receptors in neuronal survival.

4. Sphingolipids in neurodegenerative diseases

Currently, more than 30 million people are affected by neurodegenerative diseases. Alzheimer’s disease (AD) is the most common of these diseases and affects 25 million people worldwide, while 6.3 million people have Parkinson’s disease (PD), and 220,000 have amyotrophic lateral sclerosis (ALS) [48]. The accumulation or dysregulation of sphingolipids is proposed to contribute to the pathogenesis of these neurodegenerative diseases by severely affecting lysosomal enzyme activity, which triggers an accumulation of lipids within organelles of the "endosomal-autophagic-lysosomal" system [49].

4.1. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis is a fatal neurodegenerative disease that is characterized by the irrevocable degeneration of upper and lower motor neurons. The progressive loss of neurons results in debilitating motor weakness, increasing paralysis, and death usually occurs within 3–5 years from symptom onset [50]. It is becoming evident that ALS pathology extends beyond the motor system [51].

Several studies have identified metabolic alterations in ALS patients. These are mostly defined by hypermetabolism and dyslipidemia, which are clinically associated with the severity of symptoms [52–54]. Hypermetabolism is an early phenomenon that is present throughout the disease [55–57], and that appears to negatively impact prognosis [58–60]. By contrast, hyperlipidemia appears to be a protective factor in ALS, and it is known that a hyperlipidemic diet significantly improves survival of ALS mice (Sod1G93A) [61] and slows progression of disease in ALS patients [62]. Congruent with this, high levels of total cholesterol [63] and elevated levels of serum triglyceride [64] appear to be beneficial in ALS patients. More recently, the beneficial effects of high-caloric nutrition on the survival of ALS patients with fast-progressing disease were shown [65]. While the cause for metabolic dysregulation in ALS remains unknown, consistent observations of the beneficial effects of lipids has spurred extensive research into the contribution of lipid metabolism to the disease.

An abnormal increase in sphingolipid levels (sphingomyelin, ceramide, cholesterol, etc.) has been observed in the spinal cord of ALS patients, and in the spinal cord of asymptomatic and symptomatic SOD1G93A mice [66]. Subsequent studies confirming an increase in metabolites of sphingolipids (glucosylceramide, ceramide, galactosylceramide, GM3, GM1, etc.) and their associated enzymes (GBA1, GBA2, etc.) in the spinal cord of Sod1G93A mice [67], SOD1G93A mice and ALS patients [68] indicate that altered metabolism of sphingolipids could be integral to the progression of ALS pathology [69]. Interestingly, the inhibition of glucosylceramide synthesis dramatically accelerates disease progression in SOD1G93A mice while the intracerebroventricular perfusion of GM3 is able to significantly delay the onset of paralysis in SOD1G93A mice [68]. While the clinical relevance of altered sphingolipid metabolism remains to be established, levels of sphingomyelin and long chain triglycerides in the CSF of ALS patients has been shown to correlate with the progression of ALS [70,71].

The detection of antibodies targeting gangliosides, in particular GM1, in serum [72,73], brain [74] and spinal cord [75] of some patients with ALS led to clinical trials of ganglioside administration. These trials have not been conclusive [76–78]. However, Xu et al. have shown that a single injection of human IgM (rHgM12) binding gangliosides extends the survival of two ALS mouse models [79] thus emphasizing a role for glycosphingolipids in ALS.

An increase in the levels of glucosylceramide and GM1 have been observed in the CSF of ALS patients [80]. Transcriptomic studies of muscle biopsies from ALS patients have reported a significant increase in the expression of the UGCG gene, encoding the enzyme responsible for the synthesis of glucosylceramide. Similar to ALS patients, mouse models of ALS show accumulation of glucosylceramide in skeletal muscle. Lipidomic analysis revealed complete modulation of sphingolipids in skeletal muscles and spinal cords of Sod1G93A mice prior to the onset of disease phenotypes. Interestingly, the levels of GM2 and GM3 were increased in Sod1G93A mice, but also in non-transgenic mice that had undergone axotomy, suggesting that altered sphingolipid metabolic might occurs as a consequence of denervation. In addition, inhibition of glucosylceramide synthesis by an UGCG inhibitor significantly delayed functional recovery after sciatic nerve injury [67]. Conversely, inhibition of the degradation of glucosylceramide by conduritol B epoxide, an irreversible inhibitor of GBA1 and GBA2, improved functional recovery in a model of sciatic nerve compression and slowed disease progression in Sod1G93A mice [80]. Thus, current evidence indicates that sphingolipid metabolism is likely to be a key modulator of disease course in ALS.

4.2. Other neurological diseases

In recent years, there has been a growing body of evidence to indicate that the metabolism of sphingolipids contributes to the pathophysiology of other diseases such as Alzheimer’s disease, Parkinson’s disease and Gaucher disease. The gradual accumulation of protein aggregates, which leads to toxicity and neurodegeneration of cells of the nervous system, occurs in response to defects in the metabolism of sphingolipids, major players in the lysosomal degradation of cellular debris.

4.2.1. Alzheimer’s disease

Alzheimer’s disease (AD) is the most common neurodegenerative disease characterized by the accumulation of extracellular amyloid plaques and intraneuronal deposits of the Tau protein [81,82]. Although the molecular mechanism that underpins the development of the disease is not clear, numerous studies suggest that sphingolipids play a crucial role in the pathogenesis of AD [83–88]. Indeed, the levels of sphingolipids (e.g. sphingomyelin and ceramide) and their associated synthetic enzymes (sphingomyelin synthase and sphingomyelinase) are increased in the brains of AD mice and AD patients [89–91]. Several teams have also shown that elevated levels of serum ceramide in AD patients and AD model animals [92–96] may be predictive of AD and cognitive impairment [97–99]. Ceramide, produced by sphingomyelinases, stabilizes β-secretase, which leads to an increase in the cleavage of the amyloid precursor protein [100]. In this regard, increasing ceramide and sphingomyelinase levels promotes the accumulation of amyloid-β.
4.2.1. Parkinson’s disease

Parkinson’s disease is the second most common neurodegenerative disease after Alzheimer’s disease [121–124]. Stimulation of S1P by FTY720 improves memory, synaptic plasticity, reduces β-amyloid production and enhances motor function in several distinct animal models of these diseases [125–128]. More recently, FTY720 was shown to significantly improve survival in the SOD1G93A mouse model of ALS [129].

5. Therapeutic targeting of sphingolipids in neurodegeneration

With a growing body of evidence to show that sphingolipids actively participate in the pathophysiology of neurodegenerative disease [5,49,119], there is an increased focus on the targeting of the metabolism of sphingolipids for therapeutic development. Due to the complexity of the sphingolipid pathway, only the most common players in neurodegenerative diseases will be discussed.

5.1. Sphingolipids at the centre of new therapies in animal and cell models

Some molecules have proven to be promising candidates for attenuating various pathologies in vitro and in vivo models of neurodegenerative disease.

5.1.1. Fingolimod (FTY720)

Fingolimod is an analogue of sphingosine. In the brain, it is converted to fingolimod-phosphate (FTY720-P). Fingolimod-P is a siphingosine-1-phosphate (SIP) receptor agonist [120], which is reduced in Alzheimer’s disease and Parkinson’s disease [121–124]. Stimulation of SIP by FTY720 improves memory, synaptic plasticity, reduces β-amyloid production and enhances motor function in several distinct animal models of these diseases [125–128]. More recently, FTY720 was shown to significantly improve survival in the SOD1G93A mouse model of ALS [129].

5.1.2. Isofagomine

In 2009, Liebermann and co-workers demonstrated that isofagomine by acting as a chaperone of GBA1 ensured good conformational stability and activity of human GBA1 while also increasing its enzymatic activity [130] (see Table 1). In support of this, isofagomine was shown to extend the lifespan in animal models of Gaucher disease and increase the activity and levels of GBA1 in the brain and visceral tissues, although its effects on the accumulation of glucosylceramide and glycosphingosine is yet to be determined [131]. Finally, isofagomine reduces aggregates of human wildtype α-synuclein in dopaminergic neurons in the substantia nigra of a mouse model of synucleinopathy [132]. In general, current data on the use of isofagomine in Gaucher disease and synucleinopathies is encouraging.

5.1.3. Ambroxol

Ambroxol is a molecule known for its mucolytic, antioxidant and anaesthetic properties [133]. It can cross the blood-brain barrier (BBB) and improve the activity of GBA1 in Gaucher disease patient cells in vitro, and in wildtype mice in vivo [134] (see Table 1). In 2009, Magsawa and collaborators demonstrated that ambroxol stabilizes the conformation of GBA1, thereby increasing its enzymatic activity, and reducing the accumulation of glucosylceramide in fibroblasts from Gaucher disease patients [135]. Ambroxol has also been shown to enhance the activity of GBA1 in fibroblasts of Parkinson’s disease patients, and to reduce the aggregation of α-synuclein [114,136]. In wildtype and transgenic mice overexpressing human α-synuclein, treatment with ambroxol led to an increase in GBA1 mRNA and enzyme activity in the brain, and a concomitant reduction in levels of α-synuclein [137]. Finally, the activities of GBA1 and β-hexosaminidase are significantly improved in non-human primates after treatment with ambroxol [138]. In the context of ALS, our laboratory has demonstrated that ambroxol prevents the loss of muscle strength, delays disease progression, and extends survival of SOD1G93A mice. Moreover, ambroxol promotes axonal growth and neuronal network complexity in vitro and in vivo [139,140]. Overall, ambroxol appears to be highly effective in modulating GBA1 activity to exert neuroprotective effects across a number of mouse models of neurodegeneration.

5.2. Sphingolipid modulation in humans

There is growing interest in targeting the metabolism of sphingolipids as a therapeutic strategy in human neurodegenerative diseases. In this section, we will only present clinical studies for Gaucher disease and Parkinson’s disease.

5.2.1. Isofagomine

The protective activity of GBA1 in patients with Gaucher disease can be restored by isofagomine treatment. By stabilizing the binding of the GBA1 enzyme with its substrate [141], isofagomine increases GBA1 activity in Gaucher disease patient fibroblasts, thus preventing the toxic accumulation of glucosylceramide [130]. However, isofagomine has poor cell penetration and clinical trials for Gaucher disease have been inconclusive.

5.2.2. Ambroxol

In 2013, Zimran et al. showed that ambroxol was safe and had no adverse effects in patients with Gaucher disease [142]. In another pilot study, ambroxol was found to improve motor functions and neurological
yet the mechanisms by which the dysregulation in sphingolipid metabolism contributes to the degenerative process remains to be elucidated. While the targeting of sphingolipid metabolism has reached clinical stages for Parkinson’s disease, this is yet to occur in Alzheimer’s disease or ALS. Nonetheless, results in mouse models of neurodegenerative diseases, and in particular ALS, highlight that the modulation of sphingolipid metabolism may be beneficial. Future studies that clarify the role of sphingolipids in neurodegeneration, with the view to translate this knowledge into clinical trials and effective drug treatments are desperately needed.

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Patents
Henriques, Loeffler and Spedding hold a patent entitled “Inhibitors of Glucosylceramide Degradation in the Treatment of Diseases of the Motor Units”.

Conflicts of interest
The authors declare no conflict of interest.

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