Glycoconjugate Journal 21, 59–62, 2004

Biological roles of sulfoglycolipids and pathophysiology of their deficiency

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Mammalian sulfoglycolipids are comprised of two major members, sulfatide (SO_3-3Gal-ceramide) and seminolipid (SO_3-3Gal-alkylacylglycerol). Sulfatide is abundant in the myelin sheath and seminolipid is expressed on the spermatogenic cells. Cerebroside sulfotransferase (CST)-deficient mice generated by gene targeting completely lack sulfatide and seminolipid all over the body. CST-null mice manifest some neurological disorders due to myelin dysfunction, an aberrant enhancement of oligodendrocyte terminal differentiation, and an arrest of spermatogenesis, indicating that sulfation of glycolipids is essential for myelin formation and spermatogenesis. Moreover, CST-deficiency ameliorates L-selectin-dependent monocyte infiltration in the kidney after ureteral obstruction, an experimental model of renal interstitial inflammation, indicating that sulfatide is an endogenous ligand of L-selectin. Studies on the molecular mechanisms by which sulfoglycolipids participate in these biological processes are ongoing.
Published in 2004.

Keywords: **sulfotransferase, sulfatide, seminolipid, myelin, spermatogenesis**

Sulfoglycolipids and their biosynthesis

Two major sulfoglycolipids exist in the mammal: one being the sulfatide, which is a sphingolipid and the other being the seminolipid, which is an ether glycerolipid [1,2] (Figure 1). Sulfatide is a major lipid component of the myelin sheath and is synthesized in myelin-generating cells, oligodendrocyes in the central nervous system and Schwann cells in the peripheral nervous system. Seminolipid is synthesized in spermatocytes and maintained in the subsequent germ cell stages.

The carbohydrate moiety of sulfatide and seminolipid has the same structure and is biosynthesized via sequential reactions catalyzed by common enzymes: ceramide galactosyltransferase [3] (CGT, EC 2.4.1.45) and cerebroside sulfotransferase [4,5] (CST, EC 2.8.2.11) (Figure 1). CGT is located in the endoplasmic reticulum and CST is in the Golgi membranes. It is proved that both enzymes have no isozymes as evidenced by gene disruption in mice [6–8]. The tissue distribution of sulfoglycolipids is mainly regulated by the tissue-specific expression of the *CGT* gene. *CST* gene expression is also tissue-specific, but its regulation is not as strict as the *CGT* gene [9].

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Sulfatide in myelin function and oligodendrocyte differentiation

Mammals have acquired property known as myelin during the evolution so as to enhance the conductivity of the neuronal impulse. Oligodendrocytes produce vast amounts of myelin, a unique and lipid-rich biomembranes with a relatively simple array of myelin-specific proteins in the central nervous system. This membrane, an extension of the oligodendrocyte plasma membrane, forms multilamellar and spirally wrapped sheaths around neuronal axons. The gaps between adjacent myelin sheaths are referred to as nodes of Ranvier, and myelin forms lateral loops there [10] (Figure 2). These myelin loops terminate at the paranode region and engage in the formation of a septate-like adhesive junction with the axon membrane, axolemma. This specialized axo-glial junction acts as an electronical and biochemical barrier between nodal and internodal membrane compartments. Voltage-gated sodium channels concentrate in the nodal axolemma, while shaker-type K^+ channels, Kv1.1 and Kv1.2, localize within the juxtaparanodal axolemma. Saltatory conduction of the action potential is attributed to this organization. The adhesion of myelin to the axolemma plays a critical role in this clustering of ion channels. Thus myelin serves not only as a simple insulator but also as a functional platform of the neuron-glia interaction.

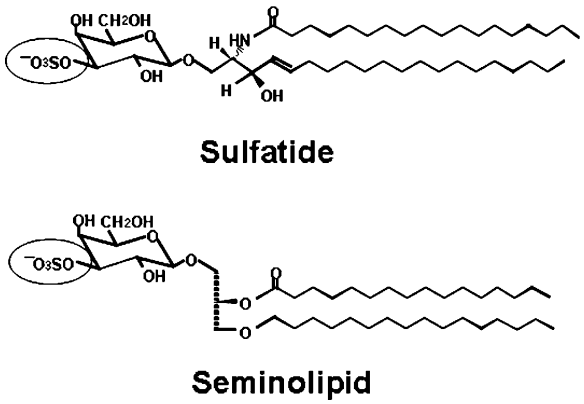

Figure 1. Structure of sulfatide and seminolipid.

GalCer and sulfatide comprise 23% and 4% of the total lipid content in the myelin sheath, respectively [1]. In order to elucidate the physiological function of sulfoglycolipids, CST-deficient mice were created by means of gene targeting [8]. CST-deficient mice show a complete loss of sulfatide in brain. CST-null mice were born healthy, but began to display hindlimb weakness by 6 weeks of age and subsequently showed a pronounced tremor and progressive ataxia. Histological analysis revealed that axons were well myelinated in CST-null mice. Electron microscopic analysis of myelinated nerve fibers, however, revealed disorganized termination of the lateral loops at the node of Ranvier, similar to that reported in CGT-deficient mice [11] (Figure 2). Furthermore, clustering of Na^+ and K^+ channels at the node is also deteriorated in CST-null mice [12] as observed in CGT-null mice [13] (Figure 2). These findings strongly suggest that sulfatide is an indispensable molecule for the adhesive junction of myelin loop and axolemma at the paranode region. It has been reported that three proteins: Caspr/paranodin, contactin, which are on the axolemma, and the 155 kDa splice isoform of neurofascin (NF155), which is on the myelin membrane, form the axo-glial adhesion apparatus at the paranodal region [10]. It is an important problem in the future how a lipid component of myelin, sulfatide, interacts with these protein complexes and maintain the adhesive junction.

Despite the significant neurological disorders, CST-null mice are able to survive to more than one year of age [8]. The phenotype of the CST-deficient mice was milder than that of CGT-deficient mice [6,7] in terms of the age of onset, life span, and the severity of symptoms. Moreover, CST-deficient mice show neither decrease of the maximal conduction velocity or demyelination unlike CGT-null mice [8,11]. This discrepancy suggests that sub-localization on myelin sheath is different between GalCer and sulfatide and that GalCer act, not only as a precursor for sulfatide synthesis, but also as a functional molecule.

Since sulfatide emerges when oligodendrocyte progenitors cease dividing and commence differentiating, it is supposed to be involved in the regulation of terminal differentiation. In fact, this terminal differentiation is enhanced in CGT-KO mice [14]. Furthermore, oligodendrocyte differentiation in wild-type mice was found to be blocked by anti-sulfatide antibody but not by anti-GalCer antibody [14]. These findings strongly suggest that sulfatide is a key negative regulator of the oligodendrocyte differentiation. This hypothesis was certified by the fact that terminal differentiation of oligodendrocytes is enhanced in CST-KO mice [15]. Hence sulfatide plays a critical role in the regulation of oligodendrocyte terminal differentiation, in addition to their eventual roles as structural components of mature myelin.

Seminolipid in spermatogenesis

Spermatogenesis occurs in the seminiferous tubules in the testis [16]. Spermatogonia that are germline stem cells sit in a special place on the basement membrane of seminiferous tubules called niche. These spermatogonial stem cells differentiate into spermatocytes, ceasing proliferation. Subsequently, spermatocytes undergo meiosis and differentiate into haploid cells referred to as spermatids, migrating from the surrounding to the lumen of the seminiferous tubules on the stroma cells termed Sertoli cells with mutual interaction. After that, they mature into spermatozoa via morphogenesis and leave the seminiferous tubules for the epididymis through the efferent ductules.

More than 90% of glycolipid in the testis consists of a unique glyceroglycolipid, seminolipid [2]. Its carbohydrate moiety is

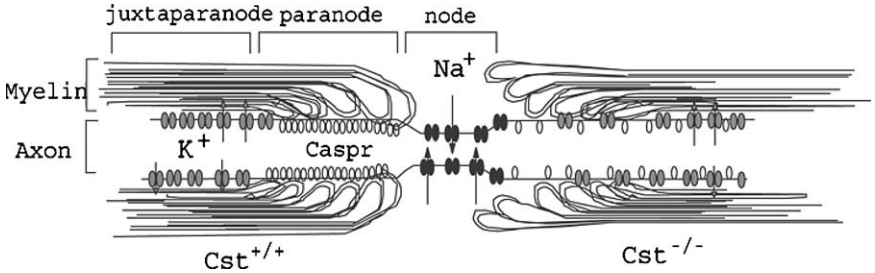

Figure 2. Organization of nodes of Ranvier and its alterations in CST-deficient mice. In wild-type mice (*left side*), myelin membranes and axon membranes form junctions at the paranode region and Na^+ and K^+ channels cluster at the node and juxtaparnode regions, respectively, separated by the septate-like axo-glial junction. In CST-deficient mice (*right side*), formation of the axo-glial junction is disordered and the clustering of Na^+ and K^+ channels is also disturbed.

the same as that of sulfatide and the synthesizing enzymes are found to be common (Figure 1). Seminolipid is synthesized at an early stage of spermatocytes.

In CGT-deficient mice, neither galactosylalkylacylglycerol (GalEAG) nor seminolipid is synthesized, and spermatogenesis is arrested prior to the meiosis [17]. On the other hand, primary spermatocytes seem to be normal in CST-deficient mice, but spermatogenesis is blocked at the metaphase of the first meiosis [8]. The arrested stage in the germ cell differentiation of CST-null mice appears to be somewhat later than that in CGT-null mice, suggesting that both GalEAG and seminolipid are successively involved in the genetic program of spermatogenesis in the same order as their biosynthesis. Since these glycolipids are expressed on the cell surface of primary spermatocytes from the end of the leptotene stage or the zygotene stage and later, the interaction between Sertoli cells and spermatocytes, which is known to be important for their differentiation, may be disrupted in these knockout mice.

Sulfatide is an endogenous L-selectin ligand in the kidney

Blood monocytes that extravasate to sites of inflammation differentiate into macrophages and induce inflammatory response. In the experimental model of kidney interstitial inflammation, unilateral ureteral obstruction (UUO) leads to infiltration of manocytes into interstitium. Monocyte infiltration is a multistep process in which chemokine and adhesion molecules play key roles. The initial step of this process involves the binding of monocytes to the endothelium of venules, mediated by various adhesion molecules such as selectins. The monocyte infiltration following UUO is inhibited by a neutralizing antibody against L-selectin [18]. Although L-selectin was originally identified as a lymphocyte homing receptor, its ligands are expressed not only in lymphoid organs but also in other tissues. The main L-selectin ligand activity in lymphoid organs is served by sialylated, fucosylated, and sulfated glycans on mucin-like molecules. On the other hand, L-selectin was found to bind to sulfatide as well [19]. Since distribution of sulfatide, which is demonstrated by anti-sulfatide monoclonal antibody, was very close to that of L-selectin ligand and exogenously added sulfatide considerably inhibited the monocyte infiltration into after UUO treatment [18], sulfatide was supposed to be involved in the monocyte infiltration mediated by interaction between L-selectin on monocytes and sulfatide in the kidney. This hypothesis was tested using CST-null mice [20]. The distributions of L-selectin ligand activity and sulfatide were relocated from the distal tubules to the endothelium of peritubular capillaries, where monocytes infiltrate, after UUO treatment in wild-type mice. In contrast, the L-selectin ligand activity was not detected in CST-null mice irrespective of UUO treatment. Compared with wild-type mice, CST-null mice showed a considerable reduction in the number of monocytes/macrophages that infiltrated into the interstitium after UUO. The number of monocytes/macrophages was also reduced to a similar extent in L-selectin-null mice. These findings indicate that sulfatide is a major L-selectin ligand in the kidney and that the interaction between L-selectin and sulfatide plays a central role in monocyte infiltration into the kidney interstitium.

Perspectives

Our studies have revealed that sulfoglycolipids are essential for organisms. Now we have to elucidate molecular mechanisms how sulfoglycolipids work in the biological processes in which sulfoglycolipids are proved to be involved. To this end, sulfoglycolipids-interacting molecules should be identified as the first step. As discussed above, sulfoglycolipids are supposed to play critical roles in cell-cell communication. Sulfoglycolipids may directly interact with molecules on the opposite cells. It is true for the L-selectin-sulfatide interaction mediating the monocyte filtration in the kidney. Alternatively, sulfoglycolipids may associate with other molecules on the same membrane. Glycoshingolipids self-associate in cellular membranes to form a microdomain reffered to as lipid raft. Glycosylphosphatidylinositol-anchor proteins, which contain ether glycerolipid like seminolipid, are also enriched in this microdomain. The fact that sulfatide and seminolipid were recovered in detergent-insoluble floating membrane fractions (data not shown) suggest that sulfoglycolipids are included in the lipid rafts. These microdomains are proposed to serve as platforms within the plasma membrane for receptor signaling and trafficking. Sulfoglycolipids may contribute in organization of such functional platforms.

Acknowledgments

This study was supported by a Grant-in-aid for Scientific Research on Priority Area No. 14082204 and by the 21st Century COE program from the Ministry of Education, Science, Culture, Sports and Technology of Japan.

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Received 31 March 2004; accepted 18 May 2004