Sphingolipids and Kidney Disease: Possible Role of Preeclampsia and Intrauterine Growth Restriction (IUGR)

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Abstract
Sphingolipids are now considered not only as constitutional components of the cellular membrane but also as essential bioactive factors regulating development and physiologic functions. Ceramide is a vital intermediate of sphingolipid metabolism, synthesized by de novo and salvage pathways, producing multiple types of sphingolipids and their metabolites. Although mutations in gene-encoding enzymes regulating sphingolipid synthesis and metabolism cause distinct diseases, an abnormal sphingolipid metabolism contributes to various pathologic conditions, including kidney diseases. Excessive accumulation of glycosphingolipids and promotion of the ceramide salvage and sphingosine-1-phosphate (S1P) pathways are found in the damaged kidney. Acceleration of the sphingosine kinase/S1P/S1P receptor (SphK/S1P/S1PR) axis plays a central role in deteriorating kidney functions. The SphK/S1P/S1PR signaling impairment is also found during pregnancy complications, such as preeclampsia and intrauterine growth restriction (IUGR). This mini-review discusses the current state of knowledge regarding the role of sphingolipid metabolism on kidney diseases, and the possible involvement of preeclampsia and IUGR conditions.

Introduction
Over several decades, studies on bioactive lipids have provided information concerning their cellular functions beyond their structural and energy-storage roles. Among a large number of bioactive lipids, sphingolipids and their metabolites have been the focus of attention (1,2). Sphingolipids are commonly distributed in the cell membrane of the eukaryotic organism and contain an 18-carbon amino alcohol, called the sphingoid base, with a fatty-acid tail or headgroup attached to the base (3,4). Sphingosine is a major sphingoid base in mammals and is often converted to a central intermediate of sphingolipid metabolism, ceramide (5). Ceramide is ester bonded with phosphatidylcholine and becomes sphingomyelin, which comprises 5%–10% of the total mammalian cell phospholipids (6). The addition of various hydrophilic headgroups to ceramide ultimately produces more complex sphingolipids. Sphingolipid variations diversify their biologic functions, and sphingolipids and their metabolites are now known to be involved in cellular signaling, regulating cell survival, growth, proliferation, differentiation, and cellular responses to inflammation by acting as cell-signaling mediators. Their mechanisms include acting as second messengers of intracellular signaling, supporting lipid-raft composition, linking transmembrane domains of the signaling protein, creating mitochondrial membrane pores, and regulating enzymatic activation as cofactors (1,7–9). Conversely, dysregulation of sphingolipid metabolism results in pathologic states, such as cancer, neurologic disease, osteoporosis, diabetes, and atherosclerosis (2,10–13).

Morbidity and mortality resulting from CKD are increasing worldwide. Abnormal lipid metabolism, including dyslipidemia and excessive accumulation of sphingolipids, has been reported to play a critical role in the pathogenesis and progression of CKD (3,14,15). Previous studies suggest a positive correlation between the onset of adult CKD and a prior history of intrauterine growth restriction (IUGR) induced by pregnancy complications (16–18). Moreover, precise sphingolipid metabolism is needed to maintain normal pregnancy. This review summarizes the pathophysiologic roles of sphingolipid metabolism in CKD and its possible role in preeclampsia and IUGR.

Sphingolipid Metabolism

De Novo Synthesis
Sphingolipid de novo synthesis occurs on the cytoplasmic side of the endoplasmic reticulum through condensation of l-serine with palmitoyl CoA (Figure 1) (19,20). The de novo sphingolipid biosynthesis is normally activated by metabolic overload of serine and/or palmitoyl CoA and by a stress stimulus, such as heat, oxidation, chemotherapeutics, cannabinoids, and TNF (21). After biosynthesis of 3-ketosphingosine and dihydrospingosine (spinganine), ceramide, a precursor and central molecule in sphingolipid biosynthesis, is formed by desaturation of dihydroceramide at carbon 4–carbon 5 of the sphingoid base (22).

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In mammalian cells, ceramide is then converted into sphingomyelin or glucosylceramide, and glucosylceramide further changes into more complex glycosphingolipids. Major glycosphingolipids are glucosphingolipids and galactosphingolipids, which are attached with glucose and galactose, respectively. Glycosphingolipids are the largest subclass of sphingolipids and are often distributed in lipid rafts; they are present in the plasma membrane's outer leaflet and, when needed, they play roles in regulating interactions between cells and protein activation (23,24).

Gangliosides are a minor class of glycosphingolipids containing complex, attached sugar chains and are essential components of plasma membranes. In some instances, glycosphingolipids may contain complex sugar chains such as N-acetylgalactosamine and N-glycolyneuraminic acid (8,25). Gangliosides with one or more N-acetylneuraminic acid linkages are labeled GM1, GM2, and GM3 (one N-acetylneuraminic acid), or GD1a, GD1b, GD2, GD3, GT1b, and GQ1 (more than one N-acetylneuraminic acid) (26).

Salvage Pathway

Ceramide is also synthesized by a salvage pathway (27). In the presence of stress stimuli, sphingomyelin is broken down to ceramide and phosphocholine. This reaction is mediated by sphingomyelinase (28,29). Undesired, complex sphingolipids—such as glycosphingolipids—may be broken down in the acidic environment of lysosomes or late endosomes to ceramide (28). Complex glycosphingolipids are degraded through sequential hydrolysis of terminal hydrophilic moieties by hydrolases, where glucose or galactose is removed by β-glucosidases or galactosidase, respectively, to produce ceramide.

Ceramide is hydrolyzed by ceramidase to a sphingosine base and free fatty acid. These two products leave the lysosomes or endosomes to become recycled substrates of ceramide biosynthesis (29). Alternatively, biosynthesized sphingosine may be phosphorylated by sphingosine kinase (SphK) to form sphingosine-1-phosphate (S1P), an essential cellular signaling molecule (30). S1P can be degraded to 2-trans-hexadecenal and phosphoethanolamine by S1P lyase (31).

SphK/S1P/S1P Receptor Axis in the Kidney

Two SphK isoforms were identified: SphK1 and SphK2. These isoforms are present in the cytosol and intracellular compartments, respectively (32). SphK1 is reported to exert antiapoptotic functions in renal mesangial cells by increasing S1P levels, whereas cultured mesangial cells isolated from SphK2-knockout mice are resistant to apoptosis (33–35). In the human proximal tubular HK-2 cell line, SphK1 overexpression protects against peroxidase-induced necrosis by increasing S1P content (36).
Sphingolipids and Kidney Diseases

Dyslipidemia, which involves high levels of LDL cholesterol and triglycerides in addition to low levels of HDL cholesterol, is a major risk factor for atherosclerotic diseases, including CKD. Alternatively, hypoalbuminemia, resulting from proteinuria and a decline in renal function, may induce the accumulation of atherogenic, triglyceride-rich lipoproteins. Recent studies have shown lipid-induced oxidation and inflammation in the kidney, so-called lipotoxicity (43). Some genetic disorders involving disruption of sphingolipid metabolism exhibit renal damage, indicating the kidney is sensitive to sphingolipid alterations. Fabry disease is caused by α-galactosidase A mutations, which result in deficient activity of a lysosomal hydrolyase and excessive accumulation of globotriaosylceramide (Gb3) in cells throughout the body, particularly cells in the kidney, heart, nervous and gastrointestinal systems, and vasculature in the skin (44). The resulting phenotype may include fatal, progressive kidney damage in hemizygous males, whereas, in some individuals, milder symptoms often appear later in life. Although the precise mechanism has not been clarified, the potential action of Gb3 has been investigated in podocytes, which accumulate more Gb3 than other renal cell types. Knocking down the α-galactosidase A gene, by RNA interference and lentiviral-transduction techniques, upregulates LC3-II and downregulates the activity of the mammalian target of rapamycin kinase in podocytes, indicating dysregulation of autophagy (45,46). The decadecylated bioactive form of Gb3 activates the NOTCH signaling pathway, leading to a proinflammatory response, dedifferentiation, and extracellular matrix accumulation via NF-κB translocation (47).

Accumulation of sphingolipids contributes to renal disorders. Although normal glomeruli express gangliosides abundantly, renal levels of glycosphingolipids (such as glucosylceramide, lactosylceramide, and ganglioside GM3) are elevated in patients with diabetic nephropathy (48–53), polycystic kidney disease, renal cell carcinoma, lupus nephritis, age-related decreased kidney function, and their experimental models (54–57). Conversely, in a model of minimal change disease induced by puromycin aminonucleoside, the amount of ganglioside GD3 and O-acetyl GD3 decreased in a time-dependent manner with the progression of proteinuria (58). Because sialoglycoproteins contribute to the glomerular filtration barrier by retaining the negative charge, decreases in gangliosides may alter glomerular permeability.

Diabetic nephropathy is characterized by albuminuria, glomerular and tubulointerstitial fibrosis, and glomerulosclerosis; this condition is a leading cause of ESKD. In patients with diabetes, high plasma levels of sphingolipids, including glucosylceramide, ceramide, sphingosine, and sphinganine, have been observed (3,59–61). Inhibition of the formation of glucosylceramide suppresses pathologic changes in diabetic rat kidneys, suggesting a pathogenic role of glucosylceramide (53). Sphingomyelinase phosphodiesterase acid-like 3b (SMPDL3b) in the membrane lipid raft activates the conversion of sphingomyelin to ceramide and phosphorycholine, purportedly by modulating acid sphingomyelinase. Glomerular expression of this enzyme is enhanced in both human and mouse diabetic nephropathy. In db/db diabetic mice, ceramide levels in the renal cortex are decreased, whereas glomerular mesangial and tubular levels of sphingosine and S1P are enhanced (62–64). Increased SMPDL3b action may induce the production of other ceramide metabolites, such as glycosphingolipids and S1P, by promoting sphingomyelin conversion to ceramide. This hypothesis is also supported by AKI studies, demonstrating that sphingomyelinase activity and ceramide content increase in proportion to the extent of injury to proximal tubule cells (65–67). Moreover, a selective S1PR1 agonist, SEW2871, attenuates proteinuria in early-stage diabetic nephropathy in rats, suggesting a beneficial property of S1PR1 stimulation (68). In the pathogenesis of type 2 diabetes mellitus, ceramide is reported to participate in islet β-cell dysfunction and apoptosis (69). This report suggests that abnormal sphingolipid metabolism deteriorates diabetic nephropathy by direct effects on the kidney and glucose intolerance. Obesity plays an essential role in the onset and progression of type 2 diabetes mellitus by releasing pathogenic adipocytokines, including inflammatory cytokines. Treatment with long-chain saturated free fatty acids promotes ceramide and diacylglycerol accumulation and blocks insulin signaling in C2C12 myotubes (70). Inhibition of de novo sphingolipid synthesis suppresses inflammatory cytokine release from murine 3T3-L1 cells (71).

Unlike the diabetic kidney, renal SMPDL3b levels are low in patients with FSGS. This suggests that the accumulation of sphingomyelin may participate in FSGS pathogenesis. Fornoni et al. (72) found that serum from patients with FSGS has decreased acid-sphingomyelinase activity and SMPDL3b levels, and FSGS is associated with increases in actin cytoskeletal remodeling and apoptosis in podocytes. FSGS is the most common cause of nephrotic syndrome and results in progressive renal dysfunction (73). Soluble urokinase plasminogen activator receptor (suPAR), which is elevated in serum from patients with FSGS, activates αVβ3 integrin in podocytes, leading to a migratory phenotype. Serum suPAR levels are also elevated in patients with diabetic nephropathy. In podocytes treated with serum from patients with diabetic nephropathy, SMPDL3b interacts with suPAR to cause the podocytes to change from a migratory to an apoptotic phenotype through increasing RhoA activity (74). Taken together, regulation of sphingolipid metabolism could be a therapeutic target for glomerular diseases.

Inflammation induces the production and release of fibrogenic cytokines and growth factors, leading to fibrosis, which results in irreversible kidney dysfunction (75). S1P has been considered to play an important role in both inflammation and fibrosis. S1P, synthesized by SphK in the cytosol, is transported to the extracellular space by transporters,
Sphingolipids are synthesized and metabolized by multiple pathways. Abnormal sphingolipid metabolism is implicated in excessive autophagy in cultured human trophoblast cells and in pregnant murine placentas, and necrosis in human choriocarcinoma JEG3 cells, primary isolated cytotoxic trophoblasts, and in human preeclamptic placentas (95–97). Progressive trophoblast cell death is a common feature of IUGR pregnancy, with or without preeclampsia. Furthermore, differences in sphingolipid metabolism may depend on trophoblast phenotypes, which are reported to be different between IUGR and preeclampsia (98). The serine palmitoyltransferase activity and the expression of sphingosine and sphingomyelin are high in choric arteries of the human preeclamptic placenta (99). In placental arterial endothelial cells, S1P content is reduced by heightened S1P phosphatase and lyase activity. Moreover, the equilibrium shift of S1PR expression/activity toward S1PR2 and lower S1PR1 promotes endothelial dysfunction in preeclampsia (99). The SphK/S1P/S1PR axis plays a crucial role in placental angiogenesis and endothelial-barrier function during pregnancy through downstream signaling of extracellular signal-regulated protein kinases 1/2 and phospholipase C (100,101). FTY720 (nonspecific agonist except for S1PR2) has been found to decrease the expression of vascular endothelial cell growth factor in human decidual natural killer (NK) cells, and to inhibit the migration and angiogenesis of decidual NK cell–mediated extravillous trophoblasts in vitro. Because S1PR5 is expressed predominantly in decidual NK cells, S1P signaling via S1PR5 may play an essential role in the angiogenic function of decidual NK cells and trophoblast migration during pregnancy (102).

The role of sphingolipids in hypertension and kidney disease in the offspring of patients with IUGR has not been investigated, despite strong evidence that IUGR is known to increase the risk of adult cardiovascular and renal diseases. IUGR often results from placental insufficiency and is related to an increase in perinatal morbidity and mortality. IUGR is commonly defined as a fetal gap—the inability to reach growth potential which is associated with a birth weight less than the tenth percentile of the average gestational age (103,104). Offspring of patients with IUGR have been shown to have low nephron numbers (105), which causes a reduction in filtration surface area, leading to systemic hypertension and progressive renal insufficiency; sequelae becomes even more severe with confounding factors such as excess dietary sodium. Clinical and animal data regarding the maturation of renal function in offspring resulting from IUGR show birth weight is positively associated with GFR and negatively associated with BP and serum creatinine (106,107). These data suggest that offspring of those with IUGR are at risk of developing hypertension and renal failure. The long-term effects of S1P on BP may involve S1P-induced modulation of renal blood flow and renal sodium handling. S1P mediates natriuresis via the activation of S1PR1 in the renal medulla of rats (108,109). S1P1 is a candidate gene that determines the response to salt in spontaneously hypertensive, stroke-prone rats (110). Given the essential role of sphingolipids on kidney function and control of BP, which are impaired in IUGR, it is possible that the sphingolipid pathway may play a role in the pathophysiology of IUGR and requires further investigation.

Summary

Sphingolipids are synthesized and metabolized by multiple pathways. Abnormal sphingolipid metabolism is implicated in...
various diseases, especially those involving the kidney. S1P is the most active sphingolipid metabolite and causes kidney inflammation and fibrosis through the SphK1/S1P/S1PRs axis. On the other hand, in fetal development, the Sphk/SIP/SIPR1 axis mediates angiogenesis, suppresses the immune response to the embryo, and is involved in uterine cell differentiation and proliferation, which are essential processes required to maintain proper placenta functions. Hence, inadequate SIP action causes IUGR, increasing the risk of adult-onset diseases—such as obesity, diabetes, hypertension, and cardiovascular and kidney diseases—and resulting in a high susceptibility to kidney injury (Figure 2) (111,112). It is possible that abnormal sphingolipid metabolism may be a result of alterations caused by these diseases. However, studies using pharmacologic inhibition and gene-deletion techniques raise the notion that sphingolipid metabolism may be one of the pivotal causes of kidney diseases. Controlling sphingolipid metabolism from the fetal period into adulthood determines our lifelong fate, including that of our kidney function. The effects of SIP on the endothelial barrier and sensitivity to vasoconstrictors also depend on its concentration (113). Further studies to determine the extent and timing of Sphk/SIP/SIPRs inhibition/activation and the conditions that regulate the effects of SIP will provide novel therapeutic targets against kidney disease, in general, and specifically against kidney disease induced by preeclampsia and IUGR.

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B. Bhunu, S. Intapad, and H. Toba were responsible for funding acquisition and validation; B. Bhunu, S. Intapad, H. Toba, and R. Yokota conceptualized the study, wrote the original draft, and reviewed and edited the manuscript; and S. Intapad provided supervision.

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