Sphingolipids and kidney disease: Possible role of preeclampsia and intrauterine growth restriction (IUGR)

Rodrigo Yokota\textsuperscript{1}, Benjamin Bhunu\textsuperscript{1}, Hiroe Toba\textsuperscript{2}, Suttira Intapad\textsuperscript{1}

\textsuperscript{1}Department of Pharmacology, Tulane University School of Medicine, New Orleans, LA 70112.
\textsuperscript{2}Department of Clinical Pharmacology, Division of Pathological Sciences, Kyoto Pharmaceutical University, Kyoto, Japan

Corresponding Author:
Suttira Intapad, PhD
Assistant Professor
Department of Pharmacology
Tulane University School of Medicine
1430 Tulane Avenue, #8683
New Orleans, LA 70112-2699
Email: sintapad@tulane.edu
PHONE: 504-988-9924
Abstract

Sphingolipids are now considered not only as constitutional components of the cellular membrane but also as essential bioactive factors regulating development and physiological 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 pathological conditions, including kidney disease. 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 metabolism 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 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 comprise 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 bio-functions, and sphingolipids and their metabolites are now known to be involved in cellular signaling regulating cell survival, growth, proliferation, differentiation, and cellular response 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 pathological states such as cancer, neurological disease, osteoporosis, diabetes, and atherosclerosis\(^2\)\(^10\)\(^-\)\(^13\).

Morbidity and mortality resulting from chronic kidney disease (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 pathophysiological roles of sphingolipid metabolism in CKD and its possible role in preeclampsia and IUGR.

Sphingolipid metabolism (See Figure 1)

1) *De novo* synthesis

Sphingolipid *de novo* synthesis occurs on the cytoplasmic side of the
endoplasmic reticulum through condensation of L-serine with palmitoyl Coenzyme A\textsuperscript{19,20}. The \textit{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 tumor necrosis factor (TNF)\textsuperscript{21}. After biosynthesis of 3-ketosphingosine and dihydrosphingosine (sphinganine), ceramide, a precursor and central molecule in sphingolipid biosynthesis, is formed by desaturation of dihydroceramide at C4-C5 of the sphingoid base\textsuperscript{22}.

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\textsuperscript{23,24}. Gangliosides are a minor class of glycosphingolipids containing complex attached sugar chains and are essential components of the plasma membranes. In some instances, glycosphingolipids may contain complex sugar chains such as N-acetylgalactosamine and N-glycolyneuraminic acid\textsuperscript{25,26}. Gangliosides with one or more N-acetylneuraminic acids (NANA) 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)\textsuperscript{27}.

2) Salvage pathway

Ceramide is also synthesized by a salvage pathway\textsuperscript{28}. In the presence of stress stimuli, sphingomyelin is broken down to ceramide and phosphocholine. This reaction is mediated by sphingomyelinase\textsuperscript{29,30}. Undesired complex sphingolipids such as glycosphingolipids may be broken down in the acidic environment of lysosomes or late endosomes to ceramide\textsuperscript{29}. 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\textsuperscript{30}. Alternatively, biosynthesized sphingosine may be phosphorylated by sphingosine kinase (SphK) to form sphingosine-1-phosphate (S1P), an essential cellular
signaling molecule. S1P can be degraded to 2-trans hexadecenal and phosphoethanolamine by S1P lyase.

**SphK/S1P/S1PRs axis in the kidney**

Two SphK isoforms were identified: SphK1 and SphK2, these are present in the cytosol and intracellular compartments, respectively. SphK1 is reported to exert anti-apoptotic function in renal mesangial cells by increasing S1P level, while cultured mesangial cells isolated from SphK2-knockout mice are resistant to apoptosis. In the human proximal tubular HK-2 cell line, SphK1 overexpression protects against peroxidase-induced necrosis by increasing S1P content.

S1P can act as an extracellular ligand for cell membrane receptors and intracellular signaling molecule. Five S1P receptors (S1PRs: S1PR1-S1PR5) were identified, S1PR1-3 are detected in renal medulla and glomeruli. The protein levels of S1PR1 and S1PR2, not S1PR3-S1PR5, are abundantly expressed in rat preglomerular microvessels. On the other hand, whole mouse kidneys express S1PR1-S1PR4 mRNA with a rank order of S1PR1>S1PR3>S1PR2>S1PR4 except S1PR5 mRNA, whereas cultured mesangial cells express all 5 receptors.

**Sphingolipids and kidney diseases**

Dyslipidemia: high levels of low-density lipoprotein-cholesterol and triglycerides in addition to a low level of high-density lipoprotein-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.

Some genetic disorders where sphingolipid metabolism is disrupted, exhibit renal damages indicating that the kidney is sensitive to sphingolipid alterations. Fabry’s disease is caused by α-galactosidase A (GLA) mutations, resulting in deficient activity of a lysosomal hydrolase and excessive accumulation of globotriaosylceramide (Gb3) in cells throughout the body, particularly cells in the kidney, heart, nervous and gastrointestinal system, and vasculature in the skin. 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 of
Gb3 action has been investigated in podocytes, which accumulate more in Gb3 than other renal cell types. The GLA gene knockdown by RNA interference and lentiviral transduction techniques upregulates LC3-II and downregulates mTOR kinase activity in podocytes, indicating dysregulation of autophagy. The deacetylated bioactive form of Gb3 activates the NOTCH signaling pathway, leading to a pro-inflammatory response, dedifferentiation, and extracellular matrix accumulation via nuclear factor-κB translocation.

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 the patients with diabetic nephropathy, polycystic kidney disease, renal cell carcinoma, lupus nephritis, age-related decreased kidney function, and their experimental models. 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. Since 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 end-stage renal disease. In patients with diabetes, high plasma levels of sphingolipids: including glycosphingolipids, ceramide, sphingosine, and sphinganine, have been observed. Inhibition of glucosylceramide formation suppresses pathological changes in diabetic rat kidneys, suggesting a pathogenic role of glycosphingolipid. Sphingomyelinase phosphodiesterase acid-like 3b (SMPDL3b) in the membrane lipid raft activates the conversion of sphingomyelin to ceramide and phosphorylcholine, 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. 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 acute kidney injury studies, demonstrating that sphingomyelinase activity and ceramide content increase in proportion to the extent of proximal tubule cell injury. Moreover, a selective S1P1R agonist, SEW2871, attenuates proteinuria in early-stage diabetic nephropathy in rats, suggesting a
beneficial property of S1PR1 stimulation. In the pathogenesis of type 2 diabetes mellitus, ceramide is reported to participate in islet β-cell dysfunction and apoptosis. 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. Long-chain saturated-free fatty acids-treatment promotes ceramide and diacylglycerol accumulation and blocks insulin signaling in C2C12 myotubes. Inhibition of de novo sphingolipid synthesis suppresses inflammatory cytokine release from murine 3T3-L1 cells.

Unlike the diabetic kidney, renal SMPDL3b levels are low in patients with focal segmental glomerulosclerosis (FSGS). This provides the possibility that the accumulation of sphingomyelin may participate in FSGS pathogenesis. Fornoni et al. revealed that serum from FSGS patients has decreased acid sphingomyelinase activity and SMPDL3b levels, FSGS is associated with increases in actin cytoskeletal remodeling and apoptosis in podocytes. FSGS is the most common cause of nephrotic syndrome and shows progressive renal dysfunction. Soluble urokinase plasminogen activator receptor (suPAR), which is elevated in serum from patients with FSGS, activates αVβ3 integrin in podocytes, leading to migratory phenotype. Serum suPAR levels are also elevated in patients with diabetic nephropathy. In podocytes treated with serum obtained from patients with diabetic nephropathy, SMPDL3b interacts with suPAR and renders podocyte change from a migratory to an apoptotic phenotype through increasing RhoA activity. 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. 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, including ATP-binding cassette (ABC) transporters. Exported S1P can bind to G protein-coupled receptors (S1PRs) on the plasma membrane in an autocrine fashion or different cell types. S1P released from glomerular mesangial cells can bind to S1PR2 and S1PR3 in the fibroblasts, activating the transforming growth factor (TGF)-β1/Smad pathway and triggering fibrogenic action and SphK1 production. Extracellular S1P can bind to S1PRs on immune cells such as macrophages and lymphocytes, leading to inflammation. This
vicious cycle is called the SphK1/S1P/S1PRs axis. Furthermore, overexpression of SphK1 and S1P is found in the diabetic kidney and high glucose-treated mesangial cells. The SphK activity and S1P level are increased in isolated glomeruli of diabetic rats. In the pathogenesis of diabetic nephropathy, differentiation of tubular epithelial cells and fibroblasts to myofibroblasts is thought to be mediated by in the SphK1/S1P/S1PRs axis, presumably via S1PR2 and subsequent Rho kinase activation. SphK2-knockout mice exhibit less renal fibrosis than wild-type and SphK1-knockout mice 14 days after acute kidney injury induced by folic acid or unilateral ischemia-reperfusion. Likewise, S1PR3 inhibition suppresses collagen deposition, myofibroblast differentiation, proteinuria, and leucocyte infiltration in the model of ureteral obstruction. FTY720, an immunosuppressive S1PRs ligand that functions as S1PRs antagonist, prevents inflammatory alterations in ureteral obstruction and angiotensin II treatment models. However, the S1P effects are diverse depending on receptor subtypes and pathological conditions. For instance, SphK1/S1P/S1PR1 axis in endothelial cells and proximal tubular cells plays important roles in protecting against renal ischemia-reperfusion injury. Bajwa et al. demonstrated the therapeutic effects of S1PR3-deficient bone marrow-derived dendritic cell transfers in renal ischemia-reperfusion injury through the expansion of splenic CD4(+)Foxp3(+)T(REG)s.

Possible role of sphingolipids in preeclampsia and IUGR?

Preeclampsia is a maternal gestational disease characterized by kidney dysfunction shown by proteinuria and hypertension after 20 weeks of gestation and is a major cause of maternal and fetal morbidity and mortality, including IUGR. The origin of the preeclampsia pathology is believed to be in the placenta, even though preeclampsia shows a high degree of heterogeneity in clinical features. Abnormal placentation (characterized with insufficient cytotrophoblast invasion of spinal arteries) and abnormal remodeling of decidual vessels limit placental perfusion, leading to release of placental factors into the maternal circulation, including soluble fms-like tyrosine kinase 1 (sFlt1). The sFlt is believed to inhibit vasodilation and induce maternal hypertension by antagonizing vascular endothelial cell growth factor (VEGF) action to produce nitric oxide.

In placentae from pregnancy complicated by IUGR without preeclampsia, low ceramide and high sphingosine levels are observed compared to age-matched controls.
Contrary to what is observed in IUGR pregnancy, acid ceramidase expression/activity and ceramide content are reported to increase in preeclampsia. In conjunction with the increase in de novo synthesis, ceramide overload causes excessive autophagy in cultured human trophoblast cells and in pregnant murine placentae and necroptosis in human choriocarcinoma JEG3 cells and primary isolated cytотrophoblasts and in human preeclamptic placentae. 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. The serine palmitoyltransferase activity and the expression of sphingosine and sphingomyelin are high in chorionic arteries of the human preeclamptic placenta. 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. The SphK/S1P/S1PR1 axis plays a crucial role in placental angiogenesis and endothelial barrier function during pregnancy through extracellular signal-regulated protein kinases (ERK) 1/2 and phospholipase C downstream signaling. FTY720 (non-specific agonist except for S1PR2) has been found to decrease the VEGF expression in human decidual natural killer (NK) cells and to inhibit decidual NK cell-mediated extravillous trophoblast migration and angiogenesis in vitro. Since S1PR5 is expressed predominantly in decidual NK cells, S1P signaling via S1PR5 may play an essential role in decidual NK cell angiogenic function and trophoblast migration during pregnancy.

The role of sphingolipids in hypertension and kidney disease in IUGR offspring 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—inability to reach growth potential associated with birth weight less than the 10th percentile of the average gestational age. IUGR offspring is associated with having low nephron numbers, causing the reduction in filtration surface area, which leads to systemic hypertension and progressive renal insufficiency; sequellae made even more severe with confounding factors such as excess dietary sodium. Clinical and animal data regarding the maturation of renal function in IUGRs shows birth weight is positively associated with glomerular filtration rate (GFR) and negatively associated with blood pressure and serum creatinine. These data suggest that IUGR individuals are at risk of
developing hypertension and renal failure. The long-term effects of S1P on blood pressure may involve S1P-induced modulation of renal blood flow and renal Na+ handling. S1P mediates natriuresis via the activation of S1PR1 in the renal medulla of rats\textsuperscript{109,110}. The \textit{s1p1} is a candidate gene that determines the response to salt in spontaneously hypertensive stroke-prone rats\textsuperscript{111}. Given the essential role of sphingolipids on kidney function and control of blood pressure, 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 of 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/S1P/S1PR1 axis mediates angiogenesis, suppresses immune response to embryos and uterine cell differentiation and proliferation, which are essential processes required to maintain proper placenta functions. Hence, inadequate S1P action causes IUGR, increasing the risk of adult-onset diseases such as obesity, diabetes, hypertension, cardiovascular and kidney diseases, and a high susceptibility to kidney injury (Figure2 )\textsuperscript{112,113}. There are possibilities that abnormal sphingolipid metabolism may be consequent alterations of the diseases. However, studies using pharmacological 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 kidney function. The effects of S1P on endothelial barrier and sensitivity to vasoconstrictors also depend on its concentrations\textsuperscript{114}. Further studies to determine the extent and timing of SphK/S1P/S1PRs inhibition/activation and the conditions which regulate the effects of S1P will provide a novel therapeutic target against kidney disease in general and specifically against kidney disease induced by preeclampsia and IUGR.
DISCLOSURES
All authors have nothing to disclose.

FUNDING
This work from S. Intapad was supported by the Norman Siegel Research Scholar Grant of the American Society of Nephrology (ASN) Foundation for Kidney Research, 16SDG27770041 from the American Heart Association (AHA), P20GM109036 from the National Institute of General Medical Sciences of the National Institutes of Health, and start-up funding from Tulane University. B. Bhunu was supported by the Fulbright Junior Staff Development Program fellowship awarded by the United States embassy in Zimbabwe. This work is also supported by grant-in-aids for scientific research from Hoansha Foundation to H. Toba.

ACKNOWLEDGMENTS
The author acknowledges Nancy Busija, M.A., for critically reading and editing of the manuscript.

AUTHOR CONTRIBUTIONS
R Yokota: Conceptualization; Writing - original draft; Writing - review and editing
B Bhunu: Conceptualization; Funding acquisition; Validation; Writing - original draft; Writing - review and editing
H Toba: Conceptualization; Funding acquisition; Validation; Writing - original draft; Writing - review and editing
S Intapad: Conceptualization; Funding acquisition; Supervision; Validation; Writing - original draft; Writing - review and editing
References

FIGURE LEGENDS

Figure 1. The Sphingolipid metabolism pathway

(de novo biosynthesis pathway): After condensation, serine palmitoyl CoA transferase (SPT) catalyzes the production of 3-ketodihydrosphingosine, which is a rate limiting step in sphingolipid metabolism\textsuperscript{115}. SPT belongs to the \(\alpha\)-axoamine synthase family and requires pyridoxal 5\(^{\prime}\) phosphate as a cofactor\textsuperscript{116}. The 3-ketodihydrosphingosine reductase converts 3-ketodihydrosphingosine to sphinganine by reducing the keto group. Sphinganine is then N-acylated in microsomes by one of the six isoforms of dihydroceramide synthase to generate different species of dihydroceramide, depending on the length of the fatty-acyl added\textsuperscript{8,117}. (Sphingomyelin pathway): Sphingomyelin is formed by the addition of phosphocholine to the C1 hydroxyl group of ceramides by sphingomyelin synthase in the Golgi apparatus\textsuperscript{118}. The phosphocholine comes from phosphatidylcholine, and diacylglycerol is released from the reaction. (Glycolipids pathway): Glycosphingolipids contain sugar residues attached to C1 hydroxyl group of the sphingoid base. Another salvage pathway to produce ceramide is mediated by sphingomyelinase, which degrades sphingomyelin (Sphingomyelin pathway). (Sphingosine-1-phosphate pathway): Ceramide biosynthesizes sphingosine-1-phosphate by sphingosine kinase-mediated phosphorylation.

Figure 2. Hypothesized roles of SphK/S1P/S1PRs axis in chronic kidney disease (CKD) and intrauterine growth restriction (IUGR).

Accerelation of SphK/S1P/S1PRs axis may induce inflammation and fibrosis, leading to CKD. On the other hand, the physiologically controlled SphK/S1P/S1PRs axis may essential in maintaining normal pregnancy through angiogenesis, suppression of the immune response to embryos, and uterine cell differentiation and proliferation.