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Critical role of the sphingolipid pathway in stroke: a review of current utility and potential therapeutic targets

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Abstract

Sphingolipids are a series of cell membrane derived lipids which act as signaling molecules and play a critical role in cell death and survival, proliferation, recognition and migration. Sphingosine 1 phosphate acts as a key signaling molecule and regulates lymphocyte trafficking, glial cell activation, vasoconstriction, endothelial barrier function and neuronal death pathways which plays a critical role in numerous neurological conditions.

Stroke is a second leading cause of death all over the world and effective therapies are still in great demand, including ischemic stroke and hemorrhagic stroke as well as the post stroke repair.

Significantly, sphingolipid activities change after stroke and correlate with stroke outcome, which has promoted efforts to testify whether sphingolipid pathway could be a novel therapeutic target in stroke.

Sphingolipid metabolic pathway, the connection between the pathway and stroke, as well as therapeutic interventions to manipulate the pathway to reduce stroke-induced brain injury are discussed in this review.

Keywords

Sphingolipids; sphingosine 1 phosphate; stroke; immune modulation

1. Introduction

The sphingolipids are a series of cell membrane derived lipids which act as signaling molecules and play a critical role in cell death and survival, proliferation, recognition and migration.(1–3) These essential lipids were first described by the Germany physician Ludwig Thudichum in his book “a treatise on the chemical constitution of the brain”.(1) The pro-apoptotic molecule ceramide (Cer) and the anti-apoptotic sphingosine-1-phosphate (S1P) act as the key signaling molecules in this pathway, whose balance is critical for

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sequelae after stressful stimulations. For example, S1P regulates lymphocyte trafficking,(4) glial cell activation,(2, 5) vasoconstriction,(6, 7) endothelial barrier function(8, 9) and neuronal death pathways,(10, 11) which act as important components in many neurological conditions.(1)

Stroke is the second leading cause of death in the world with an enormous public health burden. Effective therapies based on pathological mechanisms are still in great need.(12) Stroke causes prolonged major changes in sphingolipid signaling. This has promoted efforts examining whether the sphingolipid pathway is a novel therapeutic target in stroke.(1) This review mainly focuses on the sphingolipid metabolic pathway, the connection between the pathway and stroke, as well as tested and potential therapeutic interventions to manipulate that pathway to reduce stroke-induced brain injury.

2. The sphingolipid pathway

Sphingolipid de novo synthesis begins in the endoplasmic reticulum with the condensation of serine and palmitoyl coenzyme A (CoA) by serine palmitoyltransferase (SPT) to form 3-keto-dihydrosphingosine.(2) That compound is converted to dihydrosphingosine by 3-ketodihydrosphingosine reductase which is then N-acylated by ceramide synthases (CerS). The formed dihydroceramides are dehydrogenated to ceramide by desaturase. Ceramide can be transported from the endoplasmic reticulum to the Golgi by ceramide transfer protein (CERT) where it is used for sphingomyelin synthesis.(13) It can also be phosphorylated by ceramide kinase to form ceramide-1-phosphate (C1P).(2) Through sphingomyelin deacylase, it also can produce sphingosylphosphorylcholine (SPC) which is emerging as an important lipid mediator of cell regulation in multiple systems.(14, 15)

Although the sphingolipid family is derived from a common sphingosine backbone, the sphingosine molecule itself is not directly generated by de novo synthesis.(3) Sphingolipids have a rapid turnover and their levels are controlled by the balance between synthesis and degradation in multiple compartments, which forms a critical rheostat and their substrates are readily interconvertible.(1, 16) The production of ceramide, derived either from the membrane lipid sphingomyelin by sphingomyelinases or synthesized de novo, can be stimulated by many stressors.(1, 16) It acts as the central player of sphingolipid metabolism, being used to generate either complex sphingolipids, such as sphingomyelin and glycosphingolipid, or signaling molecules, such as C1P and S1P.(3) Ceramide is also degraded by ceramidase to sphingosine and this is the only known pathway to generate that molecule.(2) Additionally, the reutilization of sphingosine back to ceramide plays a critical role in sphingolipid homeostasis.(16) (Figure 1)

Phosphorylation of sphingosine by two isoforms of sphingosine kinase (SphK1 and SphK2) produces S1P, a crucial metabolite and intermediate in the sphingolipid pathway.(17) S1P is an anti-inflammatory, pro-proliferative and anti-apoptotic signaling molecule. S1P can be transported out of cells by spinster 2 (Spns2)(18) where it signals through a G protein-coupled receptor family (GPCRs). While originally known as endothelial differentiation genes (EDG),(19) they were renamed by IUPHAR as S1P₁₋₅.(20) S1P can then either be dephosphorylated by phosphatases, including two S1P-specific, endoplasmic reticulum-

localized phosphatases (SPP1 and SPP2), or irreversibly degraded by S1P lyase (SPL) to phosphoethanolamine and hexadecenal that can be incorporated into glycerolipids.(2) (Figure 1)

Ceramide

Ceramide is a hydrophobic backbone of sphingolipids. It participates in multiple biological processes and all of its specific functions have not yet been clearly elucidated.(2) Mammals have six Cer synthases (CERS1–6).(21) Ceramide, as a second messenger, can regulate multiple processes such as cell growth, differentiation, necrosis, proliferation and apoptosis and also play a part in angiotensin II type 2 receptor induced growth inhibition and apoptosis in cardiac and vascular tissues.(22) PP1, PP2A and PP2C are major serine/threonine protein phosphatases activated by ceramide and are responsible for pro-apoptotic functions, mainly involving PP2A derived dephosphorylation of Akt.(23–25) Stimulation of Protein kinase C (PKC) by ceramide is also supposed to relate to its membrane recruitment, which also results in Akt inhibition. Additionally, ceramide-enriched membrane microdomains (or platforms) play a critical role in cell signaling. Changes in membrane domains can alter membrane structure and interactions of lipids and signaling proteins. A role of such microdomains has been shown in numerous signaling pathways involved in immune cell activation and apoptosis induction.(2, 26) Alterations and protective functions of ceramide after stroke and correlations with preconditioning as well as subarachnoid hemorrhage (SAH) have been reported in several studies. (27–29)

Ceramide-1-phosphate (C1P)

Ceramide is phosphorylated by ceramide kinase (CERK) to form C1P. That molecule can activate cytosolic phospholipase-A2 α (cPLA2 α) and, thus, eicosanoid production.(2, 30) It can also inhibit tumor necrosis factor (TNF)-converting enzyme (TACE also known as ADAM17) which cleaves pro-TNF to release the active inflammatory form.(31)

Sphingosylphosphorylcholine (SPC)

SPC is another ceramide metabolite. It is converted from ceramide by sphingomyelin deacylase and it is now emerging as a crucial lipid mediator. It has effects on cell proliferation, migration, differentiation, metabolism and cell death in many systems including the cardiovascular system, immune system, central nervous system and skin.(14, 15) It is elevated in some pathological conditions(32–37) and was recently discovered as a pro-inflammatory mediator in cerebral arteries.(38) This, combined with previous evidence on a correlation with post-SAH vasospasm,(32, 37) makes SPC a promising novel target in stroke worthy of further investigation.

Sphingosine-1-phosphate (S1P)

S1P is a major intercellular signaling molecules controlling a wide variety of essential cellular processes such as cell growth, survival, motility, invasion into parenchyma, angiogenesis, trafficking of immune cells, endothelial barrier integrity and vascular contractility.(17, 39, 40) These functions of S1P are mediated by S1P₁-S1P₅ receptors

expressed on diverse cell types, including immune cells, endothelia and glia, which regulate multiple downstream signaling pathways.(5, 13)

S1P is present in blood and lymph in the submicromolar range but is present at much lower levels in tissues. This creates a sharp S1P gradient for crucial functions in immune cell trafficking regulation and maintenance of vascular integrity. The main source of S1P in blood is supposed to be erythrocytes and vascular endothelial cells(2, 41) while lymphatic endothelial cells mainly form lymph S1P.(2, 42) Transportation of S1P out of vascular and lymphatic endothelial cells is mainly through a specific transporter, Spns2.(18)

S1P receptors, expression and function

The multiple physiological functions of S1P are mediated by a series of specific, high-affinity GPCRs with five receptors, namely S1P₁-S1P₅. These were formerly termed endothelial differentiation genes EDG -1, EDG-5, EDG-3, EDG-6 and EDG-8, respectively. (5) Differential but overlapping expression patterns and intracellular signaling pathways for each receptor enable S1P to exert diverse functions. S1P receptors have differing expression in both the immune system and central nervous system (CNS), with versatile functions during growth and development as well as pathological processes (Table 1). Briefly, S1P₁₋₃ receptors are widely expressed but especially rich in the cardiovascular and immune systems.(5) S1P₄ is expressed in the lymphoid system(5, 43) and airway smooth muscle cells.(44, 45) S1P₅ is present in the white matter tracts of the central nervous system.(46, 47) The expression levels of S1P₄ and S1P₅ are relatively low compared to S1P₁₋₃. In the CNS, both in vivo and in vitro studies have shown that neurons principally express S1P₁ and S1P₃, microglia predominantly express S1P₁, while astrocytes and oligodendrocytes mainly express S1P₃ and S1P₅, respectively.(5)

Generally, S1P₁ couples with the G_i subunit of heterotrimeric G proteins, while S1P₂ and S1P₃ couple with G_i, G_q, and G_{12/13}, and S1P₄ and S1P₅ with G_i and G_{12/13}.(3) Signaling through G_i primarily leads to activation of the Ras/ERK pathway to promote proliferation, the PI3K/Akt pathway to prevent apoptosis, the PI3K/Rac pathway to promote cytoskeletal rearrangement and migration, and phospholipase C (PLC) activation to increase intracellular calcium and inhibit adenylyl cyclase to reduce Cyclic Adenosine monophosphate (cAMP) production. (Figure 2) Signaling through G_q could also activate the PLC pathway, while signaling through G_{12/13} promotes Rho activation to inhibit Rac and migration as well as barrier function. (3) (Figure 2)

The balance of signaling pathways downstream of each receptor plays a decisive role in the versatile function of S1P and that depends upon the expression pattern of S1P receptor subtypes in specific cell types. For example, S1P₁ and S1P₂ function competitively for cell migration. S1P₁ promotes migration via Rac activation,(48) whereas S1P₂ suppresses migration by Rho-mediated inhibition of Rac.(49, 50) Most of the S1P biological functions are considered to be mediated by S1P receptors mentioned above, but there are also several reports showing some intracellular S1P effects. Recent studies discovered that S1P binds to histone deacetylases (HDAC1 and HDAC2) inhibiting their enzymatic activity(51) and binds to TNF receptor associated factor 2 (TRAF2), a key component in Nuclear factor kappa B (NF- κ B) signaling triggered by Tumor necrosis factor alpha (TNF- α), stimulating its E3

ubiquitin ligase activity.(52) The physiological relevance of these findings remains questioned and merit further investigation.

The development of specific gene deletion models has more clearly defined the function of S1P receptors and sphingolipid pathway enzymes. (Table 1) Deletion of S1P₁ causes embryonic lethality between E12.5 and E14.5 due to excessive hemorrhage.(53) Additionally, a comparison between endothelial-specific deletion and global deletion indicates a critical role of S1P₁ in endothelial cells for vascular stabilization.(54) Dysfunction in neurogenesis can also be caused by S1P₁ depletion. Embryos at E12.5 have cell loss, increased apoptotic cells and decreased mitotic cells in different parts of the brain. (55) SphK1/SphK2 double knockout mice also show increased vascular leakage as well as neural cell death with similar outcomes to S1P₁ knockout mice.(55, 56) These results clearly demonstrate a crucial role of SphK/S1P/S1P₁ axis during neuronal/vascular development. The severest hemorrhage occurs in S1P₁-S1P₃ triple knockout mice during E10.5 and E11.5. (57) Taken as a whole, S1P₁ acts as a crucial role in vascular maturation with/without support from S1P₂ and S1P₃. Double depletion of S1P₂ and S1P₃ can cause similar dysfunction and embryonic death around E13.5 due to hemorrhage, while single depletion did not cause embryonic lethality.(57–59)

S1P₂ deficient mice are born without obvious deficits, but some will develop spontaneous, sporadic and occasionally lethal seizures between 3 to 7 weeks of age.(60) Simultaneously, a sharp increase in the excitability of neocortical pyramidal neurons was observed, indicating that S1P₂ is essential for the development and mediation of neuronal excitability. Moreover, S1P₂ is also crucial for auditory and vestibular system function.(61, 62) Several studies have also revealed that S1P₂ plays a critical role in angiogenesis and atherogenesis.(63–66) A recent study demonstrated a role of S1P₂ in vascular permeability and hemorrhagic complications after stroke.(67) Additionally, S1P₂ inhibits macrophage recruitment during inflammation.(68)

S1P₃ knockout mice also show no obvious phenotype.(59) However, the wide expression of S1P₃ in the cardiovascular system as well described functions in cardiovascular function (heart rate, blood pressure), myocardial perfusion, cardiomyocyte protection after ischemia/reperfusion (I/R) injury,(69–71) vasoconstriction(40) as well as cardiac fibrosis,(72) indicate S1P₃ is an important part of cardiovascular-related physiologic and pathologic processes. Additionally, S1P₃ also functions to stimulate of endothelial progenitor cells(73) and several reports have unfolded that S1P₃ participates in pro-inflammatory and coagulation related processes.(3) It has been reported that dendritic cell Protease activated receptor 1 (PAR1)-S1P₃ crosstalk plays a critical role in the connection between coagulation and inflammation. (3)

S1P₄ is expressed outside the CNS in lymphoid tissues.(5, 43) It has immunosuppressive effects by inhibiting T cell proliferation and secretion of cytokines. S1P₅ is mainly expressed in oligodendrocytes in the brain, but S1P₅ deficient mice do not show deficits in myelination.(47) In the immune system, S1P₅ is expressed on natural killer cells and regulates their trafficking.(5, 74, 75)

Taken together, S1P exerts its effects through specific receptors in many organ systems, most notably cardiovascular, immune and central nervous systems. Each receptor has differential but partially overlapping tissue distributions and intracellular signaling pathways, and sometimes they work cooperatively and sometimes competitively with each other (Table 1, Figure 2).

Besides the S1P receptors, changes in the enzymes involved in the sphingolipid pathway also have important roles in neurological conditions. The SphKs catalyzing S1P formation, SphK1 with cytoplasmic localization and SphK2 as the predominant SphK isoform in the brain, with primarily nuclear localization(76) as well as the S1P degrading enzymes S1P lyase (S1PL),(77) S1P phosphatases (SPP1 and SPP2), and the lysophospholipid phosphatase 3 (LPP3)(78) have been found to be altered and contribute to several neurological diseases and also represent potential targets for neurological disease intervention.(1)

3. Stroke and the sphingolipid pathway

Stroke is the second most common cause of death in the world.(12) Due to either blood vessel occlusion (ischemia) or rupture (hemorrhage), stroke leads to transient or permanent neurological deficits, leaving a heavy burden for society. In developed countries, up to 75%–80% of strokes are caused by acute ischemic stroke (AIS), while 10–15% of strokes are characterized as primary intracerebral hemorrhage (ICH) and approximately 5–10% are subarachnoid hemorrhage (SAH).(79) There are either no or very limited therapeutic options for stroke and these are desperately needed. Mounting evidence has shown an intimate relationship between stroke and sphingolipid pathway. (Table 2, Figure 3)

Cerebral Ischemia

Sudden thromboembolic occlusion of a brain vessel can cause hypoperfusion of the corresponding vascular territory leading to AIS and neurological deficits. To date, the only available therapies are tissue plasminogen activator (tPA) and thrombectomy to induce revascularization. However, due to a narrow therapeutic time window (4.5h) for tPA and unstable outcome of thrombectomy, only a small fraction of patients benefit from those therapies.(80–82) There is, therefore, an urgent need for other medical therapies for AIS. After a stroke, the central area of tissue damage gradually expands into surrounding tissue, the penumbra, and it that tissue that may be rescued.(12, 83)

The overall pathology after AIS involves many components including oxidative stress, immune activation, blood-brain barrier (BBB) damage and neuronal death.(84, 85) Mounting evidence indicates that the immune response after AIS plays a critical role in stroke outcome. The release of cell death products, such as Damage associated molecular pattern molecules (DAMPs), activates pattern recognition receptors (PRR), for instance, Toll-like receptors (TLR), resulting in both an innate and an adaptive immune response. There is activation of resident microglia as well as a serial infiltration of inflammatory components into the brain through a damaged BBB, which further aggravates brain tissue damage.(86)

Amplification of initial brain injury by inflammatory mediators continues well beyond 4.5h of stroke onset, the current tPA time window, making it a promising therapeutic target.(84, 87) Early, microglia/macrophages and neutrophils are crucial players in the inflammation. (84, 86) Cytokines, complement and, in delayed brain inflammation, T and B lymphocytes as well as Natural killer (NK) cells, have also been reported to contribute to post-ischemic inflammation.(86–93) Considering the regulatory functions of the sphingolipid signaling pathway in immune system and endothelial biology, interventions targeting that pathway are a promising approach in stroke therapy.

To date, a mounting number of *in vivo* and *in vitro* preclinical studies and pilot human studies have shown therapeutic effects of S1P receptor modulation and elucidated possible mechanisms by which the SphK-S1P axis affects stroke.(67, 94–107) For example, preclinically, fingolimod (FTY720), a S1P receptor modulator, has shown short and long-term protection in rodent models of AIS.(94–96) Reduction in lesion size and improvement in neurological outcomes as well as decreased cell death can be observed after fingolimod treatment up to 15 days after onset.(94–96) A reduction of nuclear translocation of apoptosis inducing factor (AIF), caspase-3 cleavage and TUNEL positive neurons, as well as up-regulation of anti-apoptotic pathways such as extracellular signal regulated kinase (ERK1/2), protein kinase B/Akt kinase (PKB/Akt) and Bcl-2 were observed after administration of fingolimod. This supports the hypothesis that fingolimod exerts direct neuronal protection. (95, 96) Moreover, decreased immune cell activation and infiltration (such as activated neutrophils, microglia/macrophages) and adhesion molecule (ICAM-1) expression in the ipsilateral hemisphere has been detected.(94–96) Inspired by such preclinical studies, a pilot study examined the effects of fingolimod in 22 patients at 4.5h after stroke onset. It found evidence of neuroprotection with reduced lymphocyte counts, improved neurological dysfunction, milder lesion enlargement and decreased microvascular permeability,(102) which coincides well with the previous rodent studies.

The effects of fingolimod are cell-type specific. It can regulate the polarization on microglia activation.(108) Decreased ICAM-1 expression in brain microvascular endothelial cells, an indicator of the inflammatory response, was found after fingolimod administration but protection was not found in neuronal cell cultures subjected to conditions to simulate ischemia (glutamate, H₂O₂ or hypoxia).(96, 109) Considering potential differences between *in vitro* and *in vivo* conditions, further exploration of fingolimod cell-specific effects are needed. Interestingly, a reduction of microvascular thrombosis in peri-infarct area was observed after fingolimod administration which identified a correlation between inflammation and thrombus formation, i.e. thromboinflammation.(100) Using Rag^{-/-} mice and cell culture, Kraft et al. found lymphopenia and reduced microvascular thrombosis but not direct neuroprotection after fingolimod intervention.(100) Thus, the mode of action of fingolimod on secondary occlusion and post-stroke coagulation deserves further investigation.

Considering the non-selective function of fingolimod on S1P₁, S1P₃, S1P₄ and S1P₅, and corresponding complications, the crucial sphingolipid receptor target in stroke intervention remains blurred and deserves further investigation. Hasegawa et al. used the selective S1P₁ agonist SEW2871 and S1P₁, S1P₃, S1P₄ antagonist VPC20319 to test the role of S1P₁ in

stroke intervention and found that S1P₁ activation can reduce neuronal death and plays a critical role in the overall outcome of ischemic stroke.(95) As S1P₃ has mutual functions with thrombin and PAR-1 in regulating coagulation and vascular permeability,(3) the thrombus formation after stroke may not only correlate to S1P₁ in lymphocyte but also S1P₃ in endothelial cells.

S1P₂ has also recently been found to be crucial in endothelial function. Experiments using gene knockout and a selective S1PR₂ antagonist (JTE013) both *in vitro* and *in vivo* in rodent and human specimens indicate it protects against vascular dysfunction after I/R injury.(67) Effects on BBB permeability, matrix metalloproteinase 9 (MMP-9) expression and hemorrhagic transformation were observed, but not on neuronal survival, indicating a critical role of S1P₂ in endothelial function. One area that deserves investigation is whether S1P₂ may impact glucose-induced hemorrhagic transformation. Hyperglycemia has positive relationship with I/R injury and post-stroke hemorrhagic transformation in animal models and clinically.(110, 111)

The function of SphK should also not be neglected. SphK1 and SphK2, by regulating S1P formation, are key components of the sphingolipid pathway.(2) SphK2 is essential for phosphorylation of fingolimod with its protective effects in ischemic stroke.(97) Gene deletion studies on SphK1 and SphK2 demonstrated that SphK2 provides support in stroke protection with decreased lesion size and better neurological function.(97) The specific mechanisms need further elucidation and further attention on SphK2-S1P axis in stroke intervention should be paid a high priority.

Additionally, combined use of tPA and fingolimod has raised attention and gained partially positive results,(67, 105, 112). The need for a combination approach is based on potential side-effects of tPA therapy, ischemia/reperfusion (I/R) injury and high risk of fatal hemorrhagic transformation, as well as its narrow therapeutic window.(113–115) Therefore, an ideal combination or intervention is of great significance for better use of recanalization and reperfusion by tPA. The function of S1P signal pathway in endothelial cells has been tested by administration of non-selective S1PR modulator fingolimod in rodent and human, as well as mechanism study of S1P₂ in endothelial protection.(67, 105, 112) Using a model of thromboembolic stroke, Campos et al. found that fingolimod reduced neurological deficit, infarct volume and especially reduced the risk of hemorrhagic transformation and BBB damage secondary to delayed use of tPA.(105) In contrast, in a stroke model of large hemispheric infarction, no benefit was shown, especially on functional outcome and BBB integrity, when fingolimod was administered alone or in combination with tPA.(116) A disadvantage of this study is the lack of measurement of hemorrhagic transformation and infarct volume. Considering the severe outcome of this 3h MCAO model, different measurement of neurological function may also be applied. Moreover, a multicenter pilot trial examined using fingolimod combined with tPA within 4.5h after stroke onset in patients with hemispheric ischemic stroke stemming from anterior or middle cerebral arterial occlusion. Patients had milder reperfusion injury and improved clinical outcomes.(112) The specific role of S1PR₂ in hemorrhagic transformation has been discussed and further manifested the correlation between sphingolipids and hemorrhagic transformation. (67)

Taken as a whole, the function of the sphingolipid pathway in I/R injury has gained much attention and partially positive results. It deserves further investigation and detailed dissection of mechanism. The effects of manipulating the sphingolipid pathway along with tPA administration to reduce I/R injury needs further models to mimic the exact pathology of endothelial function and neurological deterioration after I/R injury with tPA. In addition, the use of a relatively non-selective S1P receptor modulator, fingolimod, makes identification of the exact S1P signaling events involved lack of specify. The possible function of sphingolipid pathway in widening the therapeutic window and its function on large infarction patients, especially on hemorrhagic transformation, still need to be taken into consideration in future studies.

Intracerebral hemorrhage

ICH is a devastating disease with high mortality and morbidity with no current therapy. (117–122) Considering the aging population, the incidence of ICH is expected to increase. (123) ICH is classified as either primary or secondary, based on bleeding cause. Primary ICH, which occurs in ~80% of patients, results from spontaneous blood vessel rupture (primarily due to hypertension), whereas in secondary ICH the underlying cause is another condition such as brain tumors or traumatic brain injury. (124) Irrespective of type, ICH results in a hematoma, edema, inflammation, apoptosis and necrosis. (118, 125)

ICH induces both primary and secondary injury. (126) The primary injury is caused by hematoma formation and its expansion which leads to mechanical damage, compression of surrounding brain tissue, increased intracranial pressure, followed potentially by oligemia, neurotransmitter release, mitochondrial disturbances, and membrane depolarization. (127–129) Secondary injury is, however, an important component. It involves cytotoxic, excitotoxic, oxidative and inflammation pathways, and brain edema. (130–132) Peri-hematoma edema exacerbates the hematoma mass effect, aggravates secondary brain injury and cell death process through subsequent oligemia and inflammatory insults. (117, 123, 124) Edema has the potential to negatively impact ICH outcome. (133–135) Thrombin, inflammatory mediators and the release of clot components, such as hemoglobin and iron, are major causes of cerebral edema and brain injury after ICH. (117–119, 136, 137)

Thrombin is a critical factor in ICH both as a component of the coagulation cascade that limits bleeding but also as a factor that can induce injury mainly through PAR-1. (138) Thus, it can trigger inflammatory cell infiltration, BBB damage and edema, and neuron and astrocyte death. (117–119, 138) The functions of thrombin may be receptor or non-receptor (e.g. fibrinogen cleavage) mediated. There are three receptors, PAR-1, PAR-3 and PAR-4, with PAR-1 being the main subtype mediating several pathological effects. (139) And thrombin inhibition can be protective in hemorrhagic stroke models. (140, 141)

S1P₁ plays a role in activated protein C (aPC)-mediated endothelial barrier protection, either by SphK1-mediated S1P production or by EPCR-mediated direct trans-activation of S1P₁, while a differential coupling of thrombin-activated PAR-1 with S1P₃ leads to endothelial barrier disruption. (3) Thus, whether thrombin or aPC/EPCR is the activating protease in combination with the presence of S1P₁ or S1P₃ determines PAR-1 signaling specificity in endothelial cells during vascular permeability regulation. (3, 142–146) Therefore, the SphK-

S1P signaling pathway plays a critical role in thrombin-induced ICH injury and may be a potential therapeutic target.

Inflammation has emerged as a crucial component during the onset and progression of ICH, with cross-talk between multiple pathways.(87, 124, 133, 147–149) Brain-intrinsic microglia are the first inflammatory cell type to react to brain injury. By releasing inflammatory mediators, they contribute to BBB breakdown and leukocyte influx from blood. These include neutrophils, monocytes and macrophages, followed by lymphocyte populations. (150–153) Activated resident and migrant cells also produce cytokines and chemokines and that, together with the cell death products, further exacerbates the inflammatory process causing peri-hematoma edema and subsequent brain injury.(124, 154, 155) Because of the effects of the sphingolipid pathway on inflammatory and endothelial cells, it may be a target to affect these processes.

In rodents, administration of the S1PR modulator fingolimod ICH reduced the inflammatory response, decreasing CD3⁺ T lymphocyte infiltration and endothelial ICAM-1 expression, as well as interferon- γ (IFN- γ)and interleukin (IL)-17 levels in brain.(156) A number of studies have also found improvements in neurological function, as well as reductions in brain edema and neuronal death,(156–158) indicating the possible efficacy of targeting to S1PR in ICH. Investigations comparing single and consecutive doses found a better outcome with continuous dosing which suggest a prolonged therapeutic window for ICH. Further studies are needed to define the therapeutic time window and care is needed in relation to long-term dosing and immune suppression.

There has been a randomized, placebo-controlled, pilot study with 23 ICH patients with 5–30 ml hematoma volumes. Patients who were given fingolimod orally for 3 consecutive days had gained improved neurological function, decreased inflammation and peri-hematoma edema.(159) Moreover, short- and long-term benefits have been found with fingolimod administration in neurological deficits, cell death and brain atrophy.(156–158) This supports the concept of targeting the sphingolipid pathway in ICH treatment. However, no significant change in microglia/macrophages was found using CD68 as marker has been observed in a rodent experiment,(157) which leaves a question about the critical target of the S1P signaling pathway in ICH intervention.

Intraventricular hemorrhage (IVH) is bleeding into the brain ventricles. It occurs secondary to ICH and SAH (160)and it is associated with poor prognosis for hemorrhagic stroke.(161) Severe IVH leads to hydrocephalus and has a great risk of hemorrhage-associated morbidity. Unfortunately, no effective therapies exist to date.(162) As with ICH, there is a role for inflammation and the coagulation cascade in IVH-induced brain injury and there is also a role for those processes in IVH-induced hydrocephalus.(163) As the sphingolipid pathway has effects on inflammation and coagulation, it is may be a therapeutic target for IVH-induced hydrocephalus and sphingolipid pathway, but this has not been examined.

Subarachnoid hemorrhage

Although SAH only accounts for ~5% of all strokes, it has a particularly high mortality and morbidity.(164) Early brain injury after SAH, which happens before the onset of delayed

vasospasm, can cause death.(165) However, SAH has a biphasic time course, with that initial hemorrhagic insult being followed by delayed cerebral ischemia (DCI) within 4 to 12 days. (166–170) DCI may cause half of the mortality in SAH and it has been a major focus of SAH research.(166–168) Cerebral vasospasm, a potential cause of DCI, is a frequent complication of SAH and it has been regarded as a major determinant of outcome. Angiographic vasospasm is mainly driven by marked alterations in cerebrovascular response which compromise cerebral auto-regulation.(168, 171, 172) However, despite a clear correlation between hypoperfusion and vasospasm, cerebral blood flow deficits do exist in regions without vasospasm.(173) A general amplification of myogenic tone in the cerebral microcirculation may be a more meaningful regulator for SAH outcome by comprehensively altering perfusion deficits.(174) Therapeutic interventions should particularly correct the increased myogenic tone in SAH.

During the past decades, the role of the sphingolipid pathway in SAH has received considerable attention.(32, 37, 38, 175–179) S1P was first found to induce vasoconstriction in canine basilar artery and SPC was determined to be a novel mediator of Rho-kinase induced cerebral vasospasm and act as a proinflammatory mediator in cerebral arteries. Further, SPC cerebrospinal fluid (CSF) levels are elevated in SAH patients.(32, 37, 38, 176) Moreover, sphingolipids and CSF ceramide alterations after SAH have also been detected and correlate with clinical outcome. In the CSF of patients with SAH, ceramide is increased and an activation of sphingomyelinae (ASMase), S1P-lyase, and glucosylceramide synthase (GCS) lead to an alteration in the production of protective (S1P) in favor of deleterious (Ceramide) sphingolipids.(175, 177) Using SphK1^{-/-} mice and S1PR antagonism (JTE013), SAH significantly presented an increase in myogenic tone and JTE013 can fully reverse the myogenic tone augmentation both *in vivo* and *in vitro*, which reveals a critical role of S1P signaling pathway as a mediator of increased myogenic tone in SAH.(178) Interestingly, S1P₃ receptors are also reported to mediate the potent constriction of cerebral arteries by S1P,(40) thus providing a novel target of S1P₁/S1P₃ system in regulating the vascular response after SAH. Tang et al. also observed the expression of S1P on the cerebral vasospasm after SAH using a “double hemorrhage” model in rabbit,(179) and they found S1P expression a new marker for the development of cerebral vasospasm. Also, targeting either TNF- α or the S1P signaling pathway after SAH attenuated neuronal apoptosis and improved neurological outcome.(178) Furthermore, the specific role of SphK1 in vasoconstriction has also been demonstrated in isolated basilar arteries.(160, 180) Thus, the sphingolipid pathway plays an important role in SAH and especially vasospasm. However, due to the complicated pathology of SAH, more details on the pathological target and the specific functions of S1P receptors are still needed.

Preconditioning

Preconditioning is a process by which a sublethal noxious stimulation induces a robust protection or tolerance against subsequent lethal injuries.(181–184) It has been observed in multiple organs, including brain. Moreover, a wide range of stimuli can induce preconditioning, e.g. hypoxia, transient ischemia, hyperbaric oxygen, hypothermia, hyperthermia, inhalational anesthetics (isoflurane) or inflammatory agents such as bacterial lipopolysaccharide.(181) Interestingly, cross-talk exists between different stimuli which

means one type of preconditioning can protect against a wide range of other stresses.(181) Brain preconditioning involves multiple downstream pathways including: metabolic inhibition, activation of extra- and intra- cellular defense mechanisms, disturbance of neuronal excitatory/ inhibitory balance, and suppression of inflammatory sequelae.(181)

Hypoxia inducible factor (HIF) is a crucial factor involved in preconditioning. It is stabilized by hypoxia and ischemia, as well as non-hypoxia stimuli involved in preconditioning such as isoflurane or lipopolysaccharide (LPS). It exists in three isoforms which bind to hypoxia-responsive elements (HRE) of hypoxia regulated genes to promote their transcription.(1) A relationship between hypoxia preconditioning and the sphingolipid pathway has been shown by several in vivo and in vitro studies.(27–29, 185–193) Ceramide is critical in hypoxia-induced preconditioning both in cultured neurons(28) and in rat brain.(27) It plays a role as a mediator during the protective process and is capable of up-regulating B cell lymphoma 2 (Bcl-2) while decreasing TUNEL positive cells.(27) Additionally, ceramide is critical in the induction of ischemic tolerance in ischemia- and LPS-induced preconditioning.(29, 189) Elements of the sphingolipid pathway downstream of ceramide, especially SphK2, are also involved in preconditioning.(190–192) SphK2 is proposed to act as a proximal trigger to drive S1P mediating alterations in gene expression and, thus, to promote the ischemia-tolerant phenotype.(191) Moreover, SphK2 is also involved in BBB protection during preconditioning.(190) Recent evidence also suggests that cross-talk between HIF and the SphK2 produced S1P signaling can co-upregulate chemokine (C-C motif) ligand 2 (CCL2) expression, which can contribute to the stroke-tolerant phenotype established by hypoxic and S1P agonist preconditioning.(186)

Isoflurane can also induce preconditioning with protective effects in several type of stroke.(194) Those effects correlate with changes in the sphingolipid pathway,(188) especially activation of the S1P/ PI3K/Akt pathway.(187) Moreover, isoflurane can protect the BBB, an effect mainly mediated by SphK1 expression and S1P₁/S1P₃ activation.(193) A recent study also revealed that preconditioning-stimulated autophagy is mediated by activation of SphK2, and proposed to function through Beclin 1/ Bcl-2 interaction.(185)

Considering the complicated mechanisms underlying preconditioning and the possible pathways enrolled, the sphingolipid pathway has shown a close relationship to preconditioning-induced protection of preconditioning, and particularly hypoxia, ischemia and isoflurane preconditioning.(1) The possible mechanisms involved may be multi-fold and both upstream and downstream investigations are still necessary.

Post-stroke repair

Post-stroke neurogenesis plays a significant role in overall stroke outcome. It involves three major steps: proliferation, migration and differentiation into neural phenotypes, with proliferation having a major role.(195) Studies on the role of the sphingolipid pathway in recovery have mainly focused on astrogliosis (178) and neural progenitor cells.(196, 197) Brain injury induces astrogliosis which involves transcriptional, biochemical and morphological changes in astrocytes.(198) The glial scar can have both negative and positive impacts on recovery. Modulating the S1P receptor signaling with fingolimod has a protective effect on stroke recovery at later time points using due to reduced astrogliosis, modulation of

synaptic morphology and increased neurotrophic factor expression.(191) Significantly, S1P can also induce neural progenitor cells (NPC) migration and regulation of S1P₂ can promote migration towards injured brain.(196) S1P also reduces glial cell line derived neurotrophic factor levels.(199) Moreover, S1P has a versatile role in neurogenesis, possibly through a pathway involving MAP kinase (MAPK) and Rho kinase.(197) Additionally, fingolimod can increase brain-derived neurotrophic factor in Rett syndrome(200) and amyloid β -induced memory impairment.(201, 202)

4. Conclusions

Mounting evidence during the past two decades from both animals and humans, both *in vivo* and *in vitro*, has indicated a major role of the sphingolipid pathway in stroke pathology. In addition, many studies have shown that it is a potential therapeutic target for stroke, those who are inconsistent with the STAIR and RIGOR criteria are not included.(203, 204) There is much still to learn about the underlying effects of the sphingolipid pathway in stroke and more investigations are still greatly needed to elucidate novel targets and provide a solid foundation for further clinical practice in stroke management.

Search criteria

This review focuses on research in sphingolipid pathway in stroke. We searched Pubmed with the terms “sphingolipid” “sphingosine” “sphingosine 1 phosphate” “stroke” “isch(a)emic stroke” “intracerebral h(a)emorrhage” “subarachnoid h(a)emorrhage” “h(a)emorrhage” “preconditioning” “repair” and retrieved all articles published in English. We selected articles for their conceptual importance and primacy and complied with STAIR and RIGOR criteria. For controversial issues, evidence on both sides was sought.

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Figure 1. Sphingolipid metabolism pathway of S1P receptor signaling

De novo sphingolipid metabolism occurs at the endoplasmic reticulum (ER) and starts with the condensation of palmitoyl coenzyme A (CoA) and serine by serine palmitoyltransferase (SPT), which can be negatively regulated by ORM1-like protein 3 (ORMDL3), followed by series of enzyme catalyzed reactions finally forming ceramide. Ceramide, generated either from the de novo pathway or from degradation of sphingomyelin (SM) or glycosphingolipids (GluCer) can be either phosphorylated by ceramide kinase (CerK) to ceramide-1-phosphate (C1P) or transferred into sphingosylphosphorylcholine (SPC) by SM deacylase or deacylated by ceramidase to sphingosine, which is then phosphorylated to sphingosine-1-phosphate (S1P) by sphingosine kinases (SphKs). S1P can be dephosphorylated to sphingosine by a sphingosine phosphate phosphatase (SPPase) or converted to phosphoethanolamine and hexadecenal by S1P lyase (SPL) which can then be further converted to glycerolipids.

S1P is exported from cells by a specific transporter, Spns2, and carried by proteins such as high density lipoprotein (HDL)-associated apoM and albumin to the circulation.

Extracellular S1P also activates five S1P receptors, namely S1P₁₋₅ and function in diverse cell signaling pathways.

CDase, ceramidase; CerS, ceramide synthase; GCS, glucosylceramide synthase; GCCase, glucosylceramidase; ORMDL3, ORM1-like protein 3; PtdEtn, phosphatidylethanolamine; Pase, phosphatase; SMase, sphingomyelinases; SMS, sphingomyelin synthase.

Figure 2. Biological functions of S1P receptor signaling

S1P can combine with five S1P receptors which is S1P₁₋₅. These modulate diverse intracellular signals pathways dependent upon the coupled G proteins and expression of each receptor in specific cell types. S1P₁₋₅ couples with the G_i subunit and can function in vasorelaxation and cell survival, proliferation and migration as well as barrier integrity, S1P₂₋₅ can couple with G_{12/13} and play a role in cell migration barrier integrity and migration in oligodendrocyte precursor cells, and S1P₂₋₃ couple with G_q which play a part in vasoconstriction.

PI3K, phosphatidylinositol 3 kinases; PKB, protein kinase B; PLC, phospholipase C.

Figure 3. Interventions and outcomes of sphingolipid pathway in ischemic stroke

Different interventions aiming at sphingolipid pathway in ischemic stroke with outcomes summarized. Numbers represents the amount of studies aiming at this outcome and the total amount of studies of this intervention.

Table 1

Expression and function of SIP receptors and corresponding agonist and antagonist

Receptor	Coupling	mRNA distribution	Cellular expression			Knock out phenotype	Agonist	Antagonist
			CNS	Immune system	Other cells			
SIP ₁ (EDG1)	G _{i10}	Brain, heart, spleen, liver, lung, thymus, kidney, skeletal muscle, lymphoid	Neurons, astrocytes, oligodendrocyte, microglia	Lymphocytes, mast cells, eosinophils	Atrial myocytes, endothelial cells, smooth muscle cells, Schwann cells	Embryonic lethal (E12.5-E14.5), lymphopenia, vascular maturation defects, reduced lymphocyte egress, impaired astrogenesis, impaired neurogenesis	FTY720-P, KRP-203, AUY954, SEW2871, CYM-5442, Arylpropionic acids	VPC-23019, W123 (R)-W146, VPC44116
SIP ₂ (EDG5)	G _{i10} , G _q and G _{12/13}	Brain, heart, spleen, liver, lung, thymus, kidney, skeletal muscle	Neurons, astrocytes, microglia	NA	Smooth muscle cells, Schwann cells	Spontaneous seizures, increased neuronal excitability, deafness, increased cerebrovascular permeability	CYM-5520 (allostERIC)	JTE-013
SIP ₃ (EDG3)	G _{i10} , G _q and G _{12/13}	Brain, heart, spleen, liver, lung, thymus, kidney, testis, skeletal muscle	Neurons, astrocytes, microglia	NA	Atrial myocytes, endothelial cells, smooth muscle cells, lung, kidney, intestine, cartilage, Schwann cells	Increased permeability of pulmonary epithelial barrier	FTY720-P, KRP-203, CYM-5541 (allostERIC)	VPC-23019, SPM-202, SPM-354
SIP ₄ (EDG6)	G _{i10} and G _{12/13}	lymphoid, lung	NA	Leukocytes	Schwann cells	NA	FTY720-P, KRP-203, CYM-50308 (selective)	CYM-50374
SIP ₅ (EDG8)	G _{i10} and G _{12/13}	Brain, skin, spleen	Oligodendrocyte, astrocytes, microglia	NK cells	Schwann cells	Preserved myelination, aberrant NK cell homing	FTY720-P, KRP-203	NA

CNS = Central nervous system; NK = Natural Killer cells; NA=Not applicable.

Table 2

Investigations on sphingolipid pathway in stroke

Stroke subtype	Target lysophospholipids	Animal, stroke model	Human specimens / Patients	Intervention	Results	Reference	Year
AIS	GSLs	Male S-D rats, permanent MCAO	-	One week after MCAO, L-PDMP 40mg/kg, i.p., B.i.d., for 14 consecutive days	L-PDMP can improve re-acquisition of memory and learning function, indicated a neuronal protective effect of L-PDMP involving glucosylceramide biosynthesis	Hisaki et al.	2008
AIS	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	Male C57BL/6 mice, tMCAO (90min)	-	Onset of MCAO, FTY720 1mg/kg, i.p.	FTY720 reduces lesion size and improve neurological outcome	Czech et al.	2009
AIS	SIP ₁	Male S-D rats, tMCAO (2h)	-	Immediately after reperfusion, FTY720 0.25mg/kg and 1mg/kg, SEW2871 1mg/kg, VPC23019 5mg/kg, 0.5mg/kg, i.p.	FTY720 can reduce infarct volume and improve neurological score and decreased apoptosis, the protective function may mainly act through SIP ₁	Hasegawa et al.	2010
AIS	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	Male C57BL/6 mice, tMCAO (90min), permanent MCAO	-	30min after reperfusion, FTY720 0.5mg/kg and 1mg/kg, i.p.	FTY720 can reduce infarct volume and improve neurological outcome up to 15 days mainly through vasculo-protection rather than direct neuronal protection.	Wei et al.	2011
AIS	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	Male C57BL/6 mice, permanent MCAO	-	48h before or 3h after occlusion, FTY720 1mg/kg, p.o. (single dose) / 1.p. (Q.d.)	FTY720 reduces CNS lymphocyte infiltration but no effect on infarct volume alleviation or neurobehavior promotion.	Liesz et al.	2011
AIS	SphK2	C57BL/6 mice, SphK1 ^{-/-} mice, SphK2 ^{-/-} mice, tMCAO (120min)	-	Immediately after occlusion, FTY720 1mg/kg, i.p.	SphK2 ^{-/-} mice have larger ischemic lesions and more severe neurological deficit as well as abolished protective function of FTY720, indicated an endogenous protective mechanism of SphK2 in AIS	Pfeilschifter et al.	2011
AIS	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	Male C57BL/6 mice and Rag1 ^{-/-} mice, tMCAO (60min or 90min)	-	Immediately before reperfusion, FTY720 1mg/kg, i.p.	FTY720 reduces lesion size and improve neurological outcome by induction of lymphopenia and microvascular thrombosis but not direct neuronal protection	Kraft et al.	2013
AIS	SphK1, SphK2, SIP ₁	Male S-D rats, tMCAO (2h)	-	Immediately after reperfusion, FTY720 0.25mg/kg, i.p.	SIP ₁ , SphK1, and SphK2 were downregulated in the infarct cortex, but preserved in the peri-infarct cortex after MCAO, activation of the sphingosine metabolic pathway may be neuroprotective in AIS, FTY720 reduced neuronal injury possibly via SIP ₁ activation.	Hasegawa et al.	2013
AIS	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	-	22 patients with anterior circulation ischemic stroke, 4.5–72h after onset	Beginning within 1h after baseline MRI and no later than 72h after onset, FTY720 0.5mg,	FTY720 can reduce secondary lesion enlargement, microvascular permeability, and improve clinical outcomes during the acute phase and 3-mo follow-up visit.	Fu et al.	2014

Stroke subtype	Target lysophospholipids	Animal, stroke model	Human specimens / Patients	Intervention	Results	Reference	Year
AIS	SphK1	Male CD-1 mice, tMCAO (60min)	(more than 4.5h)	p.o., Q.d., for 3 consecutive days 3, 12, 24, 48 and 72h after reperfusion, SphK1 inhibitor 5C (CAY10621) 2mg/kg, i.v.	SphK1 but not SphK2 was induced in ischemic brains over 96h after stroke, inhibition or knockdown of SphK1 improved the outcomes following stroke and suppressed post-ischemic neuroinflammation. SphK1 can also reduce microglia induced inflammation in vitro	Zheng et al.	2015
AIS	SIP ₂	Male C57BL/6 mice and SIP ₂ ^{-/-} mice, tMCAO (90min or 180min)	Slice of human pial vessels and microvessels	10min or 1.7h or 4.5h or 7.5h after reperfusion, JTE013 10mg/kg or 30mg/kg, oral gavage	SIP ₂ plays a critical role in the induction of cerebrovascular permeability, development of hemorrhage and neurovascular injury in experimental stroke. SIP ₂ also correlate with MMP-9 expression and regulate endothelium response after injury.	Kim et al.	2015
AIS	SphK2	Male S-D rats, tMCAO (60min)	-	3, 6 or 9h after MCAO, Allicin 50mg/kg, i.p.	Allicin can reduce infarct volume, attenuate brain edema and improve neurological outcome as well as decrease neuronal death. SphK2 mediated mechanism was involved in allicin-induced protection	Lin et al.	2015
AIS	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	Male C57BL/6 mice, MCA thrombolytic occlusion model (Ref)	-	(1) permanent occlusion: 45min, 24 and 48h after occlusion, FTY720 0.5mg/kg, i.p.; (2) tPA 30min after thrombin injection: 30min (together with tPA), 24 and 48h after occlusion, FTY720 0.5mg/kg, i.p.; (3) tPA 3h after thrombin injection: 3h (together with tPA), 24 and 48h after occlusion, FTY720 0.5mg/kg, i.p.;	FTY720 attenuate neurological deficit and decrease infarct volume in thrombolytic occlusion model. Combined with tPA, it can improve neurological outcome and decline the risk of hemorrhagic transformation after delayed tPA administration.	Campos et al.	2013
AIS	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	-	47 AIS patients with anterior or middle cerebral artery occlusion, <3h after onset	Less than 3h after onset, tPA 0.9mg/kg, i.v., FTY720 0.5mg p.o., q.d., for 3 consecutive days and first dose co-administered with tPA.	Combination of FTY720 and tPA can attenuate reperfusion injury, infarct volume and improve clinical outcome in AIS patients.	Zhu et al.	2015
ICH	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	CD-1 mice, cICH model	-	1h after ICH induction, FTY720 1mg/kg, i.p.	FTY720 can reduce brain edema and improve neurological function.	Rolland et al.	2011

Stroke subtype	Target lysophospholipids	Animal, stroke model	Human specimens / Patients	Intervention	Results	Reference	Year
ICH	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	Male CD-1 mice, bICH / cICH model; Male S-D rats, cICH model	-	1h after ICH induction, FTY720 1mg/kg, i.p., single dose or Q.d.	FTY720 can improve neurological outcome and brain edema at short-/long-term, and induce fewer lymphocyte infiltration in blood and brain.	Rolland et al.	2013
ICH	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	Male CD-1 mice, cICH model	-	30min after surgery, FTY720 0.5mg/kg, i.p., Q.d., for 2 consecutive days.	FTY720 can enhance neurological outcome and decrease brain edema, while no difference in inflammatory (CD68 ⁺) cells	Lu et al.	2014
ICH	SIP ₁ , SIP ₃ , SIP ₄ , SIP ₅	-	23 patients with primary parenchymal ICH of 5–30ml in basal ganglia, less than 72h prior to admission	Within 1h after the baseline CT scan, FTY720 0.5mg, p.o., q.d., for 3 consecutive days	FTY720 can attenuate neurological deficit and decrease circulating lymphocyte counts, as well as decreased perihematoma edema volume and rPHE and showed better long-term recovery	Fu et al.	2014
SAH	SIP	Dog SAH model	-	SIP injected into cisterna magna	SIP induces vasoconstriction in canine basilar artery in vivo and in vitro, may act through activation of Rho/Rho-kinase pathway	Tosaka et al.	2001
SAH	SPC	Dog SAH model and single-hemorrhage model	-	After administering SPC into cisterna magna, Y27632 or EPA was injected intracisternally; During single-hemorrhage model, Y27632 or EPA was injected on day 7 after SAH	SPC is a novel mediator of Rho-kinase-induced cerebral vasospasm (CV) in vivo. The inhibition of CV induced by SPC or after SAH by eicosapentaenoic acid (EPA) suggests beneficial roles of EPA in the treatment of CV, SPC-ROK pathway may be involved in CV.	Shirao et al.	2008
SAH	SPC	Dogs SAH model	CSF of 11 patients with SAH who had undergone surgery within 72h of the onset of SAH and classified as Fisher grade 3 by CT.	SPC injected into cisterna magna	SPC concentrations elevated after SAH in CSF of patients, suggested that SPC is involved in development of cerebral vasospasm. SPC rapid diluted in canine CSF indicated a higher concentration of SPC released in CSF than previous study.	Kurokawa et al.	2009
SAH	SPC and SIP	-	-	Lysophilized SIP and SPC incubated with rat cerebral arteries.	SPC but not SIP acts as a proinflammatory mediator in cerebral arteries.	Wirtig et al.	2011
SAH	Cer	-	CSF of Fisher 3 grade SAH patients within 48h of the bleed.	-	Ceramide changes occur in SAH. Elevation of levels of ceramide, particularly C _{18:0} , correlated with the occurrence of symptomatic vasospasm and poor neurological outcome after SAH.	Testi et al.	2012
SAH	Ceramide, DHC, SIP, ASMase, NSMase, SMS,	Male S-D rats, SAH model	CSF of Fisher 3 grade SAH patients within 48h of the	-	Activation of ASMase, SIP-lyase, and GCS resulting in a shift in the production of protective SIP in favor of deleterious Cer	Testi et al.	2015

Stroke subtype	Target lysophospholipids	Animal, stroke model	Human specimens / Patients	Intervention	Results	Reference	Year
SAH	SIP-lyase, GCS SIP and TNF- α signaling pathway	C57BL/6 mice, TNF- $\alpha^{-/-}$ and SphK1 $^{-/-}$ mice, SAH model	bleed.	Immediately after SAH, JTE013, 3 mg/kg, i.p., q.d., for 3 consecutive days	after SAH Vascular smooth muscle cell TNF- α and SIP signaling significantly enhance cerebral artery tone in SAH; anti-TNF- α and anti-SIP treatment may significantly improve outcome	Yaqi et al.	2015
SAH	SIP	Rabbit, double hemorrhage SAH model	-	-	SIP expression in cerebral arteries may be a new indicator during the development of cerebral vasospasm after SAH which can act as a new therapeutic target	Tang et al.	2015

AIS = Acute ischemic stroke; Cer = Ceramide; GSLs = Glucosylceramide; CNS = Central nervous system; SphK = Sphingosine kinase; tPA = Tissue plasminogen activator; ICH = Intracerebral hemorrhage; cICH = Collagenase infusion ICH model; bICH = autologous blood infusion ICH model; rPHE = relative perihematomal edema; SAH = subarachnoid hemorrhage; SPC = Sphingosylphosphorylcholine; CSF = Cerebrospinal fluid; DHC = Dihydroceramide; SIP = Sphingosine-1-phosphate; ASMase = acid sphingomyelinases; NSMase = neutral sphingomyelinases; SMS = sphingomyelinase synthase; GCS = glucosylceramide synthase