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REVIEW ARTICLE

FTY720 (Fingolimod) Ameliorates Brain Injury through Multiple Mechanisms and is a Strong Candidate for Stroke Treatment

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Abstract: FTY720 (Fingolimod) is a known sphingosine-1-phosphate (S1P) receptor agonist that exerts strong anti-inflammatory effects and was approved as the first oral drug for the treatment of multiple sclerosis by the US Food and Drug Administration (FDA) in 2010. FTY720 is mainly associated with unique functional "antagonist" and "agonist" mechanisms. The functional antagonistic mechanism is mediated by the transient down-regulation and degradation of S1P receptors on lymphocytes, which prevents lymphocytes from entering the blood stream from the lymph node. This subsequently results in the development of lymphopenia and reduces lymphocytic inflammation. Functional agonistic mechanisms are executed through S1P receptors expressed on the surface of various cells including neurons, astrocytes, microglia, and blood vessel endothelial cells. These functions might play important roles in regulating anti-apoptotic systems, modulating brain immune and phagocytic activities, preserving the Blood-Brain-Barrier (BBB), and the proliferation of neural precursor cells. Recently, FTY720 have shown receptor-independent effects, including intracellular target bindings and epigenetic modulations. Many researchers have recognized the positive effects of FTY720 and launched basic and clinical experiments to test the use of this agent against stroke. Although the mechanism of FTY720 has not been fully elucidated, its efficacy against cerebral stroke is becoming clear, not only in animal models, but also in ischemic stroke patients through clinical trials. In this article, we review the data obtained from laboratory findings and preliminary clinical trials using FTY720 for stroke treatment.

Keywords: FTY720, fingolimod, stroke, sphingosine-1-phosphate, inflammation, sphingosine kinase.

1. INTRODUCTION

FTY720 (Fingolimod) was originally synthesized by a group of Japanese researchers in 1992 while investigating structure-activity relationships in derivatives of the fungal metabolite myriocin (ISP-I), which was isolated from *Isaria sinclairii* [1]. FTY720 shows strong immunosuppressive potential and was approved as the first oral immunomodulatory drug for multiple sclerosis (MS) by the United States food and drug administration (FDA) in 2010. It is catalyzed by sphingosine kinase (SphK) to its physiologically active phosphorylated form (FTY720-P), and this acts as a high-affinity agonist of Sphingosine-1-phosphate (S1P) receptors and uniquely exerts both functional "antagonist" and "agonist" effects through S1P receptors. The functional antagonistic mechanisms are mediated by the temporary down-regulation and degradation of S1P receptors on lymphocytes, which prevents these cells from entering the blood stream from the lymph node. This subsequently causes lymphopenia and reduces harmful lymphocytic inflammation. Functional agonistic mechanisms are executed through S1P receptors expressed on the membrane surfaces of cells including neurons, astrocytes, microglia, and vessel endothelial cells. FTY720-P binds to four of the five known G protein-coupled S1P receptors ($S1P_1$, $S1P_{3-5}$), which results in the activation of its downstream pathways *via* the transduction of G protein isoforms (G_s, G_i, G_q, and $G_{12/13}$) [2]. These pathways regulate multiple cellular events including cell proliferation and survival, mostly in a cell-protective manner [3]. Recent researches have also revealed that FTY720 may exhibit receptor-

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independent epigenetic effect which are modulated through intracellular signaling and histone deacetylases. Due to these marked protective effects, many researchers are beginning to conduct basic and clinical experiments to test the ability of FTY720 to ameliorate stroke damage. In this review, we discuss the synthetic pathway and functional mechanism associated with FTY720 and then review the current experimental and clinical data regarding its use as a promising neuroprotective drug candidate against stroke.

2. CERAMIDE, SPHINGOSINE, S1P, SPHK, FTY720, AND S1P RECEPTORS

FTY720 acts as a high affinity agonist of S1P receptors and uniquely shows both functional "antagonist" and "agonist" mechanisms. To better understand its activity, it is important to elucidate not only the mechanisms associated with FTY720 and S1P receptors, but also the metabolic and functional mechanisms related to the original ligand, S1P, and its metabolites and enzymes including sphingosine, ceramide, and SphK (Fig. **1**). Until recently, these sphingolipids and their enzymes were considered only ubiquitous components of the cell membrane with no biological function. However, they have been widely revaluated as they were shown to regulate many vital cell functions through membrane microdomains and the integration of cell signaling; thus, they play an important role in different cell processes including proliferation, inflammation, apoptosis, and migration.

The synthesis of sphingolipids starts from the conversion of serine and palmitoyl coenzyme A (CoA) into ceramide [4], and an alternative biosynthetic pathway results in the breakdown of sphingomyelin. The ceramide can then be metabolized to form sphingosine. Sphingosine is further phosphorylated into S1P by two kinases, namely SphK1 or SphK2. To be physiologically active, FYT720 needs to be phosphorylated (FTY720-P), and SphK1 and SphK2 are essential for this conversion. S1P is then catalyzed into hexadecenal and phosphoethanolamine by the S1P-lyase.

2.1. Ceramide

Ceramide is densely located in the cell membrane, and is one of the major components of the phospholipid

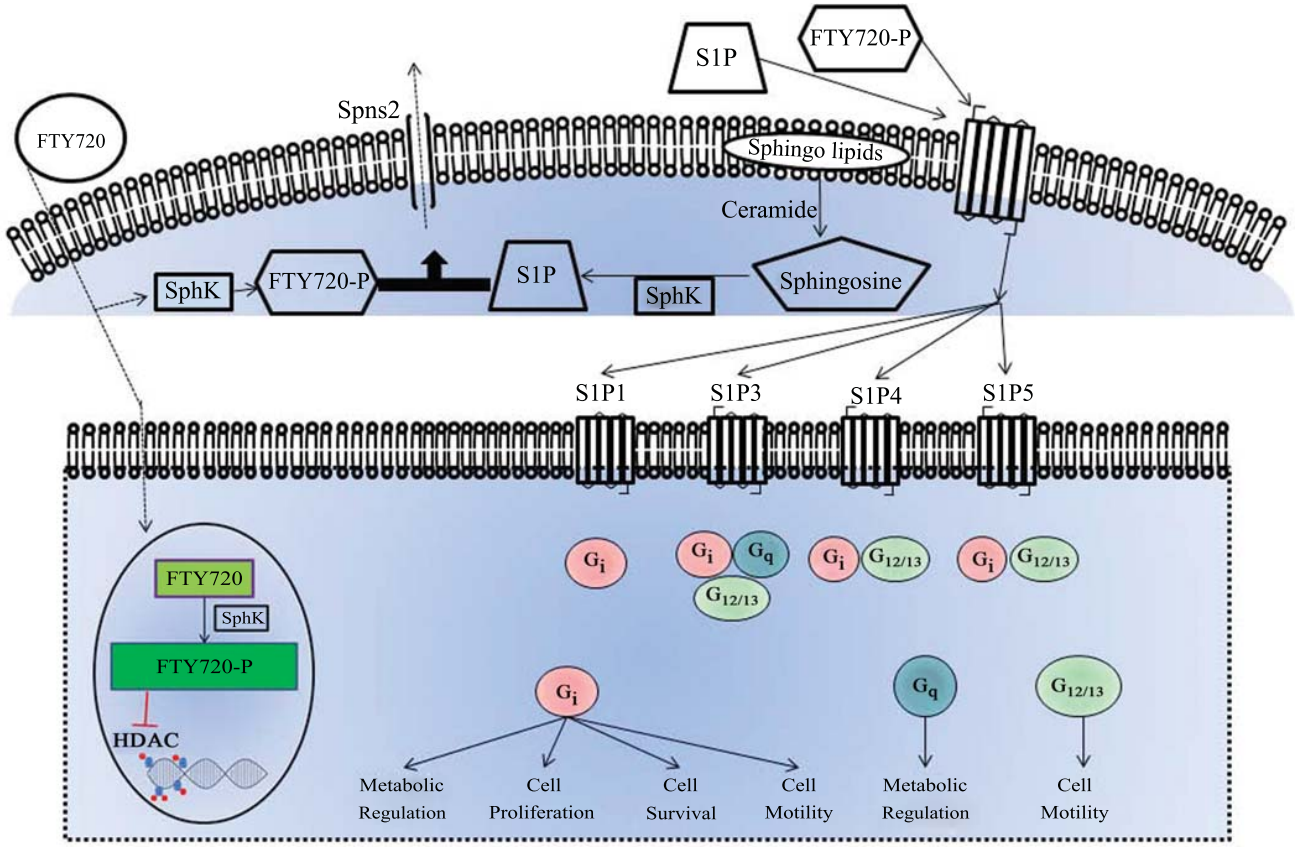

Fig. (1). Schematic outline of sphingolipid metabolism (upper) and biosynthesis and signaling through S1P receptors (lower). Sphk: sphingosine kinase, S1P: sphingosine-1-phosphate, FTY720-P: phosphorylated FTY720.

bilayer [5]. Recent data show that stress stimuli such as inflammatory mediators, heat, hypoxia, oxidative stress, ultraviolet radiation, and chemotherapeutics increase ceramide production [6]. This compound was shown to not only promote cell cycle arrest and apoptosis, but also play an important role in the regulation of autophagy, cell differentiation, and inflammatory responses [7].

2.2. Sphingosine

Sphingosines are also bioactive sphingolipids. They were first described as the physiological inhibitors of the survival signal Protein Kinase C (PKC) and were also found to up-regulate caspase 3, which plays important role in apoptosis [8]. Since then, there have been many studies showing that sphingosine is toxic to cells [8a, 8d]. However, a recent report has shown different effects, specifically, that its function might depend on its concentration, as lower concentrations (submicromolar) of sphingosine can be cardio-protective [9], whereas higher concentrations could be toxic [8c].

2.3. S1P

S1P is a bioactive lipid signaling molecule that is generated when one of two isoforms of the enzyme, SphK1 or SphK2, catalyzes sphingosine. S1P was first described as an intracellular second messenger, based on its ability to activate SphK and increase intracellular S1P levels. However, the discovery and cloning of five G protein-coupled receptors ($S1P_{1–5}$) expressed on the cell membrane has revealed that S1P can also act as an extracellular signaling ligand, regulating cellular functions such as proliferation, survival, immunomodulation, apoptosis, migration, cytoskeletal organization, and differentiation [10]. Basal plasma and serum concentration levels of S1P are generally maintained in the lower range of 200-900 nmol/L, but these concentrations can be increased rapidly when cells are exposed to various stimuli [11]. Interestingly, the concentration of S1P is controlled by two enzymes, SphK and S1P lyase, and this system is thought to be important for the regulation of cell trafficking. Whereas SphK activity can be up-regulated by a variety of growth factors, S1P lyase activity is constantly maintained at high levels, and this results in very low intracellular S1P levels in most tissues. However, erythrocytes and platelets have low S1P lyase activity and this enables high S1P concentrations in the blood plasma [12]. This concentration gradient is presumed to be integral for lymphocyte trafficking [13].

2.4. SphK

The synthesis of S1P and FTY720-P is catalyzed by SphK. There are two isozymes of SphK (SphK1 and SphK2) and these show different subcellular localizations and enzymatic properties, as well as diverse expression patterns in various tissues; specifically, SphK1 is more abundant in the lung, spleen, kidney, heart, renal proximal tubules, and cardiomyocytes, whereas SphK2 is predominant in the brain [14]. The intracellular locations of these two enzymes are also different. SphK1 is localized predominantly in the cytoplasm, whereas SphK2 mainly resides in the nucleus [15]. Genetic deletion of both isozymes results in fetal death from severe bleeding and inadequate vasculogenesis [16], whereas mice null for either SphK1 or SphK2 exhibit normal development. Since differences in their enzymatic mechanisms are not known, it is presumed that these enzymes have complementary functions.

2.5. FTY720

FTY720 is phosphorylated intracellularly to form FTY720-P, mainly by SphK2 [17]. Both S1P and FTY720-P are transported through the extracellular space by a membrane transporter (Spns2) to bind S1P receptors (Fig. **1**) [18]. FTY720 can also bind four of five S1P receptors ($S1P_1$, $S1P_3$, $S1P_4$, $S1P_5$), and mediates downstream signaling through various G protein receptors attached to S1P receptors. The main metabolic pathway of FTY720 is cytochrome P450 (CYP450)-mediated w-hydroxylation/oxidation steps in the liver; then, inactivated FTY720 is eliminated by the renal system [19]. Because FTY720 has already been clinically adopted, there are several pharmacokinetic characteristics available based on human data. Whereas FTY720 shows high plasma protein binding (> 99%) and bioavailability (93%), absorption after the oral application of FTY720 is food-independent and relatively slow, taking 12-16 hours to reach maximal plasma concentrations. The blood clearance is also slow (6.3 ± 2.3 L/h), resulting in a half-life of 6-9 days.

2.6. S1P Receptors

After the discovery of S1P receptors, there has been extensive work aimed to understand the role of S1P and FTY720-P as extracellular ligands. A schematic of the S1P receptors is shown in Fig. (**1**). S1P mediates its effects by binding G protein-coupled receptors ($S1P_{1–5}$), which activate a variety of signaling pathways

via the transduction of G protein isoforms (G_s, G_i, G_q, and $G_{12/13}$). The pro-survival phosphatidylinositol-3-kinase (PI3K)/Akt proteins comprise the downstream molecules that are regulated by $S1P_1$ receptor signaling, and Akt activation is important for the prevention of apoptosis [20]. S1P also stimulates cell growth and proliferation *via* the activation of MAP kinase Extracellular Signal-regulated Kinases (ERKs) [21]. It is believed that elevated ERK phosphorylation plays a role in cell survival and proliferation in the penumbra, and that ERK activity might block apoptosis by enhancing the level of the anti-apoptotic protein Bcl-2 through cAMP-responsive element binding protein activation [20a]. S1P is also assumed to prevent necrosis mediated by the PKCε pathway [22].

3. MECHANISMS OF FTY720

3.1. Functional Antagonist Activity

3.1.1. Lymphopenia

One of the main immunomodulatory mechanisms of the action of FTY720 is based on its effect on lymphocyte homing. It reversibly redistributes T and B cells from the circulation to secondary lymphoid organs like peripheral and mesenteric lymph nodes, and subsequently causes lymphopenia [23]. Further, FTY720 mediates sustained desensitization of the $S1P_1$ signaling pathway by inducing receptor internalization and degradation, which at the cellular level, results in unique functional "antagonist" activity. This effect of fingolimod on $S1P_1$ is unique and is different from that of the endogenous ligand S1P. S1P also mediates $S1P_1$ internalization upon binding, but then this complex dissociates in endosomes and the receptor is recycled back to the cell membrane. Similarly, S1P [3-5] are also internalized upon FTY720 binding and are then redistributed back to the cell membrane. This down-regulation of $S1P_1$ on T cells is thought to account for the immunosuppressive activity of FTY720 and was shown to be beneficial against ischemic stroke in an animal study [24]. A recent report further showed that a certain period of lymphopenia (> 24 hours) is required for efficient activity against ischemic stroke [24]. One of the concerns associated with lymphopenia is that it might increase the rate of infection after FTY720 treatment; however, no increase in bacterial lung infections have been reported even though FTY720 strongly reduces the number of circulating leukocytes [25]. In contrast, a recent report conversely reported that peripheral T cell depletion by FTY720 at an early stage might exacerbate hypoxic–ischemic brain injury in neonatal mice [26]. They speculated that leukopenia might be involved in the loss of gray and white matter. These differences could be attributed to whether the brain is developing or matured, and further analysis is necessary to assess FTY720 and lymphopenia in pediatric treatment.

3.2. Functional Agonist Activity

3.2.1. Regulating Cerebrovascular Responses

FTY720 provides vascular protection mainly in a manner related to its immunomodulatory actions. Further, this compound could mitigate microvascular dysfunction by reducing the numbers of circulating lymphocytes as mentioned, and subsequently decrease the rate of harmful lymphocyte-endothelium–platelet interactions in the brain vasculature [27]. FTY720 was shown to not only reduce the number of circulating T lymphocytes in the peripheral blood, but also to reduce lymphocytes in the intracranial cerebral blood vessels after ischemic stroke [28]. FTY720 can also decrease intracellular adhesion molecule- (ICAM-1) expression on endothelial cells. Adhesion is mediated by interactions between β2-integrins on leukocytes with ICAM-1 on cerebral endothelial cells, and FTY720 has been shown to decrease ICAM-1 expression, which contributes to the amelioration of leukocyte plugging, termed the no-reflow phenomenon [29]. Moreover, the reduction of leukocyte adhesion to the vessel walls and local platelet activation would further inhibit thrombosis and inflammation, improving microvascular function [27, 28]. FTY720 can also activate astrocytes to release granulocyte and macrophage colony-stimulating factor, which might attenuate endothelial cell death [30]. In addition, FTY720 has been shown to suppress artery contractility in the aorta and arterial pressure involving ERK activation. This property indicates that this drug could potentially facilitate increased blood flow to the brain and improve blood supply to the ischemic brain. [31].

3.2.2. Regulating Blood-Brain-Barrier Functions

The BBB is a multicellular vascular structure mainly formed by endothelial cells, astrocytes, and pericytes. It separates the Central Nervous System (CNS) from the peripheral blood circulation and maintains the ionic balance required for neurotransmission and prevents the excessive entry of immune cells [32]. Cerebral ischemia activates hypoxic-ischemia-induced inflammation in the CNS, leading to BBB disruption by increasing permeability [33]. This allows potentially toxic cells and molecules to enter the brain [34]. There are two major types of junctions that comprise the

BBB. One is the tight junctions, which exist between adjacent endothelial cells sealing inter-endothelial cell gaps, whereas the other is the adherens junctions, which maintain inner-endothelial cell contacts [35]. The function of the BBB is dependent on the appropriate localization and expression of tight junctions and adherens junctions between brain endothelial cells [36]. Tight junction proteins consist of junctional adhesion molecules such as occludin, claudins, and ZO-1 [37], and adherens junctions are mainly comprised of cadherins. During reperfusion, BBB junctions are disrupted by reactive hyperemia and the loss of cerebral autoregulation. Several studies have shown that S1P receptors are expressed on endothelial cells and that FTY720 regulates the endothelial cell barrier mostly through $S1P_1$ receptors, resulting in vascular permeability [38]. FTY720 was also shown to stimulate endothelial cells to recruit proteins for adherens junction, resulting in the preservation of endothelial barrier properties such as vascular permeability and limited neutrophil infiltration [39]. FTY720 binding to $S1P_1$ receptors on endothelial cells promotes adherens junction assembly, strengthening the endothelial barrier [40], and the tight-junction associated protein ZO-1 is important for barrier integrity [41]. However, Yanagida *et al*. recently reported a different effect of FTY720 on BBB function. Under physiological conditions, they reported that FTY720 increases brain tracer extravasation in a size-selective and reversible manner, in which the smaller tracer (approximately 1-kDa) can pass through the BBB, whereas the larger tracer cannot [42]. Since this phenomenon was reported under physiological conditions and might be different from that during ischemic conditions, further analysis would be necessary to elucidate the associated mechanisms regarding BBB and S1P receptors. Another function of $S1P_1$ and $S1P_3$ receptors is the regulation of the activity of P-glycoprotein, which is expressed in brain capillaries and involved in the expulsion of xenobiotics from the brain. This process might also contribute to the permeability of endothelial cells and the BBB [43]. FTY720 also increases endothelial barrier properties by modulating endothelial cell cytoskeletal forces [38].

3.2.3. Regulating Neural Lineages (Neurons, Astrocytes, Microglia, Oligodendrocytes, and Synapses)

S1P receptors are also expressed in neural lineages such as neurons, astrocytes, microglia, oligodendrocytes, and endothelial cells [28c, 44]. There has been conflicting evidence as to whether FTY720 is beneficial for neuronal cells in response to ischemic insults. Some researchers have found that it can protect these cells *via* the $S1P_1$ receptor, based on results from an *in vivo* stroke model [44c, 45], whereas others did not find any significant protective role for FTY720 [29]. These differences are probably caused by differences in experimental methods; however, further studies are warranted to fully delineate the effect of FTY720.

Astrocytes have supportive, metabolic, and homeostatic functions in the CNS. Activated astrocytes might resemble "reactive gliosis" after ischemia, and these cells have been observed 4-24 hours after ischemia onset, peaking at approximately 4 days. Upon injury, astrocytes invade the lesion and proliferate to create the glial scar, which has an important role, leading to both beneficial and harmful reactions [46]. This process is thought to create a barrier between damaged and healthy cells [47]. FTY720 can also induce ERK1/2 activation in cultured astrocytes, which promotes their migration [48] and the production of growth factors [49]. Further, the deletion of astrocyte $S1P_1$ remarkably reduces levels of astrogliosis [50], and treatment with FTY720 also results in similar effects on astrocytes. Because astrocytes are activated by IL-17 [51] and FTY720 can reduce pro-inflammatory T cells (Th17), which produce IL-17 [52], FTY720 is also considered to contribute to reducing the activation of astrocytes during stroke [53].

Microglia comprise the resident immune cells of the brain and are involved in modulating the inflammatory response in brain [54]. When brain damage occurs, microglia undergo division and morphological changes and migrate to the sites of injury. They then release S1P and control local concentrations of this mediator at sites of injury, together with astrocytes [55]. FTY720 was shown to limit the inflammatory response in microglia [56]. A recent report also revealed that, whereas ischemic insults can induce M1 microglia, which exert proinflammatory effects and exacerbate ischemic injury, FTY720 might shift microglia toward M2 polarization or inhibit M1 polarization. These processes have been shown to play an important role in anti-inflammatory effects that attenuate ischemic injury, and this effect is strongly correlated with the $S1P_3$ receptor [57].

Oligodendrocytes are the cells that insulate axons in the CNS to support neural transmission. $S1P_5$ receptors are thought to be involved in regulating oligodendrocyte function [58]. Mature oligodendrocytes express high levels of $S1P_5$ and relatively lower levels of $S1P_3$, which is quite different from expression patterns in neurons and astrocytes, which express undetectable or low levels of mRNA encoding $S1P_5$ [59]. Recently,

studies showed that FTY720 regulates oligodendrocyte progenitor cells with respect to multiple processes including survival, proliferation, migration, process dynamics, and differentiation [60]. FTY720 was also shown to improve hippocampal synaptic plasticity and memory deficits [61].

3.2.4. Role of FTY720 in Autophagy

Autophagy is a physiological degradation process that mediates the constitutive turnover of cytoplasmic components to maintain cellular homeostasis through the elimination of damaged cellular products [62]. However, when the cells are faced with stress such as ischemic insults, enhanced autophagy can occur to degrade proteins and organelles to produce sufficient nutrients and energy. Moreover, recent data suggest that the process of autophagy mainly occurs in neurons instead of astrocytes during the acute phase following ischemic stroke [63], and over-activated neuronal autophagy after cerebral-ischemic injury might result in detrimental effects [64]. FTY720 was shown to exert beneficial effects through the inhibition of autophagic pathways by reducing autophagosome proteins, microtubule-associated protein 1 light chain 3 (LC-3-II) and Beclin 1, and was also shown to up-regulate the mammalian Target Of Rapamycin (mTOR) signaling pathway, which is in turn essential for autophagy through its downstream target, 70-kDa ribosomal protein, S6 kinase1 (p70S6K) [63, 65].

3.2.5 Role of FTY720 in Macrophages

During cerebral ischemia, monocyte-derived macrophages comprise the predominant immune infiltrates from the periphery during the subacute phase of inflammation [66]. Moreover, macrophages can be classified as classically activated, proinflammatory M1 macrophages and alternatively activated, anti-inflammatory M2 macrophages [67]. S1P signaling was shown to shift macrophage polarization towards the M2 anti-inflammatory phenotype [68]. During acute inflammation, S1P results in the phenotypic transformation of macrophages from a proinflammatory to an anti-inflammatory phenotype, and this process is regulated by the $S1P_1$ receptor [69]. Further, S1P significantly reduces the LPS-mediated expression of proinflammatory cytokines and the enzyme iNOS and stimulates ArgI expression in macrophages, indicating that it promotes an anti-inflammatory macrophage phenotype; in addition, S1P can affect Arg and iNOS enzymatic activity in macrophages. S1P treatment also increases the production of the anti-inflammatory cytokines transforming growth factor-β and IL-10 in macrophages. Elevated S1P, which specifically activates $S1P_1$, underlies the increase in IL-6 gene expression, which in turn reciprocally up-regulates $S1P_1$ gene expression in a JAK2-dependent manner in primary mouse macrophages. S1P-mediated $S1P_1$ activation is a major mechanism underlying inflammation through the reciprocal up-regulation of $S1P_1$ and IL-6 expression in macrophages. Because macrophages are the major immune cells in multiple tissues, they are probably the key cell types for $S1P_1$–IL-6-induced damage, which leads to chronic inflammation and tissue damage by inducing $S1P_1$ on macrophages. FTY720 decreases $S1P_1$ expression in macrophages, and S1P links persistent JAK2/p-STAT3 activation, chronic intestinal inflammation, and the development of colitis-related cancers [70]. Excessive S1P is associated with a number of pathologic conditions due to its strong, proinflammatory properties and immune-stimulatory activities [71]. Recently, the use of FTY720 to inhibit $S1P_1$ receptor activation has become a major treatment for multiple sclerosis [72].

3.3 Receptor-Independent Activity

In addition to the receptor dependent activities, accumulating evidence have recently revealed that FTY720 might also act receptor-independent to modulate cell activities. These effects include binding to the specific proteins, activating intracellular signaling pathways, and modulating epigenetic transcriptions [73]. The effect of intracellular protein binding ability is well studied especially in the field of oncology that FTY720 can mimic ceremide for binding inhibitor 2 of protein phosphatase 2A (I2PP2A), which result in reactivating tumor necrosis [73f, 74]. FTY720 also showed modulating intracellular signaling pathway to activate nuclear factor of activated T-cells 1 (NAFT1), activator protein 1 (AP-1), and nuclear factor-kappa B (NFκB) aberrantly. This action is presumed to negatively affect T cell activation and gives additional effects in immunomodulation other than to avoid lymphocyte egress of T cell from lymphoid tissue [73e]. FTY720 also showed to reduce mammalian target of rapamycin (mTOR signaling) and phosphorylated p70S6k levels in the rat brain. One of the interesting discoveries of receptor-independent activity of FTY720 is its epigenetic ability. FTY720 is shown to increase acetylation of histone (H3K9, H3K18, H3K23, H4K8) in various cells [73a-e]. It inhibits histone deacetylases (HDAC) leading to increased histone acetylation, and this effect is shown to decrease T cell activation, upregulate antiepileptogenic effect, increase neurotrophic factor generation, and rescue memory deficit. However, re-

ceptor-independent activity of FTY720 is not examined in the field of stroke.

4. FTY720 IN STROKE TREATMENT BASED ON EXPERIMENTAL STUDIES AND CLINICAL TRIALS

4.1. FTY720 in an Ischemic Stroke Model

Over the past years, FTY720 has been increasingly recognized as a potential therapeutic target for ischemic stroke [2b, 14b, 28, 29, 44c, 45, 61]. Its mechanisms of actions have been previously described, and this section will provide a methodological view of ischemic stroke experiments focusing on the efficacy of this agent. It was proposed by the opinion leaders committee in the stroke therapy academic industry roundtable (STAIR) that procedural steps for conducting experimental studies should be carefully considered to apply such methods and results to clinical trials [75]. These include the use of different types of ischemic stroke models including permanent and temporary occlusion, diverse animal types using both rodent and gyrencephalic species, different types of animals including both sexes and aged animals, and various therapeutic time points from acute to chronic stages, as well as the evaluation of different dose-response effects. This section will focus on some of these important methodological aspects of FTY720 in experimental stroke models (Table **1**). Many studies have shown the efficacy of FTY720 at concentrations ranging from 0.25 to 2 mg/kg [29, 44c, 63, 76]. However, it should be noted that the doses used in these reports were much higher than the approved clinical dose for multiple sclerosis, which is 0.5 mg/body/day (approximately 0.01 mg/kg). Single use or daily administration has been examined, and there seems to be no significant difference between these groups [28c, 77]; slow blood clearance (6.3 ± 2.3 L/h) and longer half-life (6-9 days) could be the reason for this. There are conflicting results regarding the efficacy of FTY720 with permanent ischemic models. Wei *et al*. showed that FTY720 might reduce infarct volume in a permanent MCAO model compared to that in a vehicle group [29], whereas Liesz *et al*. did not show any beneficial effects [28c]. Wei *et al*. suggested the FTY720 might protect endothelial cells and decrease the expression of adhesion molecules, resulting in decreased leukocyte binding and subsequent inflammation. However, there was no report of neurological function in this article and further studies are warranted to elucidate the effect of FTY720 on permanent ischemia. Moreover, different animal conditions and species have not been fully examined, since all of the previous reports used rat or mice to generate ischemic conditions. Hypertensive or diabetic animals, resembling the actual human condition, were also not reported.

4.2 Effect of FTY720 on Intracerebral and Subarachnoid Hemorrhage

Most previous studies have been conducted using models of ischemic stroke, and few reports have tested this agent for other types of stroke including cerebral hemorrhage and subarachnoid hemorrhage. FTY720 treatment was shown to result in a significant improvement in long-term neurocognitive performance and ameliorate brain tissue loss in a neonatal hemorrhage model [78] and adult intracerebral hemorrhage model [79]. Not only lymphopenia, but also the preservation of the BBB and limiting brain edema were considered responsible for neurological recovery in these models. Secondary damage after hemorrhagic stroke can be ameliorated by anti-inflammatory effects and endothelial cell protection. There are only two reports that have focused on FTY720 in a subarachnoid hemorrhage model [80]. Xu *et al*. found that FTY720 can decrease intravascular leukocyte adhesion and ameliorate the pial arteriolar dilation response resulting in better neurological recovery [80a]. Hasegawa *et al*. [44c] also showed that FTY720 can significantly ameliorate subarachnoid hemorrhage-induced neurological deficits through peripheral immunomodulation. They concluded that the effect of FTY720 is through lymphopenia (antagonistic effect), and not *via* direct cell protection by S1P receptor activation (agonistic effect).

4.3 FTY720 to Treat Ischemic Stroke in Clinical Trials

Despite its abundant clinical use for multiple sclerosis, there have only been two clinical trials that adopted FTY720 for ischemic stroke patients and one clinical trial to test its effects on intracerebral hemorrhage [81]. Zhu *et al*. reported a multicenter randomized trial for ischemic stroke patients using FTY720 [81a]. The patients were assigned either to an alteplase-alone group (n = 25) or an alteplase and oral fingolimod group (0.5 mg daily for 3 consecutive days; n = 22). They found that patients who received the combination of FTY720 with alteplase exhibited lower circulating lymphocytes, significant smaller lesion volumes (10.1 *versus* 34.3 mL; P = 0.04), less hemorrhagic transformation (1.2 *versus* 4.4 mL; P = 0.01), and better neurological recoveries based on the National Institute of Health Stroke Scale (NIHSS) 1 day after treatment (4 *versus* 2; P = 0.02) and based on the modified Rankin Scale 90

Table 1. Comparison of preclinical methods recommended by the stroke therapy academic industry round-table (STAIR)

Stair Reccomenda-tion	Author, Year	Different Methods Adopted	Results
Dose response	Xiao Li, 2017	0.5, 1, 2mg/kg	FYT720 attenuates activation of autophagy dose-dependently
	Yang D, 2014	0.3 or 1 ug/g,	FTY720 prevent inflammation-sensitized hypoxic–ischemic brain injury in new-borns
	Wei Y, 2011	0.5 or 1.0mg/kg	FTY720 dose-dependently decreases infarct size and neurological deficit
	Wei Y, 2011	30, 100 , 1000 nM	FTY720 dose-dependently decreases apoptotic cells
	Hasegawa, 2010	0.25, 1mg/kg	FTY720 is effective, but no difference between both groups
	Wacker, 2009	0.24 or 1.0mg/kg	FTY720 dose-dependently decreases infarct size and neurological deficit
Therapeutic targeting	Chuan Qin, 2017	0.3mg/kg, 3, 10, or 30 consecutive days	FTY720 attenuate brain inflammation by skewing microglia toward M2
	Brait VH, 2016	1 mg/kg	FTY720 reduce infarct volume after ischemia/reperfusion in mice,
	Nazari M, 2016	0.5mg/kg	FTY720 improve the infarct volume and memory performance after MCAO
	Hasegawa Y, 2013	0.25mg/kg	FTY720 improve neurological function, infarction size and S1P1 expression on neurons
	Kraft P, 2013	1 mg/kg	FTY720 reduce the Induction of lymphocytopenia and concomitant
	Czech B, 2009	1 mg/kg	FTY720 reduce lesion size, improved neurological function and activated micro-glia/macrophages
Therapeutic window	Liesz, 2011	single or daily of 1mg/kg	FTY720 did not improve outcome with permanent occlusion in both groups
	Shichita, 2009	single or daily of 1mg/kg	FTY720 is effective, but no difference between both groups
Type of Stroke	Moon E, 2015	primary and recurrent stroke	FTY720 can reduce stroke damage in both groups
	Lu L, 2014	intracerebral hemorrhage	FTY720 improve brain edema, apoptotic cells, brain atrophy and neurobehavioral functions
	Liesz, 2011	Permanent MCAO	FTY720 did not improve outcome with permanent occlusion in both groups
	Pfeilschifter, 2011	1.5hr or 3hr after tMCAO	FTY720 was effective in both groups
	Wei Y, 2011	Permanent MCAO and tMCAO	FTY720 also showed smaller infarct volume in permanent MCAO
Trial	Zhang S, 2017	0.9mg/kg	FTY720 is effective
	Zhu Z, 2015	0.9mg kg	FTY720 reduce infarction size and hemorrhage
	Fu Y, 2014	0.5mg/day orally for 3 consecu-tive days	FTY720 decrease lesion size and microvascular permeability, attenuated neuro-logical deficits
In-vitro	Pang X, 2017	0.6 μM	FTY720 combined with vitamin E revealed a synergistic effect

MCAO: middle cerebral artery occlusion, t: transient

days after treatment (good prognosis: 73% *versus* 32%; $P < 0.01$). Fu *et al*. [81b] also showed the efficacy of FTY720 for stroke patients treated 4.5 hours after onset, who are not eligible for t-PA treatment. They compared a standard management group (n = 11) to a FTY720 group (0.5 mg/day oral intake for 3 consecutive days; n = 11). They reported that patients in the FTY720 group exhibited lower circulating lymphocyte counts and better neurological recovery. The reduction of the NIHSS was 4 in the FTY720 group, whereas it was −1 in the standard management group ($P = 0.0001$) and this difference was strongly profound during the first week after disease onset. They also reported that there were no drug-related serious adverse events. These results indicate the safety and feasibility of FTY720 administration to ischemic stroke patients. The effect of FTY720 on Intracerebral Hemorrhage (ICH) has also been reported [81b]. The investigators included 23 patients with primary supratentorial ICH with hematoma volumes of 5 to 30 mL. Eleven patients were randomly assigned to the FTY720 group and 12 were assigned to the control group. In this study, 0.5 mg of FTY720 was orally administered within 1 hour of the baseline computed tomography and no later than

72 hours after the onset of symptoms. As a result, patients treated with FTY720 exhibited a reduction in neurological impairment compared to that in controls. The rate of full scores based on the Glasgow Coma Scale (15 points) was significantly increased in the FTY720 groups on day 7 (100% *vs* 50%, P = 0.01), and NIHSS score reduction was also observed in the FTY720 group (7.5 *vs* 0.5, P < 0.001). Better neurological recovery was associated with lower circulating lymphocyte counts. Long-term beneficial neurological effects were also identified in the FTY720-treated group, such as higher modified Barthel Index score (ranging from 95 to 100; 63% *vs* 0%, P = 0.001) and better modified Rankin Scale score (range, 0-1; 63% *vs* 0%, P = 0.001). FTY720 groups also exhibited fewer ICH-related lung infections. These results clearly demonstrated the efficacy of FTY720 in intracerebral hemorrhagic patients. However, FTY720 was reported to be associated with cardiovascular side effects such as bradycardia and atrioventricular blockages, which were observed during the early clinical trials for multiple sclerosis, and one cardiovascular-linked death was reported in the post-marketing period [82]. FTY720 also showed potential for teratogenicity in pregnancy that 5% of congenital abnormalities (skeletal malformations and acrania) was seen in the 66 pregnant women taking FTY720 prior to or during pregnancy [83].

Therefore, the safety of FTY720 administration should be closely examined, especially in patients with current or previous heart failure, and woman who is willing to deliver baby.

CONCLUSION

In this review, we discussed the biological and functional mechanisms associated with FTY720 and reviewed the current experimental and clinical data regarding the use of this agent as a neuroprotective drug against stroke. FTY720 seems to be a promising drug to treat stroke; however, further studies are warranted to safely and efficiently bring this to clinical practice in the future.

LIST OF ABBREVIATIONS

AP-1	=	Activator Protein 1
BBB	=	Blood-brain-barrier
CNS	=	Central Nervous System
CoA	=	Coenzyme A
CYP450	=	Cytochrome P450
ERKs	=	Extracellular Signal-regulated Kinases
FDA	=	Food and Drug Administration
HDAC	=	Histone Deacetylases
I2PP2A	=	Inhibitor 2 of Protein Phosphatase 2A
ICAM-1	=	Intracellular Adhesion Molecule-1
ICH	=	Intracerebral Hemorrhage
mTOR	=	Mammalian Target Of Rapamycin
MS	=	Multiple Sclerosis
NAFT1	=	Nuclear Factor of Activated T-cells 1
NFκB	=	Nuclear Factor-kappa B
PI3K	=	Phosphatidylinositol-3-kinase
FTY720-P	=	Phosphorylated form or FTY720
PKC	=	Protein Kinase C
SphK	=	Sphingosine Kinase
S1P	=	Sphingosine-1-phosphate
STAIR	=	Stroke Therapy Academic Industry Roundtable

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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