Critical Reviews in Biochemistry and Molecular Biology, 2011; 46(1): 2–10

informa
healthcare

REVIEW ARTICLE

Roles for lysophospholipid S1P receptors in multiple sclerosis

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Abstract

Sphingosine 1-phosphate (S1P) signaling in the treatment of multiple sclerosis (MS) has been highlighted by the efficacy of FTY720 (fingolimod), which upon phosphorylation can modulate S1P receptor activities. FTY720 has become the first oral treatment for relapsing MS that was approved by the FDA in September 2010. Phosphorylated FTY720 modulates four of the five known S1P receptors ($S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$) at high affinity. Studies in human MS and its animal model, experimental autoimmune encephalomyelitis (EAE), have revealed that FTY720 exposure alters lymphocyte trafficking via sequestration of auto-aggressive lymphocytes within lymphoid organs, representing the current understanding of its mechanism of action. These effects primarily involve $S1P_1$, which is thought to attenuate inflammatory insults in the central nervous system (CNS). In addition, FTY720's actions may involve direct effects on S1P receptor-mediated signaling in CNS cells, based upon the known expression of S1P receptors in CNS cell types relevant to MS, access to the CNS through the blood–brain barrier (BBB), and *in vitro* studies. These data implicate lysophospholipid signaling – via $S1P_1$ and perhaps other lysophospholipid receptors – in therapeutic approaches to MS and potentially other diseases with immunological and/or neurological components.

Keywords: *sphingosine 1-phosphate; FTY720; fingolimod; GPCR; experimental autoimmune encephalitis*

Introduction

Sphingosine 1-phosphate (S1P) is a lysophospholipid, which exerts diverse biological activities under physiological and pathological conditions through cell surface G protein-coupled receptors (GPCRs), named $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$ (Ishii *et al.*, 2004). S1P is present at submicromolar concentrations in various biological fluids and tissues (Ishii *et al.*, 2004) and is produced intracellularly by a series of enzymatic reactions involving membrane-derived sphingolipids that ultimately provide sphingosine as a substrate for sphingosine kinase type 1 (SphK1) and type 2 (SphK2), to produce S1P, which can then act in both autocrine and paracrine fashions (Alvarez *et al.*, 2007).

This receptor-mediated signaling system, along with its sphingolipid metabolic pathway, is central to the actions of a compound known as FTY720 (fingolimod) that was recently approved by the FDA as a treatment for relapsing multiple sclerosis (MS). FTY720 was initially reported as a myriocin derivative identified through studies of fungal metabolites, which possessed immunosuppressive properties by selectively depleting mature T cells in skin allograft models (Adachi *et al.*, 1995). FTY720 is a sphingosine analog that is also phosphorylated *in vivo* by SphKs, particularly SphK2, to produce FTY720-phosphate (FTY720-P) (Paugh *et al.*, 2003; Billich *et al.*, 2003; Zemann *et al.*, 2006), whereupon it can activate four subtypes of S1P receptors, $S1P_{1/3/4/5}$, with single-digit or lower nanomolar affinities (Brinkmann *et al.*, 2002; Mandala *et al.*, 2002). FTY720 was evaluated in humans as an agent to prevent renal transplantation rejection, although these studies were ultimately terminated for lack of efficacy (Budde *et al.*, 2006). However, studies of FTY720 in experimental autoimmune encephalitis (EAE), an animal model of MS, supported therapeutic relevance to this disease (Brinkmann *et al.*, 2002; Fujino *et al.*, 2003; Webb *et al.*, 2004; Kataoka *et al.*, 2005;

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(Received 09 July 2010; revised 27 August 2010; accepted 08 September 2010)

ISSN 1040-9238 print/ISSN 1549-7798 online © 2011 Informa Healthcare USA, Inc.
DOI: 10.3109/10409238.2010.522975

Papadopoulos *et al.*, 2010), warranting further clinical evaluation.

MS is a chronic inflammatory demyelinating disease of the central nervous system (CNS) and can be associated with irreversible progression of neurological disability (Frohman *et al.*, 2006). Currently, there is no cure for MS. Until the recent approval of FTY720 as an orally bioavailable treatment for relapsing MS, FDA-approved disease modifying therapies only consisted of injectable, immunosuppressive drugs such as interferons (IFNs) ß-1a and -1b, glatiramer acetate and natalizumab (Martin, 2010). FTY720 has been assessed in extensive clinical trials for relapsing-remitting MS (Kappos *et al.*, 2010; Cohen *et al.*, 2010), where it has shown efficacy along with an acceptable safety profile, and has received an approval from FDA in September 2010. The side-effect profile consists of a range of generally rare events (Cohen *et al.*, 2010; Kappos *et al.*, 2010), the most common of which is a transient bradycardia with initial treatment, resolving with continued exposure. Rarer events include nasopharyngitis, slightly reduced pulmonary function, and reversible macular edema (reviewed in Chun and Hartung, 2010). FTY720 recently received approval from the FDA as the first oral treatment for relapsing MS.

The current view on the mechanism of action of FTY720 is that it improves MS signs and symptoms by altering immune responses, particularly through effects on lymphocyte trafficking. In addition, the fact that relevant S1P receptors are expressed within the brain, and that FTY720 can penetrate the blood–brain barrier (BBB), raises the possibility that FTY720 may have direct effects on the CNS cells as well. This review will focus on S1P receptor mechanisms and relevant cell types that could contribute to the efficacy of FTY720 in MS to produce its demonstrated *in vivo* effects on the immune system, along with possible contributions of direct CNS influences.

S1P signaling and FTY720 efficacy in the immune system

T cells

MS is thought to be caused, at least in part, by an autoimmune attack of the CNS by myelin-specific CD4-positive T cells. The pathogenesis of MS is characterized by demyelination associated with infiltration of inflammatory cells and release of various cytokines and chemokines in the CNS (Frohman *et al.*, 2006). In EAE animal models, therapeutic or prophylactic administration of FTY720 reduces the infiltration of lymphocytes into the spinal cord with a rapid reduction in lymphocyte numbers in the peripheral blood produced by sequestration of lymphocytes within primary and secondary lymphoid organs. This is thought to be the central mechanism of action of FTY720 for disease attenuation (Brinkmann *et al.*, 2002; Fujino *et al.*, 2003; Webb *et al.*, 2004; Kataoka *et al.*, 2005; Mehling *et al.*, 2008). In addition, FTY720 reduces levels of proinflammatory products, such as interleukin (IL)-17, IFN-γ, and inducible nitric oxide synthase (iNOS), in the spinal cord of EAE animals, which also may contribute to attenuating the disease state (Fujino *et al.*, 2003; Webb *et al.*, 2004; Kataoka *et al.*, 2005; Papadopoulos *et al.*, 2010).

As just noted, FTY720 reduces lymphocyte numbers in the blood and lymph by sequestering them in the thymus and the secondary lymphoid organs such as lymph nodes and Peyer's patches (Chiba *et al.*, 1998; Pinschewer *et al.*, 2000; Mandala *et al.*, 2002). Histology has shown that FTY720 treatment induces emptying of lymphoid sinuses, suggesting that lymphocytes cannot access egress structures and cannot egress into lymph (Mandala *et al.*, 2002). The effect is reversible (Pinschewer *et al.*, 2000) and observed in both naïve and activated T cells (Xie *et al.*, 2003). Thus, the mechanism of action of FTY720 in MS is believed to be a blockage of the inflammatory cell infiltration into the lesion site, resulting from sequestration of lymphocytes in the thymus and secondary lymphoid organs and a subsequent depletion of circulating autoaggressive lymphocytes.

The regulation of lymphocyte egress involves the S1P receptor subtype $S1P_1$, which allows lymphocytes to sense an S1P concentration gradient existing between blood/lymph and lymphoid tissues. This process regulates lymphocyte recirculation from within lymphoid organs back to the blood. Several lines of evidence support this model. In the normal condition, S1P concentrations are high in blood and lymph, and low in lymphoid organs (Schwab and Cyster, 2007). When S1P in lymph is lost by genetic deletion of the S1P producing enzymes SphK1/2 from lymphatic endothelial cells, lymphocytes cannot egress from lymph nodes into lymph circulation (Pappu *et al.*, 2007; Pham *et al.*, 2010). Expression level of $S1P_1$ in thymocytes increases during their maturation, and CD4 or CD8 single-positive mature T cells acquire the ability to migrate towards increasing S1P concentrations (Matloubian *et al.*, 2004). $S1P_1$ deletion from lymphocytes results in an inhibition of lymphocyte egress from the thymus and peripheral lymphoid organs. This has been shown in studies of conditional $S1P_1$ deletion from lymphocytes using a *Lck* promoter-driven Cre or transplantation of $S1P_1$-null hematopoietic cells into irradiated wild-type animals (Allende *et al.*, 2004; Matloubian *et al.*, 2004). These studies indicate that lymphocyte egress is dependent on lymphocyte expression of $S1P_1$ and requires an S1P concentration gradient. Since FTY720 treatment mimics the effect of $S1P_1$ deletion from lymphocytes, FTY720, via its active phosphorylated metabolite, may act predominantly as a functional antagonist of

lymphatic $S1P_1$ under therapeutic conditions despite its agonist properties under acute exposure conditions. In contrast to the dramatic effect on lymphocyte egress, FTY720 does not appear to affect significantly the activation, proliferation, or effector functions of T and B cells (Pinschewer *et al.*, 2000; Brinkmann *et al.*, 2001).

B cells

Although investigations into MS pathophysiology have focused mainly on T cells, growing evidence suggests a contribution of B cells which act as antigen presenting cells to T cells and secrete proinflammatory cytokines, chemokines and autoantibodies targeting structures on the myelin sheath and the axon (McLaughlin and Wucherpfennig, 2008). Rituximab (rituxan), a monoclonal antibody against CD20, has provided direct evidence of B cell involvement in MS pathology. Rituximab inhibits MS-related inflammation by specific depletion of B cells and B cell-producing autoantibodies (Hauser *et al.*, 2008). In addition, several studies have demonstrated that $S1P_1$-mediated signaling regulates trafficking of B cells. Deletion or downregulation of $S1P_1$ in developing bone marrow B cells inhibits the release of newly generated immature B cells from the bone marrow into the blood (Allende *et al.*, 2010). Either FTY720 treatment or $S1P_1$-deletion reduces the number of IgG- and IgA-secreting mature B cells in blood and bone marrow by sequestration in secondary lymphoid organs (Kabashima *et al.*, 2006; Kunisawa *et al.*, 2007). Indeed, B cell numbers in the blood of EAE animals decrease following FTY720 treatment (Kataoka *et al.*, 2005). Thus, it is likely that S1P signaling in B cells, as well as T cells, is altered during FTY720 exposure by regulating the distribution of B cells, and possibly altering the release of cytokines, chemokines and autoantibodies. Other S1P receptor subtypes may be involved in this process based on a report identifying $S1P_3$ as contributing to B cell positioning (Cinamon *et al.*, 2008).

Other immune cells

Natural killer (NK) cells have been shown to play a role in MS, but controversy exists as to whether they are protective or pathogenic (Morandi *et al.*, 2008). $S1P_5$ is highly expressed in NK cells and is required for NK cell egress from bone marrow and lymph nodes (Walzer *et al.*, 2007; Jenne *et al.*, 2009). In addition, $S1P_1$ is expressed in NK cells and may be involved in the regulation of NK cell egress (Jenne *et al.*, 2009). $S1P_5$ deficiency severely blocks NK cell egress, whereas $S1P_1$ deficiency does not, indicating that NK cell egress is regulated mostly by $S1P_5$. Thus, targeting S1P receptors in NK cells may influence the pathogenesis of MS by altering their tissue distribution, although the outcome could be either protective or pathogenic. Whether FTY720 efficacy involves alterations of NK cells through $S1P_5$ remains unclear.

Antigen presenting cells, such as dendritic cells (DCs), macrophage/microglia, and astrocytes, are also involved in MS pathology (Slavin *et al.*, 2010). FTY720 treatment affects DC features such as migration and cytokine production *in vitro*, which are essential as antigen presenting cells (Muller *et al.*, 2005), and modulates DC trafficking *in vivo* (Czeloth *et al.*, 2005; Lan *et al.*, 2005). It is possible that S1P signaling in DCs may be involved in MS pathogenesis and could therefore be a therapeutic target.

S1P signaling and FTY720 efficacy in the CNS

S1P receptors are expressed in the CNS. FTY720 can penetrate the BBB and enter the CNS where it can be phosphorylated to its bioactive form, FTY720-P (Meno-Tetang *et al.*, 2006; Foster *et al.*, 2007). Brain levels of FTY720 and FTY720-P increase dose-dependently, and over time, exceed levels present in blood by several fold (Meno-Tetang *et al.*, 2006; Foster *et al.*, 2007). In addition, studies have demonstrated effects of FTY720 on CNS cell types as described below, consistent with their expression of S1P receptors. These observations raise the possibility that FTY720 efficacy for MS may involve direct actions on CNS cell types, in addition to effects on the immune system.

Astrocytes

Astrocytes are glial cells involved in the maintenance of the BBB, CNS metabolism, and synaptic functioning, as well as responding to pathological insults in the CNS. Recent evidence suggests a dual role of astrocytes in CNS inflammatory diseases such as MS. Astrocytes not only have the ability to enhance immune responses and inhibit myelin repair by forming a glial scar and preventing migration and maturation of oligodendrocyte progenitor cells, but can also be protective and limit CNS inflammation while supporting oligodendrocyte and axonal regeneration in some experimental systems (Williams *et al.*, 2007; Nair *et al.*, 2008).

There is evidence for the involvement of S1P signaling in astrocytes relevant to the pathogenesis of MS. Activation of S1P signaling induces astrogliosis *in vivo*, a prominent feature of CNS injury and neurodegenerative diseases, including MS (Sorensen *et al.*, 2003), and promotes proliferation of astrocytes *in vitro* (Pebay *et al.*, 2001; Sorensen *et al.*, 2003; Yamagata *et al.*, 2003; Bassi *et al.*, 2006). An animal model of Sandhoff disease, another neurodegenerative disease associated with astrogliosis, can be attenuated by genetic deletion of either SphK1 or $S1P_3$ (Wu *et al.*, 2008). S1P is released from astrocytes in an SphK dependent manner and acts

in both autocrine and paracrine manners (Riboni *et al.*, 2000; Anelli *et al.*, 2005). Upon injury, S1P production is locally increased and is associated with reactive astrocytes and microglia, suggesting S1P production from these cell types at the injury site (Kimura *et al.*, 2007). In cultured astrocytes, FTY720 exposure activates G_i-mediated signaling cascades, such as decreases in cyclic AMP, inositol phosphate formation and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation, and stimulates migration (Mullershausen *et al.*, 2007; Osinde *et al.*, 2007). Many of these cell culture effects are mimicked by $S1P_1$ agonists (S1P, SEW2871 and AUY954) and are attenuated by $S1P_1$ antagonists (VPC23019, W123 and W146) or genetic deletion of $S1P_1$ (Mullershausen *et al.*, 2007; Osinde *et al.*, 2007; Dev *et al.*, 2008), suggesting the involvement of $S1P_1$. Astrocytes in culture preferentially express $S1P_1$ and $S1P_3$, and a low level of $S1P_2$ (Pebay *et al.*, 2001; Rao *et al.*, 2003; Anelli *et al.*, 2005). By comparison, $S1P_5$ expression appears to be below detectable limits under basal conditions, but can be upregulated in culture when cells are exposed to growth factors (Rao *et al.*, 2004). *In vivo* effects in EAE models as well as direct and indirect actions of signaling on astrocytes remain to be determined.

Oligodendrocytes

Oligodendrocytes are myelin-forming glial cells of the CNS. Loss of CNS myelin and a failure of remyelination by oligodendrocytes are a characteristic of the disease and likely contribute to subsequent irreversible disability in MS (Miller and Mi, 2007). Thus, overcoming remyelination failure could be a therapeutic strategy in MS. Remyelination requires proliferation and migration of oligodendrocyte progenitor cells into demyelinated lesion sites and subsequent differentiation into mature myelin-forming cells (Miller and Mi, 2007).

In vivo, therapeutic administration of FTY720 reduces the area of demyelination in the spinal cord of animals with EAE (Kataoka *et al.*, 2005; Papadopoulos *et al.*, 2010). In organotypic culture where the systemic immune system is absent, FTY720 treatment enhances remyelination following lysolecithin-induced demyelination. This includes an increase in the number of oligodendrocyte progenitor cells, membrane outgrowth and elaboration of processes, as well as increases in microglia number and immunoreactivity for the astrocyte marker glial fibrillary acidic protein (GFAP). Both microglia and astrocytes can create an environment permissive for remyelination. Enhanced remyelination and associated astrogliosis are thought to be mediated through $S1P_{3/5}$, whereas microgliosis may occur through $S1P_{1/5}$, based upon *in vitro* experimental studies (Miron *et al.*, 2010). Other *in vitro* studies have demonstrated direct effects of FTY720 on oligodendrocytes and progenitor cells that include survival, proliferation, migration, and differentiation, all of which are involved in the process of remyelination. In cultured oligodendrocytes and progenitor cells, FTY720 exposure activates ERK1/2 and Akt, which are involved in cell survival signals (Coelho *et al.*, 2007; Jung *et al.*, 2007). Indeed, exposure to FTY720 or FTY720-P protects these cells from apoptosis induced by deprivation of serum/growth factor, as well as apoptosis induced by inflammatory cytokines (e.g. tumor necrosis factor (TNF)-α and IFN-γ) and microglial activation, which have all been implicated in the pathogenesis of MS (Coelho *et al.*, 2007; Jung *et al.*, 2007; Miron *et al.*, 2008). A study using primary cells prepared from $S1P_5$-null animals has shown that $S1P_5$ is required for survival of mature, but not immature, oligodendrocytes (Jaillard *et al.*, 2005). In addition, FTY720 can synergistically increase platelet derived growth factor-dependent cell cycle progression of oligodendrocyte progenitor cells (Jung *et al.*, 2007), inhibit migration of oligodendrocyte progenitor cells through $S1P_5$ (Novgorodov *et al.*, 2007), and induce process retraction in oligodendrocytes, although the effect is transient and followed by subsequent re-extension (Jaillard *et al.*, 2005; Miron *et al.*, 2008). FTY720 can either promote or inhibit differentiation of oligodendrocyte progenitor cells into oligodendrocytes depending on its dose (Coelho *et al.*, 2007; Jung *et al.*, 2007). Thus, direct action of FTY720 exposure on oligodendroglial lineage cells can be both beneficial (promotion of survival, proliferation, and differentiation) and detrimental (inhibition of migration and differentiation) for remyelination. However, FTY720 seems to enhance remyelination in conjunction with other CNS cells combined with altering immune system influences.

Gene expression studies have identified S1P receptors, along with other lysophospholipid receptors, on oligodendrocytes and/or their precursor cells (Weiner *et al.*, 1998; McGiffert *et al.*, 2002), and oligodendroglial lineage cells preferentially express $S1P_5$, with lower levels of $S1P_1$, $S1P_2$, and $S1P_3$ (Terai *et al.*, 2003; Yu *et al.*, 2004; Miron *et al.*, 2008). Overall, $S1P_5$ gene expression is prominent in oligodendroglial lineages, but it is still unclear if the FTY720-$S1P_5$ signaling axis is actually involved in remyelination of MS lesions. It is of note that $S1P_5$-deficient mice do not show deficits in myelination (Jaillard *et al.*, 2005).

Microglia

Microglia, brain-resident, non-neural cells, play a role in MS throughout the disease process. They are rapidly activated and recruited to inflammatory sites within the CNS, and function as antigen-presenting cells, initiating and propagating immune responses, phagocytosing damaged tissues and debris, and producing various factors that are both tissue-toxic and protective (Jack *et al.*, 2005).

S1P signaling in microglia may be involved in migration and enhancement of the inflammatory response, but its *in vivo* role for MS remains unclear. *In vitro*, S1P treatment increases the expression of proinflammatory cytokines such as TNF-α and IL-1ß and nitric oxide in lipopolysaccharide (LPS)-activated microglia (Tham *et al.*, 2003; Nayak *et al.*, 2010). *In vivo*, FTY720 treatment attenuates the infiltration of reactive macrophages/microglia into lesion sites produced by traumatic brain injury (Zhang *et al.*, 2007). Gene expression levels of S1P receptors in microglia vary depending on their activation state. Microglia in inactive states express $S1P_1$ and $S1P_3$, with little $S1P_2$, and very low $S1P_5$ (Tham *et al.*, 2003). Upon activation, downregulation of $S1P_1$ and $S1P_3$ and upregulation of $S1P_2$ occur (Tham *et al.*, 2003). S1P may be produced from activated microglia, as described above (Kimura *et al.*, 2007). The effects of *in vivo* FTY720 exposure on microglial responses, as mediated by identified receptors, remain to be established, particularly with respect to MS therapeutic effects.

Neurons

S1P receptors $S1P_{1\text{-}3}$ are expressed in the developing brain (McGiffert *et al.*, 2002), and can influence neurogenesis (Mizugishi *et al.*, 2005). Mice with constitutive deletion of either SphK1/SphK2 or $S1P_1$ show neurogenic defects (Mizugishi *et al.*, 2005). In primary cultures of neural progenitor cells, S1P treatment induces survival, proliferation, and morphological changes, and enhances nerve growth factor (NGF)-induced neurite extension (Edsall *et al.*, 1997; Harada *et al.*, 2004; Toman *et al.*, 2004). In primary dorsal root ganglion neurons, S1P treatment affects NGF-induced neurite extension and enhances NGF-induced neuronal excitability (Toman *et al.*, 2004; Zhang *et al.*, 2006). In *Xenopus*, S1P signaling can influence axon guidance (Strochlic *et al.*, 2008). In addition, S1P signaling may promote neuronal repair after injury: neural stem/progenitor cells transplanted into the injured spinal cord migrate toward injured sites in an $S1P_1$-dependent manner (Kimura *et al.*, 2007).

FTY720 has been reported to have neuroprotective effects. Treatment with FTY720 may reduce sequelae in an ischemic stroke rat model (Hasegawa *et al.*, 2010) and may reduce inflammation and promote functional recovery after spinal cord injury (Lee, KD *et al.*, 2009). However, it is unclear whether these effects involve direct actions on neurons or secondary effects of immunosuppression. Uncertainties inherent to these models, particularly their inability to predict efficacy in humans, support further studies to ascertain both possible neuroprotective functions, as well as direct versus indirect mechanisms.

Blood–brain barrier (BBB)

The pathogenesis of MS includes the penetration of inflammatory cells across the BBB into the CNS parenchyma (Correale and Villa, 2007). The penetration occurs through (a) adherence of activated T cells and other lymphocytes to endothelial cells; (b) subsequent degradation of endothelial basement membrane; and (c) migration through the endothelium into the CNS parenchyma (Correale and Villa, 2007). S1P receptors are expressed on the endothelium and could therefore participate in aspects of the BBB since vascular endothelial cells express $S1P_1$ and $S1P_3$ (Lee, MJ *et al.*, 1999). $S1P_1$ expression within endothelial cells is essential for embryonic blood vessel development, which was shown by a study using conditional mutants with specific deletion of $S1P_1$ from endothelial cells (Allende *et al.*, 2003). Furthermore, S1P enhances physical barrier properties of endothelial cells by inducing adherens junction assembly and tight junction formation (Lee, MJ *et al.*, 1999; Sanchez *et al.*, 2003; Lee, JF *et al.*, 2006). It also attenuates vascular permeability induced by thrombin, vascular endothelial cell growth factor (VEGF) or LPS-mediated acute lung/renal injury (Sanchez *et al.*, 2003; Schaphorst *et al.*, 2003; Peng *et al.*, 2004). Like S1P, FTY720 exposure can also induce adherens junction assembly and attenuate vascular leakage induced by VEGF or in LPS-mediated acute lung injury (Sanchez *et al.*, 2003; Peng *et al.*, 2004). In addition, both S1P and FTY720 can activate G_i/Akt/ERK cell survival signals in endothelial cells, and can protect endothelial cells from apoptosis induced by serum deprivation or C_2-ceramide (Lee, MJ *et al.*, 1999; Sanchez *et al.*, 2003). Thus, unlike lymphocyte trafficking in which FTY720 exposure may result in functional antagonistic activities, FTY720's effects on endothelial cell functions seem to be agonistic. Interestingly, S1P-induced barrier enhancement and survival of endothelial cells appear to be mediated through $S1P_1$ and $S1P_3$ (Lee, MJ *et al.*, 1999; Schaphorst *et al.*, 2003; Dudek *et al.*, 2007), while FTY720-induced barrier enhancement is likely through non-$S1P_1$, G_i-coupled receptor(s) (Dudek *et al.*, 2007). Additionally, an integral cellular element of the BBB is the astrocyte through its documented interactions with endothelial cells (Abbott *et al.*, 2006), which may have particular relevance to FTY720's effects in view of the aforementioned S1P receptor-mediated activities influenced by FTY720 exposure.

In MS patients and EAE animals, lesion sites, cerebrospinal fluid and/or serum exhibit evidence of upregulation for vascular cell adhesion molecules (i.e. ICAM-1, P-selectin, and VCAM-1) and matrix metalloproteinases (i.e. MMP-2, -3, -7, and -9), the former facilitating cell adhesion and the latter, basement membrane degradation (Cuzner and Opdenakker, 1999; Waubant *et al.*, 1999; Foster *et al.*, 2009). FTY720

treatment could conceivably reduce or reverse the BBB breakdown that occurs in MS/EAE, as evidenced by therapeutic FTY720 treatment on EAE animals that displayed a reduction in immunoglobulin precipitation that reflects BBB damage in the spinal cord (Foster *et al.*, 2009). Moreover, both prophylactic and therapeutic treatment of FTY720 normalized upregulated gene expression of vascular cell adhesion molecules (ICAM-1, P-selectin, and VCAM-1) and MMP-9 in the spinal cord of EAE animals, suggesting at least a partial recovery of the BBB (Foster *et al.*, 2009). A recent study using a BBB model with isolated human brain endothelial cells has suggested the involvement of $S1P_1$ in protection from oxygen/glucose deprivation (Zhu *et al.*, 2010). Additional evidence is needed to establish effects of FTY720 on BBB regulation.

Receptor mechanisms

Several studies have shown that FTY720 can inhibit S1P signaling by inducing prolonged receptor internalization and degradation (Matloubian *et al.*, 2004; Graler and Goetzl, 2004). These effects can be attributed to the irreversible internalization of bound FTY720-P that results in ubiquitination and proteosomal degradation of at least $S1P_1$ (Oo *et al.*, 2007). As a result of this irreversible internalization, $S1P_1$ is unavailable to sense the S1P gradient that is necessary for lymphocytes to egress out of the immune compartment via the efferent lymph (e.g. within lymph nodes) (Schwab and Cyster, 2007). This mechanism, referred to as functional antagonism as noted above, may be more complex based on a report of persistent intracellular signaling from internalized $S1P_1$ by FTY720-P (Mullershausen *et al.*, 2009), although the biological significance of such signaling on lymphocyte trafficking remains to be determined. The endogenous levels of FTY720 within tissues may dictate the actual modulatory effects observed within lymphoid organs (Sensken *et al.*, 2009). These data indicate that the precise definition of whether FTY720-P functions as an agonist or an antagonist in an experimental disease setting may vary depending on experimental conditions. In humans, reductions of peripheral blood lymphocytes, indicative of reduced lymphocyte egress, clearly follow a dose-response (higher FTY720 concentrations are proportional to a reduction in peripheral blood lymphocytes) (Tedesco-Silva *et al.*, 2005), indicating that the dominant effect of FTY720 on lymphocytes is likely to be through functional antagonism of $S1P_1$ and possibly other S1P receptors, at least with respect to lymphocyte trafficking. In contrast, FTY720's effects on endothelial cells are most consistent with agonism of non-$S1P_1$, G_i-coupled receptor(s).

Conclusion

The discovery of FTY720 and establishment of its efficacy in humans for the treatment of relapsing-remitting MS have revealed the relevance of receptor-mediated S1P signaling to MS. A majority of *in vivo* functional studies have demonstrated that FTY720, through identified S1P receptors, affects lymphocyte trafficking, which in turn has been inferred as the major mechanism for ameliorating MS signs and symptoms. In addition, experimental data support the actions of FTY720 exposure on CNS components that could theoretically contribute to efficacy in MS. However, CNS functional *in vivo* data relevant to MS remain to be established. Further studies will elucidate the mechanism of action of FTY720 in MS, including a more complete view of affected cell types, S1P receptor subtypes, downstream signaling pathways, and interactions between the immune system and the CNS. With the recent FDA approval of FTY720 (fingolimod) as the first orally bioavailable therapy for relapsing MS, a new chapter in the treatment of MS could be opening, based upon S1P lysophospholipid receptor signaling.

Acknowledgements

The authors thank Yasuyuki Fujii and Ji Woong Choi for vital discussions and Danielle Letourneau for editorial assistance.

Declaration of interest

This work was supported by NIH grants NS048478 and DA019674 to JC. KN is a recipient of a postdoctoral fellowship from Novartis Pharma, AG. JC is a consultant for Novartis Pharmaceutical Corp.

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Editor: Michael M. Cox