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Review

# Sphingosine 1-phosphate chemical biology

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ABSTRACT

A dozen years ago, the term 'S1P' (sphingosine 1-phosphate) was not in the lexicons of scientific literature databases. By early 2008, this query term retrieved well over 1000 citations from PubMed — about 225 of these appeared in 2007. Indeed, S1P is arguably the most heavily studied lipid molecule at present. What happened to distinguish S1P among many other signaling lipids? We believe that the seminal event was the linking of the investigational drug, FTY720 (fingolimod), to S1P signaling. This realization profoundly altered understanding of S1P biology, revealing both that S1P is prominent in lymphocyte trafficking and that mimicking S1P signaling with an agonist drug can modulate the immune system to considerable therapeutic benefit. Neither fact was known prior to FTY720; indeed, this molecule is testament to the power of chemical biology. In this communication, we attempt to summarize progress to date in S1P chemical biology.



## 1. S1P biosynthesis and degradation

In mammals, the long chain base sphingosine is formed by amidase catalyzed hydrolysis of ceramides. Sphingosine is phosphorylated by sphingosine kinase types 1 or 2 (SPHK1, SPHK2) to form S1P, which is either converted back to sphingosine by lipid phosphatases or degraded irreversibly by S1P lyase [1]. S1P synthesis occurs in cells (but see reference [2]), thus the existence of S1P in plasma indicates some efflux system is responsible for S1P's appearance. A small fraction of long chain bases lack a double bond (sphinganine (dihydrosphingosine), which is the precursor to ceramide in mammalian sphingolipid anabolism) [3]. Sphinganine is a substrate of SPHK and the product, sphinganine 1-phosphate, is for the most part indistinguishable from S1P in its biologic effects (but see reference [4]). The S1P biosynthetic pathway is widespread among mammalian tissues.

S1P concentrations in human and mouse plasma are 200–800nM, where the molecule is nearly all protein-bound. S1P introduced into the mouse vasculature is degraded quickly (T1/2 15min [5]), which indicates a rapid flux of sphingosine through the pathway outlined above. Mice lacking either SPHK1 or SPHK2 have decreased plasma S1P concentrations [6–8], but the reduction is more pronounced in SPHK1 null animals [6]. Disruption of both *Sphk1* and *Sphk2* gene loci is embryonic lethal in mice [9]. Characterization of the phosphatase(s) that hydrolyze the S1P phosphate monoester has been problematic. Leading candidates for this enzyme are the integral membrane lipid ectophosphatase LPP3 (lipid phosphate phosphohydrolase type 3) [10] and distantly-related members of the same enzyme family that are selective for sphingoid lipids (SPP1, SPP2) [11]. The paucity of selective substrates for, and inhibitors of, these enzymes, as well as the lack of useful mutant mice, leaves the identity of S1P phosphatase uncertain at present.

## 2. S1P receptors

S1P signals cells through a set of five, rhodopsin family G-protein coupled receptors named S1P1–5 (formerly EDG1, EDG5, EDG3, EDG6, EDG8) (see reference [12] for review). S1P1, S1P2, and S1P3 are expressed by a wide variety of tissues in mice and humans while S1P4 and S1P5 expression are largely limited to cells of hematopoietic origin. S1P5 is expressed also by oligodendrocytes. The affinity constants of S1P (or dihydro S1P) for the S1P receptor/G-protein complex are mostly in the single digit nanomolar range [13]. S1P has a lower affinity for the S1P4 receptor; in strict receptor nomenclature terms, S1P4 is a phytoS1P (rather than S1P) receptor because this minor S1P form (phytosphingosine lacks a 4–5 double bond, rather it has a 4-hydroxyl group) has about 10-fold higher affinity for the S1P4 receptor than S1P [14]. S1P receptors couple to a variety of heterotrimeric G-proteins with the exception of Gαs. The ability of pertussis toxin to interdict many S1P signaling events *in vitro* illus-

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trates the prominence of signaling via Gαi/o. Spiegel has invoked an additional, intracellular S1P receptor (see, for example, [15]), but the identity of this molecule(s) remains unknown.

Germ line disruption of the S1P1 receptor gene is embryonic lethal (E13.5) because of a failure of vascular maturation [16]. This defect is phenocopied by disruption of *S1p1* in the endothelial cell lineage [17] and, satisfyingly, by SPHK1/SPHK2 null mice [9]. S1P2 null mice are seizure-prone [18] and the inner ear does not develop normally, rendering these animals deaf [19,20]. S1P3 null mice are phenotypically unremarkable [21] as are, apparently, S1P5 null mice [22]. S1P4 null mice have not been reported.

## 3. FTY720

FTY720 was discovered in the course of a structure–activity relationship (SAR) study using myriocin (ISP-1) as the lead (see Fig. 1). Myriocin, which is a fungal-derived phytosphingosine analog with a connection to Chinese herbal medicine [23], is an inhibitor of serine palmitoyl CoA transferase (SPT, the first enzyme in sphingolipid biosynthesis). Initially studied as a potential anti-fungal drug, myriocin was found to be an immunosuppressant in mice [24]. The impetus for FTY720 discovery was a need to avoid the gastrointestinal toxicity of myriocin and to eliminate the chiral centers in that densely functionalized lead compound. Unlike myriocin, FTY720 does not inhibit SPT. FTY720 prolongs skin allografts in mice while evoking a profound lymphocytopenia [24]. We know now that this hematologic abnormality is a biologic signature of S1P1 receptor agonist drugs.

FTY720 is a potent drug; the ED50 for lymphopenia in mice after oral dosing is about 0.1mg/kg (mpk). Curiously, FTY720 was found to be without effect on lymphocytes *in vitro* until concentrations in excess of 1mM are used whereon apoptosis is induced [25]. The anomaly between *in vivo* and *in vitro* potencies was resolved when two groups discovered that FTY720 is phosphorylated rapidly *in vivo* and the product, FTY720-P, is an agonist that is equipotent to S1P at the S1P3, S1P4, and S1P5 receptors and about one log order more potent at S1P1 [13,26]. *In vitro* assays predicted that SPHK2 catalyzes the activation of FTY720 [27] and this prediction was verified when SPHK2 null mice were generated [7,8].

Introduction of either the parent (alcohol) or active (phosphate) drug into rodents results in a rapid equilibrium in blood with the phosphate:alcohol ratio of 3:1 [13]. FTY720 invades the sphingosine biosynthetic pathway to the extent that it is a substrate for SPHK2 and at least one phosphatase. Interestingly, rat blood *ex vivo* converts FTY720 to FTY720-P, but the reverse reaction does not occur, indicating that whatever phosphatase is responsible for the hydrolysis of FTY720-P, it is not active in blood. The ratio of parent to active drug is a summation of import and export activities as well as SPHK2 and phosphatase activities in a specific tissue environment. Thus, it is to be expected that this ratio will be different in various tissues, biologic fluids (*e.g.* CSF) and inside and outside of cells. In stable renal transplantation patients, the elimination half-life of FTY720 was found to be from 89–157h and volume of distribution was 1116 to 1737l, indicating a widespread tissue distribution [28].

The use of S1P3 null mice, FTY720 analogs, and receptor type selective agonists (see below 'Other S1P receptor agonists' section) led quickly to the realization that agonist activity at the S1P1 receptor is responsible for the lymphopenia [29]. The lymphocyte depletion from blood is the result of sequestration of lymphocytes in secondary lymphoid tissue by inhibition of egress [30]. Current thinking is that effector T-lymphocytes are thus prevented from moving to sites of inflammation such as allografts and autoimmune disease. The blockade of lymphocyte trafficking, whilst sparing lymphocyte activation mechanisms, probably underlies FTY720's minimally immunosuppressant effects (e.g. opportunistic infections, malignancies) as compared to, for example, calcineurin inhibitors such as tacrolimus or cyclosporin.

The molecular mechanism whereby FTY720 inhibits lymphocyte egress remains uncertain. One proposal is that FTY720-P, although a receptor agonist, acts as a 'functional (physiologic) antagonist', that is the drug causes desensitization of S1P1 signaling pathways in lymphocytes. These cells are thus rendered effectively S1P1 null and are unable to sense the gradient of S1P across lymphoid tissue that is supposedly necessary for proper egress. This idea is supported by the behavior of S1P1 null thymocytes, which fail to egress properly from lymphoid tissue [30,31]. Further, an S1P lyase inhibitor evokes lymphopenia (see below, 'S1P lyase inhibitors' section), perhaps because S1P accumulates within lymphoid tissues and thus disrupts the S1P gradient [32]. Counter to this argument is the inability of S1P1 receptor antagonists to evoke lymphopenia (although very few S1P receptor antagonists have been described). Further, the model presumes the chemoattractant nature of S1P for lymphocytes yet there is a paucity of evidence for the S1P/S1P1 axis functioning in lymphocyte migration; indeed, S1P is a rather anemic chemoattractant of T lymphocytes. A competing idea is that S1P1 receptor agonists influence 'stromal gates' that control lymphocyte egress [33]. Readers wishing to explore the intricacies of this debate are referred to two excellent recent reviews on the topic [34,35].

FTY720 is efficacious in a wide variety of autoimmune disease and allograft models (see [12] for review). Although the drug failed to meet primary endpoints in phase III renal transplantation studies (i.e. was not significantly better than the comparison drug, mycophenolate mofetil, in combination with cyclosporine [36,37]), FTY720 has exhibited remarkable efficacy in a placebo controlled, phase II trial of relapsing remitting multiple sclerosis [38]. The adverse events associated with FTY720 in humans are a transient bradycardia (common), dyspnea (4% of patients) and macular edema (rarely). In rodents, the bradycardia is not observed with S1P3 receptor-sparing agonists and it was not observed in S1P3 receptor null mice [29,39]. However, the use of S1P3 receptor-sparing agonists in humans or non-human primates has not been reported, thus the relative roles of various S1P receptor types in controlling heart rate in humans are not known.

Finally, the remarkable generosity of Novartis' scientists in distributing FTY720 to academic laboratories has catalyzed numerous studies. In addition to the disease models mentioned above, FTY720 has been documented to be efficacious in models of neoplastic disease [40], atherosclerosis [41], renal ischemia reperfusion injury [42–44], pain [45], angiogenesis [46], acute lung injury [47], and others. It is worth noting that FTY720 might have actions independent of its phosphorylation to FTY720-P. These include antagonism at the CB1 cannabinoid receptor [48] and activation of protein phosphatase 2A [49].

## 4. Other S1P receptor agonists

The motivation for identifying additional S1P receptor compounds is two-fold. First, more selective compounds will help to parse the plethora of FTY720's actions to smaller sets of S1P receptors. Second, more selective S1P receptor agonists might recapitulate the efficacy of FTY720 while avoiding some adverse events such as bradycardia. Regarding the latter goal, S1P1 receptor selective agonists modulate lymphocyte trafficking and this biology might be solely responsible for the therapeutic efficacy of FTY720. However, FTY720 is a 'dirty' drug in that it is an agonist at four S1P receptors. It is important to be mindful that all the clinical data, and nearly all the animal model data, have been developed with this compound. One of the few animal models that run with a more selective compound, AUY954, (an amino carboxylate that is a selective S1P1 agonist) demonstrated that this molecule was efficacious in a rat heart transplant model [50]. It will be interesting to learn whether an S1P1 receptor selective agonist can replicate the success of FTY720 in the clinic.