BIOCHEMICAL PHARMACOLOGY 75 (2008) 1893–1900

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New players on the center stage: Sphingosine 1-phosphate and its receptors as drug targets

Andrea Huwiler [a,*], *Josef Pfeilschifter* [b]

[a] *Institute of Pharmacology, University of Bern, Friedbühlstrasse 49, CH-3010 Bern, Switzerland*
[b] *Pharmazentrum Frankfurt, Klinikum der Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany*

ARTICLE INFO

Article history:
Received 5 November 2007
Accepted 31 December 2007

Keywords:
Sphingolipids
Sphingosine 1-phosphate
S1P receptors
Cancer
Drug therapy

ABSTRACT

The recent identification of a cellular balance between ceramide and sphingosine 1-phosphate (S1P) as a critical regulator of cell growth and death has stimulated increasing research effort to clarify the role of ceramide and S1P in various diseases associated with dysregulated cell proliferation and apoptosis.

S1P acts mainly, but not exclusively, by binding to and activating specific cell surface receptors, the so-called S1P receptors. These receptors belong to the class of G protein-coupled receptors that constitute five subtypes, denoted as $S1P_1$–$S1P_5$, and represent attractive pharmacological targets to interfere with S1P action. Whereas classical receptor antagonists will directly block S1P action, S1P receptor agonists have also proven useful, as recently shown for the sphingolipid-like immunomodulatory substance FTY720. When phosphorylated by sphingosine kinase to yield FTY720 phosphate, it acutely acts as an agonist at S1P receptors, but upon prolonged presence, it displays antagonistic activity by specifically desensitizing the $S1P_1$ receptor subtype.

This commentary will cover the most recent developments in the field of S1P receptor pharmacology and highlights the potential therapeutic benefit that can be expected from these novel drug targets in the future.

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Sphingolipids represent together with glycerophospholipids and cholesterol important constituents of biological membranes. However, it has emerged that some sphingolipid species exert additional functions as signalling molecules and much effort has been invested in the last years to unravel the detailed mechanisms by which such bioactive sphingolipids act. Especially sphingosine 1-phosphate (S1P) has evolved as a highly versatile molecule regulating many cell responses such as cell proliferation and apoptosis, cell differentiation, cell migration and also immunological responses [1–3].

S1P is derived from plasma membrane sphingomyelin by the sequential actions of sphingomyelinases that generate ceramide, ceramidases that generate sphingosine and, ultimately, sphingosine kinases to yield S1P [1,4]. This pathway has also been denoted as the sphingomyelin cycle. For all steps reverse reactions may take place which are catalyzed by specific enzymes such as S1P phosphatases, ceramide synthase and sphingomyelin synthase.

Regarding the sphingosine kinases two subtypes have been described denoted as SphK1 and SphK2 (for review, see [5]). Their in vivo functions are still not clear and, surprisingly, first data from cell culture experiments have revealed that SphK1 and SphK2 have opposite cellular functions in that SphK1 may promote cell growth, whereas SphK2 may rather trigger cell death. A mitogenic effect of S1P was first reported by Olivera and Spiegel [6]. S1P may act via two different mechanisms:

* *Corresponding author*. Tel.: +41 31 632 32 14; fax: +41 31 632 49 92.
E-mail address: Huwiler@pki.unibe.ch (A. Huwiler).
0006-2952/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2007.12.018

either via surface S1P receptors which implies that S1P generated inside the cell by a SphK is released from cells and acts from the outside, or via a still ill defined intracellular mode of action. To date, five different S1P receptors have been identified, denoted as $S1P_{1–5}$. Originally discovered as endothelium differentiation gene (Edg) receptors belonging to the same superfamily of G protein-coupled receptors as the lysophosphatidic acid (LPA) receptors.

Since overexpression of SphK1 in 3T3 fibroblasts leads to increased colony formation in soft agar and an injection of these cells into SCID mice results in tumor formation, an oncogenic potential of SphK1 and its product S1P was suggested [7]. This was further substantiated by the finding that in many solid tumors SphK1 mRNA expression is enhanced when compared to healthy tissue [8,9].

Due to this obvious critical role of S1P in cell growth and survival, an association of S1P to various diseases such as cancer and autoimmune diseases has been discussed [10–12]. Consequently, targeting of S1P to treat such diseases has evolved as a new possible therapeutic strategy.

When considering the details of S1P action, various approaches can be envisioned to target this signalling pathway. From a pharmacological point of view, targeting the G protein-coupled receptors is most attractive as the drugability of this particular class of cell surface receptors is well established. Thus, S1P receptor subtype selective agonists or antagonists could serve as novel and innovative therapeutics. An additional promising approach is the inhibition of the S1P generating enzymes SphK1 and/or SphK2. Finally, in the dynamically growing era of ''biologicals'', neutralizing antibodies against S1P could serve as useful drugs to block the action of extracellular S1P. Each of these strategies has distinct advantages and disadvantages and a broad diversity may be needed in the beginning to solve the problem of designing an optimal treatment for cancer and other diseases.

1. FTY720

It has become evident that the potent immunomodulatory agent FTY720 (fingolimod; 2-amino-2-(2-(4-octylphenyl) ethyl)-1,3-propanediol hydrochloride) (Fig. 1) has strong affinity to the S1P receptors [13,14]. From a cellular aspect, FTY720 reduces the number of circulating lymphocytes in blood by triggering homing of lymphocytes to secondary lymphoid tissues. It was suggested that FTY720 by itself is a prodrug and only its phosphorylation by SphKs, but most efficiently by SphK2, renders it to an active drug acting as a S1P receptor agonist [15,16]. Of the five S1P receptor subtypes, FTY720 phosphate was shown to bind to and activate four, i.e. $S1P_{1,3–5}$ but not $S1P_2$ [13,14]. Most interestingly, the immunomodulatory action of FTY720 is not due to its agonistic effect on S1P receptors, but rather due to its desensitizing effect. For FTY720, it was reported that especially the down-regulating effect on the $S1P_1$ receptor on lymphocytes is responsible for the immunomodulatory action [17,18]. By $S1P_1$ downregulation, lymphocytes are retained in secondary lymphoid organs resulting in a depletion of lymphocytes from the periphery.

It was further proposed that the extracellular concentration of S1P inversely correlates to the $S1P_1$ receptor expression level on lymphocytes. A high concentration of S1P coincides with a low $S1P_1$ receptor expression whereas a low S1P concentration leads to high $S1P_1$ receptor expression. This was recently confirmed in vivo by Pappu et al. [19]. They generated conditional SphK1 and SphK2 double knockout mice. This approach became necessary, because conventional double knockout mice are embryonically lethal. The double KO phenotype was induced 3–5 days after birth and led to undetectable levels of S1P in plasma and lymph. Surprisingly, mice survived without an obvious phenotype. However, $S1P_1$ receptor expression in lymph nodes was strongly upregulated due to S1P absence. More surprising, the egress of T-lymphocytes was inhibited in double knockout lymph nodes and resembled the situation of FTY720 treated mice which have reduced $S1P_1$ receptor expression in T-lymphocytes [17]. Thus, either an upregulation of $S1P_1$ or a downregulation of $S1P_1$ on T-lymphocytes both result in T cell homing in the lymph nodes.

Considering the down-regulating effect of FTY720 on S1P receptors [17,20] and the suggested involvement of S1P in cell growth [6], it was speculated that FTY720 could also be useful for targeted cancer therapy. In this context, studies in prostate cancer cells [21], breast cancer cells [22], liver cancinoma cells [23], renal cancer cells [24], pancreatic cancer cells [25], and leukemia cells [26–28] indeed showed that FTY720 was able to induce apoptosis of the cells. Furthermore, when injected into mice FTY720 significantly reduced tumor growth and metastasis formation without serious side effects [22]. The pro-apoptotic effect of FTY720 was associated with an activation of caspase-3 [21] and PKB/Akt dephosphorylation caused by a FTY720-activated protein phosphatase [26,29]. Whether the anti-tumor activity of FTY720 is also mediated by down-regulating $S1P_1$ receptors or by another mechanism remains to be investigated.

Moreover, it is worth mentioning that FTY720 phosphate also serves to maintain the integrity and function of vascular endothelial cells in a pertussis toxin-sensitive manner [30,31]. This clearly points at a G protein-coupled receptor-mediated event involved in FTY720 phosphate action. FTY720 phosphate was further shown to potently reverse the effect of VEGF on vascular permeability and inhibit the growth of primary and metastatic tumors in a melanoma mouse model [31].

The most common side effect occurring after FTY720 administration is the induction of a transient bradycardia [32]. This is due to the unspecific effect of FTY720 on S1P receptor subtypes other than the $S1P_1$ receptor. In this context, Sanna et al. [33] showed that treating mice with the non-selective S1P receptor agonist and FTY720-phosphate derivative AFD-(R) induced a transient bradycardia, that was not seen in $S1P_3$ deficient mice. Furthermore, the $S1P_1$-selective agonist SEW2871 did not induce bradycardia [33]. These data clearly suggested that especially the activation of the $S1P_3$ receptor subtype contributes to the slowing down of heart rate. In addition, $S1P_3$ also regulates a G protein-gated potassium channel I_{KACh} in atrial myocytes [33] which is activated by FTY720 [34] and further may explain the negative chronotropic effect of FTY720.

An additional severe side effect of FTY720 that finally led to its withdrawal from clinical trials of organ transplantation is the occurrence of a macula degeneration. The mechanism

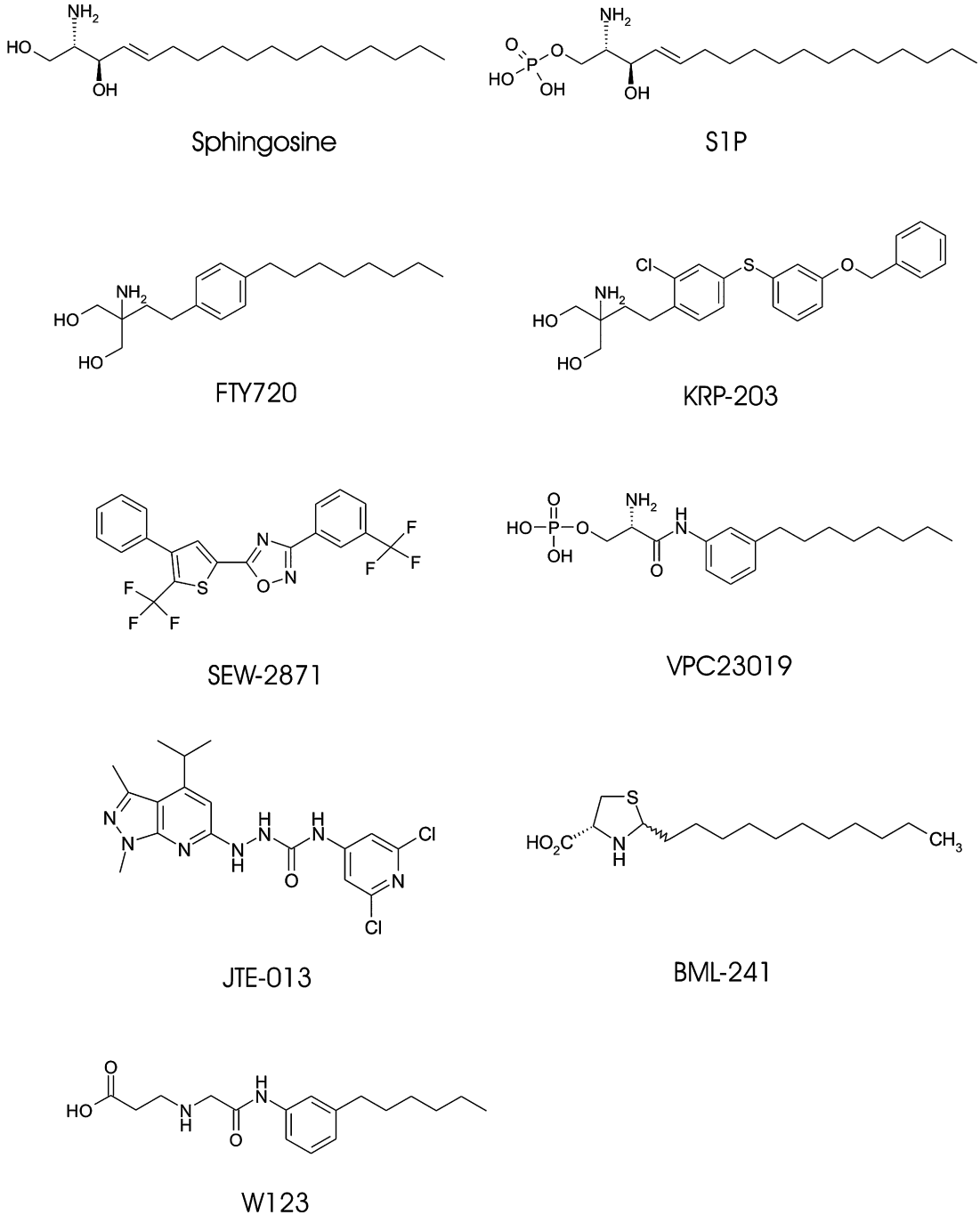

Fig. 1 – Chemical structures of some developed S1P-receptor agonists and antagonists. BML-241, 2(*R*,*S*)-undecylthiazolidine-4(*R*)-carboxylic acid; FTY720, 2-amino-2-(2-(4-octylphenyl) ethyl)-1,3-propanediol hydrochloride; JTE-013, 1-[1,3-Dimethyl-4-(2-methylethyl)-1H-pyrazolo[3,4-b]pyridin-6-yl]-4-(3,5-dichloro-4-pyridinyl)-semicarbazide; KRP-203, 2-amino-2-{2-[4-(3-benzyloxyphenylthio)-2-chlorophenyl]ethyl}-1,3-propanediol hydrochloride; S1P, sphingosine 1-phosphate; SEW2871, (5-(4-Phenyl-5-trifluoromethylthiophen-2-yl)-3-(3-trifluoromethylphenyl)-(1,2,4)-oxadiazole); VPC23019, (*R*)-phosphoric acid mono-[2-amino-2-(3-octyl-phenylcarbamoyl)-ethyl]-ethyl] ester; W123, 3-{[(3-hexyl-phenylcarbamoyl)-methyl]-amino}-propionic acid.

underlying this phenomenon is still unclear. However, angiogenesis caused by an abnormal growth of new blood vessels from existing blood vessels, is responsible for vision loss in various eye diseases [35]. Regarding angiogenic mechanisms a number of factors have been suggested to participate, but increasing evidence is now presented that especially VEGF-A is one major factor responsible for neovascular and exudative diseases of the eye. Since also S1P has been clearly associated with endothelial cell migration and angiogenesis [36], it could be speculated that in this case, FTY720 or its active phosphate

derivative act as pure agonists on one of the S1P receptors to trigger the formation of new blood vessels in the eye. However, this would be in contrast to various reports indicating an anti-angiogenic potential of FTY720 mainly in cancer cells [31,37].

Furthermore, it was shown on the cellular level that FTY720 is able to stimulate the TGFβ/Smad signalling pathway and thereby mimics TGFβ-mediated cell responses including both, anti-inflammatory and pro-fibrotic responses. Thus, in renal mesangial cells, FTY720 is able to reduce cytokine-triggered prostaglandin production by suppressing the group IIA secretory phospholipase A_2 ($sPLA_2$) gene expression [38] and to stimulate the pro-fibrotic connective tissue growth factor (CTGF) expression [39]. Similarly, FTY720 induced myofibroblast differentiation by involving the $S1P_3$ receptor, which is also predictive of a fibrotic event [40].

Recently, Payne et al. [41] reported that FTY720 is able to directly inhibit the cytosolic PLA_2 activity and thereby reduces arachidonic acid release and subsequent prostaglandin D_2 and thromboxane formation in human mast cells. Significant effects were already seen at the very low concentration of 1 nM of FTY720. Another direct target of FTY720 was identified as the cannabinoid receptor CB_1 receptor subtype [42]. In binding studies, FTY720 exerted a competitive antagonistic potential at the CB_1 receptor. Since a CB_1 specific antagonist, rimonabant, is already approved by the European Medicines Agency (EMEA) for treatment of obesity and for smoking cessation, FTY720 may have significant impact in this context, too.

2. KRP-203

Recently, a new immunosuppressant was synthesized, KRP-203 (2-amino-2-{2-[4-(3-benzyloxyphenylthio)-2-chlorophenyl]ethyl}-1,3-propanediol hydrochloride) (Fig. 1), that has structural similarity with FTY720 and, therefore, was speculated to act via the same molecular mechanisms. This substance also induced lymphocytopenia but, in contrast to FTY720, had considerably less effect on the heart rate when tested in guinea pig [43] and rat [44]. This was explained by the differential affinities of the two compounds for the $S1P_3$ receptor, which had previously been shown to be involved in the regulation of the heart rate [33]. Both substances had a similar high affinity for the $S1P_1$ receptor with an ED_{50} in the nM range [44]. However, for $S1P_3$ the phosphorylated form of FTY720 displayed an ED_{50} of 1.74 nM whereas the phosphorylated form of KRP-203 had an ED_{50} of >1 μM [44]. Thus, when using KRP-203 in rats, a 10 times higher concentration of KRP-203 than FTY720 was needed to induce a comparable transient fall in heart rate [44].

To date, KRP-203 has proven beneficial in rat organ transplantation [43–45], in rat autoimmune myocarditis [46], and in chronic colitis in mice [47]. However, so far, the effect of KRP-203 to promote macula degeneration, or to mimic other FTY720-stimulated cellular events, has not yet been reported and still awaits further investigations.

3. SEW2871

With the development of the highly selective $S1P_1$ receptor agonist SEW2871 (5-(4-Phenyl-5-trifluoromethylthiophen-2-yl)-3-(3-trifluoromethylphenyl)-(1,2,4)-oxadiazole) (Fig. 1) a valuable tool is now available to identify specifically $S1P_1$-mediated cell responses. SEW2871 possesses an ED_{50} of 13 nM for the $S1P_1$ receptor [48,49]. Like endogenous S1P, SEW2871 activates the $S1P_1$ receptor and thereby induces $S1P_1$ receptor internalization and recycling. This is in contrast to the unselective receptor agonist FTY720 phosphate, which not only induces receptor internalization but, additionally, triggers receptor degradation. SEW2871 is unable to downregulate the $S1P_1$ receptor expression level [49]. However, similar to the in vivo effect of FTY720, SEW2871 also induces lymphopenia 5–6 h after injection into mice [33].

In a study of two-photon imaging of living T cells in explanted mouse lymph nodes, it was shown that explant treatment with SEW2871 caused reduced velocity and motility of T cells specifically in the medullary region of the lymph node and reduced transmigration across the lymphatic endothelium [49]. The authors suggested that not the $S1P_1$ desensitization or downregulation, but $S1P_1$ activation is responsible for lymphocyte accumulation in lymph nodes and that the agonist signal is weak or absent during constitutive lymphocyte trafficking. They forwarded the hypothesis that SEW2871 and related immunosuppressants inhibit transendothelial migration by acting on stromal gates that are constitutively open, but close in response to signalling events mediated by $S1P_1$ receptors on sinus endothelial cells [49].

Furthermore, SEW2871 turned out to ameliorate ischemic acute renal failure in a mouse model of renal ischemia/reperfusion injury [50]. SEW2871 also acted in an anti-inflammatory manner in type 1 diabetic vessels to prevent monocyte/endothelial interaction by down-regulating VCAM-1 expression [51]. It was even speculated that SEW-2871 could have a therapeutic impact in the prevention of vascular complications of type 1 diabetes.

4. VPC23019

To date, the development of highly selective and direct S1P receptor subtype antagonists has proven difficult. Still, promising results come from the unselective $S1P_1$/$S1P_3$ antagonist VPC23019 ((R)-phosphoric acid mono-[2-amino-2-(3-octyl-phenylcarbamoyl)-ethyl]-ethyl] ester) (Fig. 1) [52]. This substance was able to inhibit agonist-induced cell migration in bladder and thyroid cancer cells [52,53].

Furthermore, VPC23019 reversed SEW2871-stimulated mitogen-activated protein kinase activation, cell migration and ligand-induced receptor internalization in T cells [49]. In the two-photon imaging study of living T cells in explanted lymph nodes, VPC23019 rapidly reversed the SEW2871-triggered slowing down of T cell motility and transmigration [49].

5. W123

A similar reversal of the SEW2871-triggered effect on T cell motility and endothelial transmigration was also seen with the very recently developed $S1P_1$ receptor antagonist W123 (3-{[(3-hexyl-phenylcarbamoyl)-methyl]-amino}-propionic acid)

(Fig. 1) [49]. However, still the specificity of W123 for other S1P receptor subtypes needs to be shown.

6. JTE-013

The synthetic compound JTE-013 (1-[1,3-Dimethyl-4-(2-methylethyl)-1H-pyrazolo[3,4-b]pyridin-6-yl]-4-(3,5-dichloro-4-pyridinyl)-semicarbazide) (Fig. 1) was characterized as a competitive antagonist of the $S1P_2$ receptor with no effect on the other S1P receptor subtypes [54]. This allowed to specifically study the involvement of $S1P_2$ in various cell responses. It turned out that in the presence of S1P, JTE-013 induced an enhanced migratory capacity of various cell types including endothelial cells [54], smooth muscle cells [54] and also glioma cells [55] suggesting a negative regulatory role of the $S1P_2$ in the process of migration, whereas a positive role was attributed to $S1P_1$ and $S1P_3$ [56,57].

Consistent with this negative role of $S1P_2$ in endothelial cell migration in vitro, JTE-013 also potentiated S1P-induced angiogenesis in vivo in a Matrigel implant assay [58].

JTE-013 was further shown to reduce vascular permeability and improve barrier integrity in a rat lung vascular permeability model [59]. Finally, in a wound healing model in diabetic mice, it was shown that injection of S1P subcutaneously into skin wounds increased the healing process and induced increased blood vessel formation within the granulation tissue consistent with the idea that S1P stimulates cell proliferation, differentiation and also angiogenesis [60]. Interestingly, a combined injection of S1P together with JTE-013 further accelerated the healing process [60].

In contrast to these data, Skoura et al. [61] suggested that in mouse retina, $S1P_2$ is positively involved in hypoxia-triggered pathological angiogenesis and therefore proposed that $S1P_2$ antagonism may be a novel therapeutic approach for the prevention or treatment of pathologic ocular neovascularization.

Recently, $S1P_2$ deficient mice were generated that at first glance had no obvious phenotype [62]. Later it became evident that the $S1P_2$ deficient mice were deaf due to multiple inner ear pathologies [63]. The authors also showed that the treatment of isolated canulated spiral modiolar arteries with JTE-013 inhibited S1P-triggered vasoconstriction of the artery. Since the regulation of vasoconstriction may be an important mechanism to protect adjacent capillary beds from high pressure, it may be speculated that a therapeutic use of JTE-013 is hampered by adverse side effects especially in the ear.

7. BML-241

In an attempt to find novel inhibitors of S1P-stimulated intracellular Ca^{2+} mobilization in HeLa cells overexpressing S1P receptors, a new lead compound was identified, BML-241 (2(*R*,*S*)-undecylthiazolidine-4(*R*)-carboxylic acid). It was shown that BML-241 at 10 μM inhibited the $S1P_3$ induced Ca^{2+} mobilization by 40%, but the $S1P_1$ triggered response by less than 10% [64]. However, another group failed to show a selectivity of BML-241 for the $S1P_3$ receptor [65]. Nevertheless, the compound may serve as a promising lead compound for the development of more selective antagonists.

8. S1P-neutralizing antibodies

As the trend in pharmaceutical sciences is clearly heading towards biological products, it is not surprising that also for S1P, the generation of neutralizing antibodies has been considered. Visentin et al. [66] have recently reported on the successful generation of a neutralizing monoclonal anti-S1P antibody and its effect in vitro and in vivo. They tested the antibody in various mouse xenograft models and found a very impressive tumor reducing efficacy of the antibody in all models tested. In addition, they showed that the release of various pro-angiogenic factors from tumor cells into serum was drastically reduced by treating mice with the anti-S1P antibody. However, the characterization of the antibody revealed that it not only recognized S1P, but also dihydro-S1P and even sphingosylphosphorylcholine. Thus, care must be taken when interpreting data with this antibody as the specificity is not as clear-cut as hoped.

9. Sphingosine kinase inhibitors

An alternative approach to reduce S1P action is to reduce its generation by inhibiting SphK1 or SphK2 enzyme activities. For a long time only crude and unspecific compounds were available to block SphK activity including dimethylsphingosine (DMS) and DL-threo-dihydrosphingosine (DHS, safingol) which were originally developed as protein kinase C inhibitors. Thus, although safingol exerted beneficial effects in an animal model of tumor growth and importantly, showed no dose-limiting toxicity [67], it remains unclear whether the therapeutic effect was due to inhibition of SphK or protein kinase C.

More recently, French et al. developed a series of novel and more specific SphK inhibitors denoted as SKI I to V [8,9]. These inhibitors were more selective for SphK and did not inhibit either protein kinase C or phosphoinositide 3-kinase. In addition, some of these inhibitors including SKI V (2-(3,4-dihydroxy-benzylidene)-benzofuran-3-one) were able to significantly reduce tumor growth in mice [8,9].

Furthermore, one of these SphK 1 inhibitors, SKI II, was able to overcome chemoresistance of prostatic adenocarcinoma cells [68] suggesting that SphK1 and its product S1P are also involved in cancer cell survival.

10. Future perspectives

Research in the last years has uncovered that sphingolipid-derived mediators, particularly S1P, are key molecules regulating many cell responses including normal and aberrant mitogenic signalling, chemoattractant functions in immune cell migration, tumor metastasis and angiogenesis which are

Table 1 – List of S1P receptor agonists and antagonists and their properties

Compound	Selectivity	In vitro effects	In vivo effects
Agonists			
FTY720 (fingolimod)	$S1P_{1,3-5}$ activation [13,14]	Inhibition of $sPLA_2$ and PGE_2 in MC [38]	Lymphocyte homing [17,18]
	$S1P_1$ downregulation [17,18]	Inhibition of $cPLA_2$ and PGD_2 in mast cells [41]	Reduced tumor cell growth [21–28]
		Antagonism of CB1 receptor [42]	Reduced vascular permeability [30,31]
		Stimulation of CTGF expression in MC [39]	Bradykardia [32]
		Myofibroblast differentiation [40]	Macula degeneration
KRP-203	$S1P_{1\gg3\ (4,5?)}$ activation [43,44]		Lymphocyte homing in rat/guinea pig [43–45]
	$S1P_1$ downregulation [44]		Ameliorates rat autoimmune myocarditis [46]
			Ameliorates chronic colitis in mice [47]
			Less bradykardia than FTY720 [44]
SEW2871	$S1P_1$ activation [48]	MAPK activation in T cells [49]	Lymphopenia in mice [33]
	No $S1P_1$ downregulation [49]	T cell migration [49]	Ameliorates ischemic acute renal failure in mice [50]
		Downregulation of VCAM-1 in EC [51]	Prevents monocyte/EC interaction in murine diabetic vessel [51]
Antagonists			
JTE-013	$S1P_2$ inhibition	Increased migration of EC [54], SMC [54] and glioma cells [55]	Potentiates angiogenesis in vivo [58]
		Vasodilatation of isolated canulated spiral modiolar arteries (deafness?) [63]	Reduces vascular permeability in a rat lung model [59]
			improves wound healing in diabetic mice[60]
VPC23019	$S1P_{1+3}$ inhibition	Inhibits agonist-stimulated migration of T cells and cancer cells [49,52,53]	
		Inhibits SEW2871-stimulated MAPK in T cells [49]	
		Reverses SEW2871-triggered lymphopenia in explanted lymph nodes [49]	
W123	$S1P_1$ inhibition	Reverses SEW2871-triggered lymphopenia in explanted lymph nodes [49]	
BML-241	$S1P_{3>1}$ inhibition	Blocks agonist-triggered Ca^{2+} mobilization [64]	

fundamental processes in cancer development and progression, but also in diabetes and other autoimmune diseases. Our emerging knowledge of this field, along with the first promising drug candidates place the targeting of S1P on the center stage of pharmacological interest aiming for an attractive new strategy in the treatment of such diseases.

To this end, a number of pharmacologically active substances have been developed, including agonists and antagonists of S1P receptors (see Table 1), neutralizing anti-S1P antibodies and inhibitors of sphingosine kinases, which have proven very promising in in vitro and first in vivo studies. Translating the sphingolipid-based therapy to the human system will represent a formidable challenge, not only because of the tremendous complexity of human physiology and pathophysiology but also because of the seemingly less obvious drugability of lipid mediators. A strategy for determining the most promising candidates will be crucial.

Acknowledgments

Work in the authors laboratories is supported by the Swiss National Foundation (FOG 784, GRK 757, GRK 1172, EXC 147, HU 842/4-1, PF 361/6-1), the German Research Foundation, the Norwegian Research Council and Avexxin, the European Union (FP6: LSHM-CT-2004-005033), the Wilhelm Sander-Foundation, the Novartis Foundation, and the Hochschulstiftung der Universität Bern.

REFERENCES

[1] Huwiler A, Kolter T, Pfeilschifter J, Sandhoff K. Physiology and pathophysiology of sphingolipid metabolism and signaling. Biochim Biophys Acta 2000;1485:63–99.
[2] Spiegel S, Milstien S. Sphingosine-1-phosphate: an enigmatic signalling lipid. Nat Rev Mol Cell Biol 2003;4:397–406.
[3] Hait NC, Oskeritzian CA, Paugh SW, Milstien S, Spiegel S. Sphingosine kinases, sphingosine 1-phosphate, apoptosis and diseases. Biochim Biophys Acta 2006;1758:2016–26.
[4] Le Stunff H, Milstien S, Spiegel S. Generation and metabolism of bioactive sphingosine-1-phosphate. J Cell Biochem 2004;92:882–99.
[5] Alemany R, van Koppen CJ, Danneberg K, ter Braak M, Meyer zu Heringdorf D. Regulation and functional roles of sphingosine kinases. Naunyn Schmiedebergs Arch Pharmacol 2007;374:413–28.
[6] Olivera A, Spiegel S. Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. Nature 1993;365:557–60.
[7] Xia P, Gamble JR, Wang L, Pitson SM, Moretti PA, Wattenberg BW, et al. An oncogenic role of sphingosine kinase. Curr Biol 2000;10:1527–30.
[8] French KJ, Schrencengost RS, Lee BD, Zhuang Y, Smith SN, Eberly JL, et al. Discovery and evaluation of inhibitors of human sphingosine kinase. Cancer Res 2003;63:5962–9.
[9] French KJ, Upson JJ, Keller SN, Zhuang Y, Yun JK, Smith CD. Antitumor activity of sphingosine kinase inhibitors. J Pharmacol Exp Ther 2006;318:596–603.
[10] Huwiler A, Pfeilschifter J. Altering the sphingosine-1-phosphate/ceramide balance: a promising approach for tumor therapy. Curr Pharm Des 2006;12:4625–35.
[11] Milstien S, Spiegel S. Targeting sphingosine-1-phosphate: a novel avenue for cancer therapeutics. Cancer Cell 2006;9:148–50.
[12] Huwiler A, Zangemeister-Wittke U. Targeting the conversion of ceramide to sphingosine 1-phosphate as a novel strategy for cancer therapy. Crit Rev Oncol Hematol 2007;63:150–9.
[13] Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, et al. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 2002;277:21453–7.
[14] Mandala S, Haidu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science 2002;296:346–9.
[15] Billich A, Bornancin F, Devay P, Mechtcheriakova D, Urtz N, Baumruker T. Phosphorylation of the immunomodulatory drug FTY720 by sphingosine kinases. J Biol Chem 2003;278:47408–15.
[16] Paugh SW, Payne SG, Barbour SE, Milstien S, Spiegel S. The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2. FEBS Lett 2003;554:189–93.
[17] Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor. Nature 2004;427:355–60.
[18] Chiba K, Matsuyuki H, Maeda Y, Sugahara K. Role of sphingosine 1-phosphate receptor type 1 in lymphocyte egress from secondary lymphoid tissues and thymus. Cell Mol Immunol 2006;3:11–9.
[19] Pappu R, Schwab SR, Cornelissen I, Pereira JP, Regard JB, Xu Y, et al. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. Science 2007;316:295–8.
[20] Gräler MH, Goetzl EJ. The immunosuppressant FTY720 down-regulates sphingosine 1-phosphate G-protein-coupled receptors. FASEB J 2004;18:551–3.
[21] Wang JD, Takahara S, Nonomura N, Ichimaru N, Toki K, Azuma H, et al. Early induction of apoptosis in androgen-independent prostate cancer cell line by FTY720 requires caspase-3 activation. Prostate 1999;40:50–5.
[22] Azuma H, Takahara S, Ichimaru N, Wang JD, Itoh Y, Otsuki Y, et al. Marked prevention of tumor growth and metastasis by a novel immunosuppressive agent, FTY720, in mouse breast cancer models. Cancer Res 2002;62:1410–9.
[23] Ho JW, Man K, Sun CK, Lee TK, Poon RT, Fan ST. Effects of a novel immunomodulating agent, FTY720, on tumor growth and angiogenesis in hepatocellular carcinoma. Mol Cancer Ther 2005;4:1430–8.
[24] Ubai T, Azuma H, Kotake Y, Inamoto T, Takahara K, Ito Y, et al. FTY720 induced Bcl-associated and Fas-independent apoptosis in human renal cancer cells in vitro and significantly reduced in vivo tumor growth in mouse xenograft. Anticancer Res 2007;27:75–88.
[25] Shen Y, Cai M, Xia W, Liu J, Zhang Q, Xie H, et al. FTY720, a synthetic compound from Isaria sinclairii, inhibits proliferation and induces apoptosis in pancreatic cancer cells. Cancer Lett 2007;254:288–97.
[26] Matsuoka Y, Nagahara Y, Ikekita M, Shinomiya T. A novel immunosuppressive agent FTY720 induced Akt dephosphorylation in leukemia cells. Br J Pharmacol 2003;138:1303–12.
[27] Neviani P, Santhanam R, Oaks JJ, Eiring AM, Notari M, Blaser BW, et al. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. J Clin Invest 2007;117:2408–21.
[28] Liu Q, Zhao X, Frissora F, Ma Y, Santhanam R, Jarjoura D, et al. FTY720 demonstrates promising pre-clinical activity for chronic lymphocytic leukemia and lymphoblastic leukemia/lymphoma. Blood 2008;111:275–84.
[29] Lee TK, Man K, Ho JW, Sun CK, Ng KT, Wang XH, et al. FTY720 induces apoptosis of human hepatoma cell lines through PI3-K-mediated Akt dephosphorylation. Carcinogenesis 2004;25:2397–405.
[30] Brinkmann V, Cyster JG, Hla T. FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. Am J Transplant 2004;4:1019–25.
[31] LaMontagne K, Littlewood-Evans A, Schnell C, O'Reilly T, Wyder L, Sanchez T, et al. Antagonism of sphingosine-1-phosphate receptors by FTY720 inhibits angiogenesis and tumor vascularization. Cancer Res 2006;66:221–31.
[32] Budde K, Schmouder RL, Brunkhorst R, Nashan B, Lucker PW, Mayer T, et al. First human trial of FTY720, a novel immunomodulator, in stable renal transplant patients. J Am Soc Nephrol 2002;13:1073–83.
[33] Sanna MG, Liao J, Jo E, Alfonso C, Ahn MY, Peterson MS, et al. Sphingosine 1-phosphate (S1P) receptor subtypes S1P1 and S1P3, respectively, regulate lymphocyte recirculation and heart rate. J Biol Chem 2004;279:13839–48.
[34] Koyrakh L, Roman ML, Brinkmann V, Wickman K. The heart rate decrease caused by acute FTY720 administration is mediated by the G protein-gated potassium channel I. Am J Transplant 2005;5:529–36.
[35] Michels S, Schmidt-Erfurth U, Rosenfeld PJ. Promising new treatments for neovascular age-related macular degeneration. Expert Opin Investig Drugs 2006;15:779–93.
[36] Panetti TS. Differential effects of sphingosine 1-phosphate and lysophosphatidic acid on endothelial cells. Biochim Biophys Acta 2002;1582:190–6.
[37] Schmid G, Guba M, Ischenko I, Papyan A, Joka M, Schrepfer S, et al. The immunosuppressant FTY720 inhibits

angiogenesis via the sphingosine 1-phosphate receptor 1. J Cell Biochem 2007;101:259–70.
[38] Xin C, Ren S, Eberhardt W, Pfeilschifter J, Huwiler A. FTY720 suppresses interleukin-1β-induced secretory phospholipase A_2 expression in renal mesangial cells by a transcriptional mechanism. Br J Pharmacol 2007;150:943–50.
[39] Xin C, Ren S, Eberhardt W, Pfeilschifter J, Huwiler A. The immunomodulator FTY720 and its phosphorylated derivative activate the Smad signalling cascade and upregulate connective tissue growth factor and collagen type IV expression in renal mesangial cells. Br J Pharmacol 2006;147:164–74.
[40] Keller CD, Rivera Gil P, Tolle M, van der Giet M, Chun J, Radeke HH, et al. Immunomodulator FTY720 induces myofibroblast differentiation via the lysophospholipid receptor S1P3 and Smad3 signaling. Am J Pathol 2007;170:281–92.
[41] Payne SG, Oskeritzian CA, Griffiths R, Subramanian P, Barbour SE, Chalfant CE, et al. The immunosuppressant drug FTY720 inhibits cytosolic phospholipase A2 independently of sphingosine-1-phosphate receptors. Blood 2007;109:1077–85.
[42] Paugh SW, Cassidy MP, He H, Milstien S, Sim-Selley LJ, Spiegel S, et al. its analog, the immunosuppressant 2-amino-2-(2-[4-octylphenyl]ethyl)-1,3-propanediol, interact with the CB1 cannabinoid receptor. Mol Pharmacol 2006;70:41–50.
[43] Shimizu H, Takahashi M, Kaneko T, Murakami T, Hakamata Y, Kudou S, et al. KRP-203, a novel synthetic immunosuppressant, prolongs graft survival and attenuates chronic rejection in rat skin and heart allografts. Circulation 2005;111:222–9.
[44] Fujishiro J, Kudou S, Iwai S, Takahashi M, Hakamata Y, Kinoshita M, et al. Use of sphingosine-1-phosphate 1 receptor agonist, KRP-203, in combination with a subtherapeutic dose of cyclosporine A for rat renal transplantation. Transplantation 2006;82:804–12.
[45] Fujishiro J, Suzuki C, Kudou S, Yasue T, Hakamata Y, Takahashi M, et al. Change from cyclosporine to combination therapy of mycophenolic acid with the new sphingosine-1-phosphate receptor agonist, KRP-203, prevents host nephrotoxicity and transplant vasculopathy in rats. J Heart Lung Transplant 2006;25:825–33.
[46] Ogawa R, Takahashi M, Hirose S, Morimoto H, Ise H, Murakami T, et al. A novel sphingosine-1-phosphate receptor agonist KRP-203 attenuates rat autoimmune myocarditis. Biochem Biophys Res Commun 2007;361:621–8.
[47] Song J, Matsuda C, Kai Y, Nishida T, Nakajima K, Mizushima T, et al. A novel sphingosine 1-phosphate receptor agonist, KRP-203, regulates chronic colitis in IL-10 gene-deficient mice. J Pharmacol Exp Ther 2008;324:276–83.
[48] Jo E, Sanna MG, Gonzalez-Cabrera PJ, Thangada S, Tigyi G, Osborne DA, et al. S1P1-selective in vivo-active agonist from high throughput screening: off-the-shelf chemical probes of receptor interactions, signaling and fate. Chem Biol 2005;12:703–15.
[49] Wei SH, Rosen H, Matheu MP, Sanna MG, Wang SK, Jo E, et al. Cahalan MD. Sphingosine 1-phosphate type 1 receptor agonism inhibits transendothelial migration of medullary T cells to lymphatic sinuses. Nat Immunol 2005;6:1228–35.
[50] Lien YH, Yong KC, Cho C, Igarashi S, Lai LW. S1P1-selective agonist, SEW2871, ameliorates ischemic acute renal failure. Kidney Int 2006;69:1601–8.
[51] Whetzel AM, Bolick DT, Srinivasan S, Macdonald TL, Morris MA, Ley K, et al. Sphingosine-1 phosphate prevents monocyte/endothelial interactions in type 1 diabetic NOD mice through activation of the S1P1 receptor. Circ Res 2006;99:731–9.
[52] Davis MD, Clemens JJ, Macdonald TL, Lynch KR. Sphingosine 1-phosphate analogs as receptor antagonists. J Biol Chem 2005;280:9833–41.
[53] Balthasar S, Samulin J, Ahlgren H, Bergelin N, Lundquist M, Toescu EC, et al. Sphingosine 1-phosphate receptor expression profile and regulation of migration in human thyroid cancer cells. Biochem J 2006;398:547–56.
[54] Osada M, Yatomi Y, Ohmori T, Ikeda H, Ozaki Y. Enhancement of sphingosine 1-phosphate-induced migration of vascular endothelial cells and smooth muscle cells by an EDG-5 antagonist. Biochem Biophys Res Commun 2002;299:483–7.
[55] Young N, van Brocklyn JR. Roles of sphingosine-1-phosphate (S1P) receptors in malignant behavior of glioma cells. Differential effects of S1P2 on cell migration and invasiveness. Exp Cell Res 2007;313:1615–27.
[56] Allende ML, Yamashita T, Proia RL. G-protein-coupled receptor S1P1 acts within endothelial cells to regulate vascular maturation. Blood 2003;102:3665–7.
[57] Waeber C, Blondeau N, Salomone S. Vascular sphingosine-1-phosphate S1P1 and S1P3 receptors. Drug News Perspect 2004;17:365–82.
[58] Inoki I, Takuwa N, Sugimoto N, Yoshioka K, Takata S, Kaneko S, et al. Negative regulation of endothelial morphogenesis and angiogenesis by S1P2 receptor. Biochem Biophys Res Commun 2006;346:293–300.
[59] Sanchez T, Skoura A, Wu MT, casserly B, Harrington EO, Hla T. Induction of vascular permeability by the sphingosine-1-phosphate receptor-2 (S1P2R) and its downstream effectors ROCK and PTEN. Arterioscler Thromb Vasc Biol 2007;27:1312–8.
[60] Kawanabe T, Kawakami T, Yatomi Y, Shimada S, Soma Y. Sphingosine 1-phosphate accelerates wound healing in diabetic mice. J Dermatol Sci 2007;48:53–60.
[61] Skoura A, Sanchez T, Claffey K, Mandala SM, Proia RL, Hla T. Essential role of sphingosine 1-phosphate receptor 2 in pathological angiogenesis of the mouse retina. J Clin Invest 2007;117:2506–16.
[62] Kono M, Mi Y, Liu Y, Sasaki T, Allende ML, Wu YP, et al. The sphingosine-1-phosphate receptors S1P1, S1P2, and S1P3 function coordinately during embryonic angiogenesis. J Biol Chem 2004;279:29367–73.
[63] Kono M, Belyantseva IA, Skoura A, Frolenkov GI, Starost MF, Dreier JL, et al. Deafness and stria vascularis defects in S1P2 receptor-null mice. J Biol Chem 2007;282:10690–6.
[64] Koide Y, Hasegawa T, Takahasji A, Endo A, Mochizuki N, Nakagawa M, et al. Development of novel EDG3 antagonists using a 3D database search and their structure-activity relationships. J Med Chem 2002;45:4629–38.
[65] Jongsma M, Hendriks-Balk MC, Michel MC, Peters SL, Alewijnse AE. BML-241 fails to display selective antagonism at the sphingosine-1-phosphate receptor, S1P(3). Br J Pharmacol 2006;149:277–82.
[66] Visentin B, Vekich JA, Sibbald BJ, Cavalli AL, Moreno KM, Matteo RG, et al. Validation of an anti-sphingosine-1-phosphate antibody as a potential therapeutic in reducing growth, invasion, and angiogenesis in multiple tumor lineages. Cancer Cell 2006;9:225–38.
[67] Schwartz GK, Ward D, Saltz L, Casper ES, Spiess T, Mullen E, et al. A pilot clinical/pharmacological study of the protein kinase C-specific inhibitor safingol alone and in combination with doxorubicin. Clin Cancer Res 1997;3:537–43.
[68] Leroux ME, Auzenne E, Evans R, Hail Jr N, Spohn W, Ghosh SC, et al. Sphingolipids and the sphingosine kinase inhibitor, SKI II, induce BCL-2-independent apoptosis in human prostatic adenocarcinoma cells. Prostate 2007;67:1699–717.