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The involvement of blood cells in the phosphorylation of the selective sphingosine-1-phosphate receptor 1 agonist SYL-927

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SYL-927 is a selective sphingosine-1-phosphate receptor 1 ($S1P_1$) agonist for autoimmune diseases. It undergoes phosphorylation to the active SYL-927-P *in vivo*, which activates $S1P_1$ on lymphocytes, causing lymphopenia by retention of lymphocytes in the lymph nodes. The aim of this study was to identify the involvement of blood cells in the phosphorylation of SYL-927. In addition, pharmacokinetics of SYL-927 and SYL-927-P in blood and plasma were compared in rats. The results demonstrated that SYL-927 can be converted to SYL-927-P in rat blood, but not in rat plasma. However, both rat blood and plasma are capable of dephosphorylating SYL-927-P to SYL-927. SYL-927-P generation and release were observed after incubating SYL-927 with rat and human erythrocytes and platelets. The addition of sphingosine kinases (SPHKs) inhibitors *N*,*N*-dimethylsphingosine (DMS) and FTY720 significantly inhibited SYL-927-P generation, indicating the involvement of SPHKs. In addition, SYL-927 and SYL-927-P levels in blood were significantly higher than those in plasma after oral administration of SYL-927 in rats, suggesting the blood cells for the production of SYL-927-P. In summary, the blood cells such as erythrocytes and platelets contribute to the generation and release of SYL-927-P, which is important for maintaining plasma active phosphate levels for prolonged effects.

1. Introduction

Sphingosine-1-phosphate (S1P), a lysophospholipid signaling molecule generated from sphingosine by sphingosine kinases (SPHKs), is involved in various physiological processes ranging from vascular maturation/angiogenesis and heart development to lymphocyte trafficking through the activation of a family of five G-protein-coupled receptors ($S1P_1$-$S1P_5$) (Hara-Yokoyama et al. 2013), and it is also associated with many pathologies including cancer, atherosclerosis, inflammation, and autoimmune disorders such as multiple sclerosis. Consequently, S1P provides a target for the therapy of such diseases (Huwileret an Pfeilschifter 2008). FTY720, an analog of sphingosine, is phosphorylated *in vivo* to FTY720-phosphate (FTY720-P) which functions as an agonist for $S1P_1$, causing lymphopenia through sequestration of circulating lymphocytes within lymph nodes and Peyer patches (Kihara and Igarashi 2008). It has been approved in the United States as a therapy for multiple sclerosis. However, a number of side effects of FTY720 such as bradycardia and hypertension were observed in preclinical and clinical trials due to its unspecific effect on other S1P receptor subtypes, especially $S1P_3$ (Sanna et al. 2004). Therefore, it is interesting to discover new selective $S1P_1$ agonists with low potency on $S1P_3$ in order to retain the positive therapeutic properties while attenuating the potential risk of adverse effects.
SYL-927 is a selective $S1P_1$ agonist and *in vivo* transferred to its active phosphorylated form, SYL-927-P, which is the direct modulator of $S1P_1$. SYL-927-P can activate $S1P_1$-dependent modulation of lymphocyte traffic without producing $S1P_3$-mediated effects in preclinical autoimmune diseases models (Xiao et al. 2016). However, it has been unclear where and how SYL-927-P is produced. It is known that SPHK1 and SPHK2, are responsible for the production of S1P, and FTY720-P is synthesized from FTY720 mainly by SPHK2 (Hanel et al. 2007). Plasma S1P is supplied from erythrocytes and platelets, whereas FTY720-P is produced mainly by platelets (Anada et al. 2007). In this study, we tested the involvement of blood cells in the generation of SYL-927-P. We found that both erythrocytes and platelets can generate, store and release SYL-927-P, and SPHKs may be involved in the conversion of SYL-927 to SYL-927-P. Accumulation and consistent phosphorylation of SYL-927 in blood cells may contribute to maintain plasma SYL-927-P levels for persisted effect.

2. Investigations and results

2.1. Interconversion of SYL-927 and SYL-927-P in rat blood and plasma

Firstly we tested the metabolic conversion of SYL-927 and SYL-927-P in rat blood and plasma. Incubation of SYL-927 with rat blood could generate SYL-927-P. As shown in Fig. 1A, the added 1 μM SYL-927 (100 pmol) was decreased by 49 % while the generated SYL-927-P reached a maximum level of 40 pmol 2 h after incubation. However, SYL-927 was found to be totally unchanged in rat plasma after 2 h incubation with no significant SYL-927-P detected. Analysis of plasma and cells fraction after incubation of SYL-927 with rat blood demonstrated that SYL-927-P levels in both fractions were increased in a time-dependent manner (Fig. 1C). This indicated that blood cells may be involved in the conversion of SYL-927 to SYL-927-P and provide plasma SYL-927-P. However, SYL-927-P (100 pmol) was metabolically unstable and converted to SYL-927 in both rat blood and plasma (Fig. 1B). It diminished by 15 % and 35 % in rat blood and plasma after 2 h incubation, resulting in SYL-927 generation of 12 and 30 pmol, respectively. The lower conversion rate of SYL-927-P in blood compared with that in plasma may be due to the capacity of blood cells to convert generated SYL-927 back to SYL-927-P. Thus it seems that SYL-927 participated in a cycle of phosphorylation/dephosphorylation in blood.

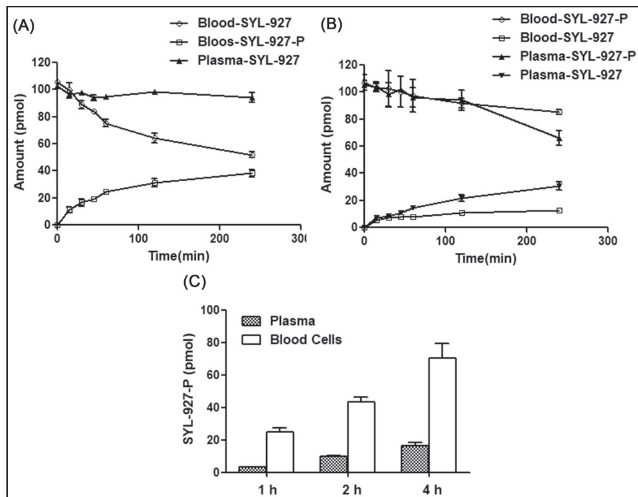

Fig. 1: Interconversion of SYL-927 and SYL-927-P in rat blood and plasma. (A) SYL-927 and (B) SYL-927-P at 1 μM were incubated with rat blood or plasma at 37°C for 15, 30, 45, 60, 120 and 240 min. (C) In addition, the blood samples of SYL-927 at 1, 2 and 4 h were treated by centrifugation to obtain plasma and cell fraction. SYL-927 and SYL-927-P in blood, plasma and cells were analyzed by LC-MS/MS. Data were expressed as mean ± SD (n = 3).

2.2. *SYL-927-P formation in rat/human erythrocytes and platelets*

Next, the ability of two major types of blood cells, erythrocytes and platelets, to generate and release SYL-927-P was investigated. After rat or human erythrocytes and platelets were incubated with SYL-927 at concentrations ranging from 1 to 500 μM, cell fraction and the medium were separated by centrifugation and SYL-927-P amounts in both fractions were analyzed by the LC-MS/MS method (Tables 1 and 2). In most cases, cellular SYL-927-P levels were

Table 1: Generation and release of SYL-927-P in rat erythrocytes and platelets. Data are shown as mean±SD (n = 3)

Conc (μM)	Rat erythrocytes			Rat platelets		
	SYL-927-P levels (pmol)		Released SYL-927-P (%)	SYL-927-P levels (pmol)		Released SYL-927-P (%)
	Medium	Cell		Medium	Cell	
1	2.99±1.18	6.83 ± 0.9	30.4	4.11 ± 0.79	9.04 ± 2.6	31.3
2	3.67±1.06	19.81 ± 7.95	15.6	5.82 ± 0.51	15.29 ± 2.08	27.6
10	8.89±3.36	50.75 ± 6.36	14.9	11.98 ± 1.89	31.95 ± 5.39	27.3
20	12.21±0.74	92.36 ± 25.09	11.7	9.36 ± 0.71	37.32 ± 3.17	20.1
50	13.71±2.92	127.14 ± 19.64	9.7	13 ± 1.49	39.34 ± 13.82	24.8
100	8.28±0.26	127.89 ± 14.16	6.1	10.92 ± 2.23	27.55 ± 3.2	28.4
200	4.78±0.42	153.69 ± 56.94	3.0	10.43 ± 0.96	24.98 ± 2.86	29.5
400	2.55±0.55	167.79 ± 11.19	1.5	9.25 ± 0.67	24.01 ± 3.33	27.8
500	4.84±0.89	149.14 ± 13.06	3.1	7.18 ± 0.78	19.76 ± 0.81	26.7

Table 2: Generation and release of SYL-927-P in human erythrocytes and platelets. Data are shown as mean±SD (n=3)

Conc (μM)	Human erythrocytes			Human platelets		
	SYL-927-P levels (pmol)		Released SYL-927-P (%)	SYL-927-P levels (pmol)		Released SYL-927-P (%)
	Medium	Cell		Medium	Cell	
1	0.26 ± 0.02	0.77 ± 0.39	25.2	0.34 ± 0.03	0.55 ± 0.23	38.2
2	0.76 ± 0.27	1.15 ± 0.6	39.8	0.57 ± 0.15	0.66 ± 0.36	46.3
10	1.9 ± 1.83	9.62 ± 4.14	16.5	6.55 ± 0.57	5.19 ± 0.42	55.8
20	2.74 ± 2.05	19.23 ± 9.66	12.5	9.54±0.68	9.56 ± 1.24	49.9
50	3.8 ± 2.11	34.78 ± 13.69	9.8	8.74 ± 1.51	14.51 ± 1.28	37.6
100	3.83 ± 3.62	78 ± 28.85	4.7	9.63 ± 0.31	16.41 ± 1.09	37.0
200	1.17 ± 0.87	166.77 ± 95	0.7	5.56 ± 2.42	17.78 ± 1.02	23.8
400	1.46 ± 1.05	177.74 ± 102.63	0.8	3.61 ± 0.65	17.62 ± 1.47	17.0
500	0.8 ± 0.57	167.34 ± 102.48	0.5	2.55 ± 0.35	17.5 ± 0.95	12.7

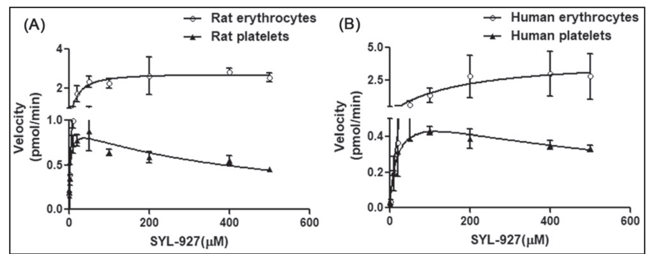

Fig. 2: Kinetic analysis of SYL-927-P generation in (A) rat and (B) human erythrocytes and platelets. Erythrocytes (8×10^8/ml) and platelets (3×10^7/ml) were incubated with 1 to 500 μM SYL-927 at 37°C for 60 min. Cells and incubational medium were separated by centrifugation and analyzed by LC-MS/MS. Velocity of SYL-927-P generation was calculated by the sum of SYL-927-P in both cells and medium. Data were expressed as mean ± SD (n = 3).

higher than those in the medium. This indicated that the two types of blood cells are capable of converting SYL-927 to SYL-927-P, and release it into extracellular space. The diminished SYL-927-P release in response to increased SYL-927 incubational concentration in erythrocytes, and the substrate inhibition curve for SYL-927-P generation (Fig. 2) in platelets suggested a possible inhibition of SYL-927-P release and generation by the substrate. The plot of the velocity of SYL-927-P generation versus substrate concentration in erythrocytes and platelets were shown in Fig. 2. The kinetic parameters were calculated by nonlinear regression analysis of the velocity of SYL-927-P generation *versus* SYL-927 concentration using the 1~500 μM in rat and human erythrocytes, 1~50 μM in rat platelets, and 1~100 μM in human platelets, respectively (Table 3). Although the V_{max} values in rat and human platelets were 3- and 7-fold lower than in erythrocytes, the K_m values in rat and human erythrocytes were 5- and 9-fold higher than in platelets, respectively. Thus, it is estimated from the Cl_{int} (V_{max}/K_m) values that platelets were slightly more efficient at phosphorylating SYL-927 than erythrocytes. However, calculations based on cell numbers (8×10^8 erythrocytes per ml, and 3×10^7 platelets per ml) indicated that the catalytic efficiency of one rat and human platelet was about 42- and 34-fold higher than that of one erythrocyte, respectively. Compared with rat erythrocytes and platelets, those of human were about 7.9- and 10-fold less efficient at converting SYL-927 to SYL-927-P, mainly due to the dramatically higher K_m values.

Table 3: Kinetic parameters of SYL-927-P generation in rat and human erythrocytes and platelets. Data are shown as mean±SD (n=3).

Species	Cell type	Km (μM)	Vmax (pmol/min)	Cl_{int} (μl/min)
Rat	Erythrocytes	15 ± 4.9	2.8 ± 0.3	0.19 ± 0.06
	Platelets	3 ± 0.73	0.93 ± 0.06	0.31 ± 0.08
Human	Erythrocytes	170.8 ± 113.1	4.15 ± 1.08	0.024 ± 0.01
	Platelets	17.79 ± 2.76	0.56 ± 0.03	0.031 ± 0.01

2.3. *Inhibition of SYL-927-P formation in rat/human erythrocytes and platelets*

The effect of two non-selective SPHK inhibitors, DMS and FTY720, which can inhibit both SPHK1 and SPHK2, on SYL-927-P formation in rat and human erythrocytes and platelets was tested. SYL-927-P generation in rat erythrocytes and platelets were inhibited by DMS and FTY720 in a dose-dependent manner. Although no significant inhibitory effect was observed with inhibitors at 1 μM, DMS and FTY720 at 10 μM significantly inhibited SYL-927-P generation in rat erythrocytes by 23% and 33%, respectively, and that in rat platelets by 50 % and 49 %, respectively (Fig. 3A). However, SYL-927-P generation in human erythrocytes was not significantly affected by two inhibitors. In human platelets, DMS exhibited no significant inhibitory effect, while FTY720 at 10 μM significantly inhibited SYL-927-P generation by 49 % (Fig. 3B). The results indicated that SPHKs may be involved in the SYL-927-P formation in rat erythrocytes and platelets, and human platelets. However, selective SPHK1 and SPHK2 inhibitors are required to identify the specific SPHK isoform.

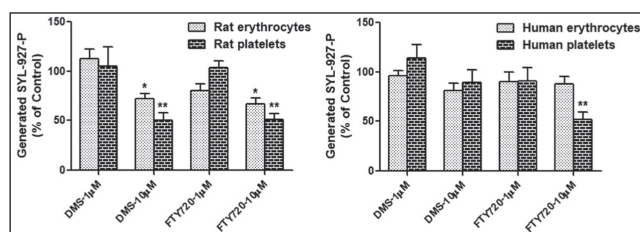

Fig. 3: Inhibition of SYL-927-P formation by DMS and FTY720 in (A) rat and (B) human erythrocytes and platelets. SYL-927 (10 μM) was incubated with erythrocytes (8×10^8/ml) and platelets (3×10^7/ml) in the presence of DMS and FTY720. The cells and medium were separated for analysis. Generated SYL-927-P consisting of that in cells and medium in the presence of inhibitors were normalized to that in controls in the absence of inhibitors. Data were expressed as mean ± SD (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$, compared to the control group.

2.4. *Pharmacokinetic study of SYL-927 in rat*

After its oral administration to rats, SYL-927 was rapidly converted to SYL-927-P, which was the predominant form in both blood and plasma (Fig. 4). However, the $AUC_{0-\infty}$ values of SYL-927 and SYL-927-P in blood were about 5- and 2-fold higher than those in plasma (Table 4), suggesting that blood cells may be the site for the accumulation and phosohorylation of SYL-927 *in vivo.*

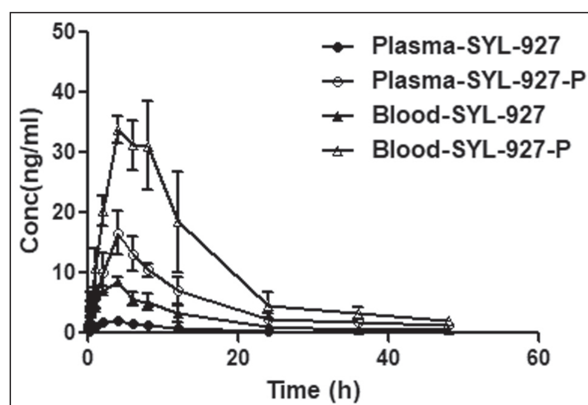

Fig. 4: Plasma and blood concentration-time profiles of SYL-927 and SYL-927-P after a single oral dose of SYL-927 at 3 mg/kg. Data were expressed as mean ± SD (n = 5).

Table 4: Pharmacokinetic parameters of SYL-927 and SYL-927-P in plasma and blood after a single oral dose of SYL-927 at 3 mg/kg. Data are shown as mean±SD (n=5).

Parameters	Unit	Values			
		Plasma		Blood	
		SYL-927	SYL-927-P	SYL-927	SYL-927-P
AUC(0-∞)	ug/L*h	24.2±6.83	245.17±34.98	121.77±51.33	555.17±126.5
$t_{1/2z}$	h	6.32±3.02	14.38±4.25	15.49±8.84	12.68±3.59
T_{max}	h	3.5±1	4±0	3.5±1	5±2
CLz/F	L/h/kg	131.01±33.54	12.43±1.78	27.93±10.81	5.65±1.43
C_{max}	ug/L	2.05±0.48	16.65±3.53	8.6±0.78	35.75±2.08

3. Discussion

SYL-927 is a prodrug and its conversion to the corresponding phosphate SYL-927-P is essential for the pharmacological effects. Therefore, understanding where and how SYL-927 gets phosphorylated is of high importance. This study demonstrated that SYL-927 and SYL-927-P can be converted to each other in rat blood. However, their incubation with rat plasma revealed no obvious SYL-927 phosphorylation but detecting the dephosphorylation of SYL-927-P. Therefore, SYL-927-P detected in plasma after incubating SYL-927 with whole blood may be supplied from blood cells. It is known that S1P is irreversibly degraded by S1P lyase or reversibly dephosphorylated by two specific S1P phosphatases (SPP1 and SPP2) (Chi 2013). In addition, LLP3, a member of lipid phosphatase family (LPP1-3), is reported to be involved in the dephosphorylation of plasma FTY720-P (Mechtcheriakova et al. 2007). Whether theses enzymes are involved in the dephosphorylation of SYL-927-P warrants studied.

Incubation of SY-927 with erythrocytes and platelets demonstrated that both two types of blood cells can convert SYL-927 to SYL-927-P and release it. Although one platelet is more efficient at phosphorylating SYL-927 than one erythrocyte, the number of erythrocytes found in the whole blood was about 20-fold higher than that of platelets (Ito et al. 2007). Thus both erythrocytes and platelets are important contributors to SYL-927-P generation in blood and provide plasma SYL-927-P. It should be noted that blood cells including erythrocytes and platelets can be damaged in pathological states such as thrombotic thrombocytopenic purpura or disseminated intravascular coagulation (Yang et al. 1999), which may affect the functional activation of SYL-927 *in vivo*. In addition, whether other types of blood cells such as lymphocytes and monocytes are responsible for SYL-927-P generation needs to be further evaluated.

Since DMS and FTY720, two non-selective SPHK inhibitors, significantly inhibit SYL-927-P generation in rat erythrocytes and platelets, and human platelets, SPHKs may be involved in SYL-927-P generation in the blood. SPHK is a highly conserved lipid kinase and two mammalian SPHKs, SPHK1 and SPHK2, have been identified to data (Hait et al. 2006). SPHK1 and SPHK2 are associated with cell growth, survival and apoptosis, and also involved in variety of diseases including cancer, asthma and atherosclerosis. Pharmacological modulation of SPHKs for treatment of such diseases has been a subject of interest both in academic and pharmaceutical industry (Patwardhan et al. 2015). Therefore this class of SPHK inhibitors may affect the function of SYL-927 when used in combination. Additionally, the identification of particular SPHK isoform involved in SYL-927-P formation using specific SPHK1 or SPHK2 inhibitor deserves to be carried out in the future studies.

Pharmacokinetic studies revealed the constantly higher levels of SYL-927 and SYL-927-P in blood compared with those in plasma after oral administration of SYL-927 in rats. This indicated that similar to FTY720 (Mandala et al. 2002; Zemann et al. 2006), SYL-927 accumulated and was consistently converted to active SYL-927-P in blood cells, contributing to long duration of SYL-927-P for drug effect to persist.

In summary, blood cells including erythrocytes and platelets are involved in the conversion of SYL-927 to SYL-927-P and its release, providing plasma SYL-927-P constantly for prolonged action.

4. Experimental

4.1. *Materials*

SYL-927, SYL-927-P, SYL-930 (internal standard) and FTY720 were provided by the Institute of Materia Medica of Chinese Academy of Medical Sciences. DMS were purchased from Sigma Chemical Co. (St Louis, MO, USA). Fresh human blood from healthy adult volunteers was provided by Peking Union Medical College Hospital. Sprague-Dawley (SD) rats (200±20 g, male) were obtained from Beijing Vital River Experimental Animal Co., Ltd. All other chemicals were purchased from reliable commercial sources.

4.2. *Preparation of erythrocytes, platelets and plasma*

Heparinized rat blood was collected from femoral vein and then plasma was obtained by centrifugation at 2000 g for 10 min. Rat and human erythrocytes and platelets were prepared as described before (Yang et al. 1999). Erythrocytes and platelets were finally suspended in a buffer comprising of 138 mM NaCl, 3.3 mM NaH_2PO_4, 2.9 mM KCl, 1 mM $MgCl_2$, 1 mg/ml glucose, 20 mM HEPES and 1% fatty acid-free BSA (pH 7.4).

4.3. *Interconversion of SYL-927 and SYL-927-P in rat blood and plasma*

Rat blood and plasma were incubated with SYL-927 or SYL-927-P (1 μM) at 37 °C in a shaking water bath in a total volume of 100 μl. The reactions were terminated at different time points (15, 30, 45, 60, 120 and 240 min) by adding two volumes of ice-cooled acetonitrile containing 50 ng/ml of SYL-930 as internal standard. The mixtures were vortexed for 30 s followed by centrifugation at 14,000 rpm for 5 min

and the supernatants were subjected to liquid chromatography/tandem mass spectrometer (LC-MS/MS) analysis. The blood samples of SYL-927 at 60, 120 and 240 min were also treated by centrifugation at 1200 g for 5 min at 4 °C to separate plasma and cell fraction. The cell fraction were washed twice with ice-cold PBS and then homogenized in 100 µl RIPA lysis buffer. The plasma and cell lysates were treated and analyzed as that of blood.

4.4. SYL-927-P formation in rat/human erythrocytes and platelets

Rat or human erythrocytes (8×10^8/ml) and platelets (3×10^7/ml) were incubated with SYL-927 at concentrations ranging from 1 to 500 µM at 37 °C for 60 min in 100 µl total volume. The cell fraction and medium were separated and analyzed as described before. Total generated SYL-927-P amounts represent the sum of SYL-927-P in the medium and cells. Velocity of SYL-927-P generation was reported as picomoles per minute. Enzyme kinetic parameters of K_m and V_{max} were obtained by fitting the Michaelis-Menten equation using Prism 5.0 software (GraphPad Software Inc., San Diego, CA).

4.5. Inhibition of SYL-927-P formation in rat/human erythrocytes and platelets

Incubation of SYL-927 (10 µM) with rat/human erythrocytes (8×10^8/ml) and platelets (3×10^7/ml) were performed in the presence of DMS (1 and 10 µM) and FTY720 (1 and 10 µM). Cell fraction and medium were separated by centrifugation and analyzed as described before. The total generated SYL-927-P amount was calculated as the sum of that in the medium and cells. SYL-927-P generation in the presence of inhibitors was normalized to that in controls in the absence of inhibitors (%).

4.6. Pharmacokinetic study of SYL-927 in rats

The SD rats (n = 5) were orally treated with SYL-927 (3 mg/kg) dissolved in ultrapure water. Then 250 µl of blood samples in each animal were collected *via* the orbital plexus into the heparinized tubes at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36 and 48 h after oral dosing. The plasma was obtained by centrifugation, and 100 µl of blood or plasma samples were combined with 20 µl of SYL-930 (500 ng/ml) and 180 µl of acetonitrile. The mixtures were centrifuged and analyzed as described before. Data fitting and pharmacokinetic parameter calculations were carried out using DAS 2.0 pharmacokinetic program (Chinese Pharmacological Society).

4.7. LC-MS/MS analysis

The quantification was performed on an API 4000 triple quadruple mass spectrometer (AB SCIEX, USA) equipped with an Agilent 1260 series HPLC system (Agilent, USA) consisting of a quaternary pump, automatic solvent degasser, autosampler and an automatic thermostatic column compartment. The LC-MS/MS system was operated in positive electrospray ionization (ESI) mode. The following were the optimum ESI conditions: ion spray voltage, 5500 V; temperature, 500 °C; CAD gas, 6 psi; CUR gas, 25 psi; both GS1 and GS2, 50 psi.
The separation was carried out on a reversed-phase Zorbax SB-C18 analytical column (3.5 µm, 2.1×100 mm, Agilent, Santa Clara, USA) with a 0.5 online filter (Upchurch Scientific Ltd.). The mobile phase was a mix of solvent A (0.1 % formic acid in water) and solvent B (0.1 % formic acid in methanol) and the flow rate was 0.2 ml/min with an operating temperature of 25 °C. The initial condition for the HPLC gradient was 70:30 (A:B). From 0.5 to 0.6 min, the mobile phase composition changed linearly to 5:95 (A:B). This condition was held until 2.5 min. The gradient was returned in a linear fashion to 70:30 (A:B) from 2.5 to 2.6 min and re-equilibrated until 9 min.
The MS recordings were carried out in multiple reaction monitoring (MRM) mode. Transitions were monitored at *m/z* 367.2→350.1 for SYL-927, *m/z* 447.1→320.1 for SYL-927-P and *m/z* 381.2→364.2 for internal standard of SYL-930. Automated data acquisition and data analysis were performed using Analyst 1.5.2 software.

4.8. Data analysis

Data are expressed as mean±SD. The differences between two groups were evaluated by Student's *t* test. $p < 0.05$ was considered to be statistically significant.

Conflicts of interest: The authors report no declarations of interest.

References

Anada Y, Igarashi Y, Kihara A (2007) The immunomodulator FTY720 is phosphorylated and released from platelets. Eur J Pharmacol 568: 106-111.
Chi H (2011) Sphingosine-1-phosphate and immune regulation: trafficking and beyond. Trends Pharmacol Sci 32: 16-24.
Hait NC, Oskeritzian CA, Paugh SW, Milstien S, Spiegel S (2006) Sphingosine kinases, sphingosine 1-phosphate, apoptosis and diseases. Biochim Biophys Acta 1758: 2016-2026.
Hanel P, Andreani P, Graler MH (2007) Erythrocytes store and release sphingosine 1-phosphate in blood. FASEB J 21: 1202-1209.
Hara-Yokoyama M, Terasawa K, Ichinose S, Watanabe A, Podyma-Inoue KA, Akiyoshi K, Igarashi Y, Yanagishita M (2013) Sphingosine kinase 2 inhibitor SG-12 induces apoptosis via phosphorylation by sphingosine kinase 2. Bioorg Med Chem Lett 23: 2220-2224.
Huwiler A, Pfeilschifter J (2008) New players on the center stage: sphingosine 1-phosphate and its receptors as drug targets. Biochem Pharmacol 75: 1893-1900.
Ito K, Anada Y, Tani M, Ikeda M, Sano T, Kihara A, Igarashi Y (2007) Lack of sphingosine 1-phosphate-degrading enzymes in erythrocytes. Biochem Biophys Res Commun 357: 212-217.
Kihara A, Igarashi Y (2008) Production and release of sphingosine 1-phosphate and the phosphorylated form of the immunomodulator FTY720. Biochim Biophys Acta 1781: 496-502.
Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thornton R, Shei GJ, Card D, Keohane C, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H (2002) Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science296: 346-349.
Mechtcheriakova D, Wlachos A, Sobanov J, Bornancin F, Zlabinger G, Baumruker T, Billich A (2007) FTY720-phosphate is dephosphorylated by lipid phosphate phosphatase 3. FEBS Lett 581: 3063-3068.
Patwardhan NN, Morris EA, Kharel Y, Raje MR, Gao M, Tomsig JL, Lynch KR, Santos WL (2015) Structure-activity relationship studies and in vivo activity of guanidine-based sphingosine kinase inhibitors: discovery of SphK1- and SphK2-selective inhibitors. J Med Chem 58: 1879-1899.
Paugh SW, Payne SG, Barbour SE, Milstien S, Spiegel S (2003) The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2. FEBS Lett 554: 189-193.
Sanna MG, Liao J, Jo E, Alfonso C, Ahn MY, Peterson MS, Webb B, Lefebvre S, Chun J, Gray N, Rosen H (2004) Sphingosine 1-phosphate (S1P) receptor subtypes S1P1 and S1P3, respectively, regulate lymphocyte recirculation and heart rate. J Biol Chem 279: 13839-13848.
Yang L, Yatomi Y, Miura Y, Satoh K, Ozaki Y (1999) Metabolism and functional effects of sphingolipids in blood cells. Br J Haematol 107: 282-293.
Xiao Q, Jin J, Wang X, Hu J, Xi M, Tian Y, Yin D (2016) Synthesis, identification, and biological activity of metabolites of two novel selective S1P1 agonists. Bioorg Med Chem 24: 2273-2279.
Zemann B, Kinzel B, Muller M, Reuschel R, Mechtcheriakova D, Urtz N, Bornancin F, Baumruker T, Billich A (2006) Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. Blood 107: 1454-1458.