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MINI-REVIEW

The signalling roles of sphingosine-1-phosphate derived from red blood cells and platelets

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Sphingosine-1-phosphate (S1P) is an essential, bioactive lysophospholipid mediator that regulates various physiological functions such as lymphocyte trafficking, inflammation and behavioural characteristics of the vascular system. S1P signalling is mediated *via* a family of five GPCRs, which are expressed in various cell types and tissues. S1P concentration is maintained in a gradient through the activity of S1P degrading enzymes, and this gradient is critical for lymphocyte egress. To exert its extracellular signalling roles, S1P must be secreted out of the cells by protein transporters. The recent discovery of S1P transporters has shed light on the sources of S1P. However, these transporters still need to be clarified as they are important in defining the S1P gradient for lymphocyte recirculation and the source of S1P for maintenance of blood vessels. Here, we review the current understanding of S1P sources, highlighting the roles of S1P transporters with an emphasis on haematopoietic cells as a major source of circulatory S1P.

Abbreviations

ABC, ATP binding cassette; ApoM, alipoprotein M; CLEC-2, C-type lectin domain family 2; HEV, high endothelial venules; MFS, major facilitator superfamily; RBC, red blood cells; S1P, sphingosine 1-phosphate; SGPP, sphingosine 1-phosphate phosphatase; SPHK, sphingosine phosphate kinase; Spns2, protein spinster homologue 2

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Introduction

During mammalian development, blood cells and blood vessels develop concurrently. Multipotent haematopoietic cells that emerge from haemogenic endothelium form blood cells. Overlapping genes in blood cells and endothelium induce them to act synergistically (Zovein *et al.*, 2008). **Sphingosine-1-phosphate (S1P)** is a lipid molecule generated by both blood cells and endothelial cells, which has important signalling roles. In the circulation system, blood cells release this lipid mediator to maintain blood vessel integrity (Camerer *et al.*, 2009). Furthermore, lymphatic endothelia release this molecule to guide T and B lymphocyte recirculation (Fukuhara *et al.*, 2012). Recent findings on the role of platelet-derived S1P in thrombus formation (Urtz *et al.*, 2015) and platelet biogenesis (Kaushansky, 2005) further emphasize the potential of this mediator. In this review, we present some of the major signalling roles of S1P derived from the haematopoietic system. We also investigate the various animal models deficient in S1P used to explore its physiological effects. Additionally, we highlight the S1P transporters involved in regulating plasma S1P levels, focussing on their potential as pharmacological targets for various diseases.

S1P signalling roles

S1P metabolism is closely regulated by two **sphingosine kinases** (**SPHK1** and **SPHK2**) (Maceyka *et al.*, 2013), which are involved in its production, and two **S1P-phosphatases (SGPP1 and SGPP2)** (Bourquin *et al.*, 2010), an S1P-lyase and three lysophospholipid hydrolases implicated in its degradation (Serra and Saba, 2010). S1P exerts its extracellular effects through high-affinity binding GPCRs, **S1P receptors 1–5 ($S1P_{1\text{-}5}$)** (Van Brocklyn *et al.*, 1998; Van Brocklyn *et al.*, 2000). The binding of S1P to each of these receptors induces various signalling effects depending on the heterotrimeric G proteins coupled to each of them. The different receptors have been suggested to have a role in diverse developmental and disease-related processes. Two major functions of S1P signalling are the regulation of lymphocyte trafficking and blood vessel integrity (Proia and Hla, 2015). For instance, deletion of **$S1P_1$ receptor** (Liu *et al.*, 2000) or SPHK1/2 (Mizugishi *et al.*, 2005) results in embryonic lethality with severe haemorrhages at early developmental stages, highlighting the critical roles of S1P signalling for blood vessel functions. In addition, S1P signalling is also vital for cell motility, and has been regarded as the central mediator for lymphocyte egress (Matloubian *et al.*, 2004). The activation of $S1P_1$ signalling and the maintenance of S1P gradient between tissues and the systemic circulation are vital for the egress of newly formed T cells from the thymus and mature T and B cells from secondary lymphoid organs (Nijnik *et al.*, 2012). A disruption of the S1P gradient can be induced by inhibiting S1P degrading enzymes or S1P transporters (Fukuhara *et al.*, 2012). Targeting S1P signalling by **fingolimod (FTY720)** has been successfully used to treat multiple sclerosis. This prodrug suppresses immune responses and causes peripheral lymphopenia by blocking lymphocyte egress from the thymus and lymph nodes (Chun and Hartung, 2010; Nijnik *et al.*, 2012). Pharmacological studies on FTY720 showed that it inhibits the expression of $S1P_1$ receptors on lymphocytes in its phosphorylated form (Brinkmann *et al.*, 2002; Pham *et al.*, 2010). FTY720 and other S1P receptor modifying compounds have confirmed the importance of S1P signalling in the recruitment of inflammatory cells. Nevertheless, FTY720 causes side effects such as bradycardia (Budde *et al.*, 2003). The S1P-S1P receptor signalling axis is important for regulating innate and adaptive immune responses. S1P signalling through **$S1P_4$ receptor** promotes neutrophil trafficking (Allende *et al.*, 2010) whereas signalling through **$S1P_5$ receptor** is necessary for natural killer T cell egress from lymph nodes and bone marrow (Jenne *et al.*, 2009). Recently, S1P-dependent signalling was detected in innate lymphoid cells in response to infection (Huang *et al.*, 2018), emphasizing the role S1P plays in cell motility.

S1P carriers and transporters

As a lipophilic molecule, extracellular S1P is associated with carriers. Plasma S1P binds to alipoprotein M (ApoM) in HDL and LDL, and albumin (Christoffersen *et al.*, 2011). HDL-bound S1P represents the major S1P fraction in plasma (Blaho *et al.*, 2015). S1P is also present in interstitial fluid in tissue and brain (Nagahashi *et al.*, 2016). However, its carriers in these tissues are unknown. In blood, the majority of S1P is bound to ApoM in HDL (Kono *et al.*, 2004). Mice lacking ApoM exhibited reduced S1P levels in plasma. However, S1P production is intact in ApoM deficient mice, suggesting that ApoM stabilizes S1P in blood (Christoffersen *et al.*, 2011). In contrast, the role of albumin-bound S1P has been little studied.

Much knowledge about S1P source was gained from studies of knockouts of S1P kinases (SPHK1/2) (Xiong *et al.*, 2014). Deletion of both SPHK1/2 results in loss of both the intracellular and extracellular sources of S1P (Mizugishi *et al.*, 2005). Yet it is difficult to discern the intracellular and extracellular signalling effects. One of the major challenges in the S1P signalling field is the identification of S1P transporters. ATP-dependent transporters such as **ABCC1** and **ABCC5** were implicated as potential S1P transporters (Mitra *et al.*, 2006; Kobayashi *et al.*, 2009). However, none of these ATP transporters yielded a significant amount of plasma S1P, obscuring their physiological functions as S1P transporters. The discovery of protein spinster homologue 2 (Spns2) as the first *bona fide* S1P transporter has shed light on the source of S1P (Kawahara *et al.*, 2009). Spns2 exports S1P from lymphatic endothelial cells and accounts for approximately 25–50% of plasma S1P (Fukuhara *et al.*, 2012; Nagahashi *et al.*, 2013). Notably, Spns2 is not the S1P transporter in haematopoietic cells, which are the major source of S1P (Mendoza *et al.*, 2012).

Solute carrier proteins represent one of the most abundant proteins in mammals. Most of these transporters including a sub-category of transporters, namely, **major facilitator superfamily (MFS)** domains transport soluble substrates. A recent finding by Vu *et al.* identified Mfsd2b, an orphan transporter that is expressed in erythrocytes and platelets. This transporter provides approximately 50% of the S1P in plasma as observed in $Mfsd2b^{-/-}$ mice (Vu *et al.*, 2017). This corroborates well with previous determinations of S1P sources from haematopoietic cells (Gazit *et al.*, 2016). $Mfsd2b^{-/-}$ mice exhibited phenotypes that are linked to abnormal S1P signalling, such as low circulating lymphocytes and sensitivity to vascular stress (Vu *et al.*, 2017).

Roles of erythrocyte-derived S1P

Compelling studies have indicated that erythrocytes are the major source of plasma S1P (Hanel *et al.*, 2007; Pappu *et al.*, 2007). Erythrocyte-specific deletion of both SPHK1 and SPHK2 led to embryonic lethality, and these embryos have severe vascular defects. This suggests that red blood cell (RBC)-derived S1P is crucial during embryonic development, specifically for vascular stabilization, maturation and remodelling (Xiong *et al.*, 2013). A detailed analysis of embryonic vascular defects further emphasizes the involvement of RBC-specific S1P in angiogenesis and also erythropoiesis. Similarly, a global deletion of both SPHK1 and 2 resulted in vascular defects, and these embryos were not viable after E13.5 (Mizugishi *et al.*, 2005). Additionally, these mutant embryos also exhibited severe defects in neurogenesis whereby, exencephaly was observed. This further confirms that S1P is vital during embryonic development especially for neurogenesis and angiogenesis. However, it is unknown whether Mfsd2b is the S1P provider for neurogenesis that was observed in double knockouts of SPHK1/2.

Deletion of both S1P synthesis enzymes SPHK1/2 has demonstrated that plasma S1P is critical for several physiological functions of blood vessels. The postnatal deletion of SPHK1/2 using Mx1-cre showed that plasma S1P is essential for protection of mice against anaphylactic shock and the vascular leakage associated with inflammation (Camerer *et al.*, 2009). Transfusion of wild-type RBC reversed these pathological conditions, indicative of the essential roles of RBC for S1P homeostasis. In another S1P depleted model, ApoM$^{-/-}$, vascular leakage was observed followed by increased inflammatory responses to LPS-induced sepsis (Zhu *et al.*, 2018). Inversely, administration of an engineered ApoM-S1P complex protects mice from cerebral and cardiac ischaemic damage and hypertension (Swendeman *et al.*, 2017). These results indicate the dual role of S1P in blood vessels in normal and pathological conditions. Furthermore, in endothelium, S1P is thought to regulate vascular tone. Cantalupo *et al.* (2017) found that autocrine activation of $S1P_1$ in endothelial cells lowered BP emphasizing its importance in vasodilatation. Nevertheless, it is unclear whether erythrocytes are responsible for this S1P pool. The identification of Mfsd2b holds great significance as its specific deletion could elucidate the role of S1P in extracellular signalling (Figure 1).

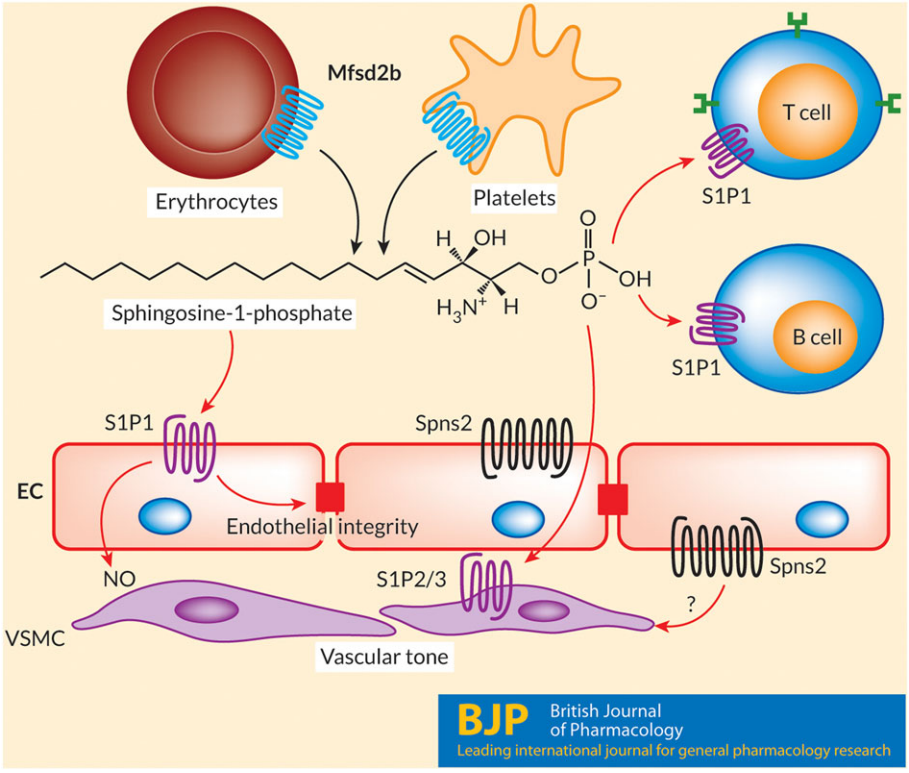

Figure 1

Extracellular signalling roles of S1P. Sphingosine-1-phosphate is transported out from haematopoietic cells by Mfsd2b and by Spns2 from endothelial cells. S1P plays a role as an extracellular signalling lipid in which it activates five different GPCRs, namely, $S1P_{1-5}$. The $S1P_1$ receptor (S1P1) is one of the most abundant receptors expressed in blood endothelial cells and T and B lymphocytes. In blood, S1P is mainly generated by erythrocytes and platelets. Mfsd2b is the transporter for S1P in these cells. In the endothelium, S1P is released *via* Spns2, especially in the lymphatic system. Both Mfsd2b and Spns2 contribute to blood S1P, which is critical for maintaining the functions of blood vessels *via* activation of $S1P_1$ receptors on endothelial cells or $S1P_{2/3}$ receptors (S1P2/3) on vascular smooth muscle cells. An active S1P signalling pathway is essential for blood vessel integrity and vascular tone. It is not known whether Spns2 releases S1P on both sides of endothelium. EC, endothelial cells; VSMC, vascular smooth muscle cells.

Release of S1P by platelets to the circulation

Platelets can produce and store large amounts of S1P, synthesis by SPHK2 being the major source of their S1P production (Urtz *et al.*, 2015), but they lack both the *de novo* sphingolipid biosynthetic pathway and the S1P catabolic enzyme S1P lyase. Also, even though platelets store large amounts of S1P, they do not contribute a significant amount of S1P to the circulation at homeostasis. However, the observation that serum S1P was increased twofold to threefold during clot formation suggest that platelets do release S1P upon aggregation (Ono *et al.*, 2013). The increase in plasma S1P concentrations by activated platelets has a critical role in the repair of endothelial vessels during injury. This mechanism is operated by receptors on vascular smooth muscle cells, which are not saturated by the plasma levels of S1P provided normally by other cell types.

Platelets are prone to activation by exogenous stimuli *via* GPCRs (Offermanns, 2006). The release of S1P can be induced by activation of platelets with potent agonists, such as thrombin, selective PAR-activating peptides (PARAPs) or a high concentration of collagen. This process results in the release of platelet mediators like **ADP**, **arachidonic acid** derivatives, **TXA_2** and **PGE_2** from their storage granules. TXA_2 and ADP are able to stimulate S1P release from platelets *in vitro* (Gazit *et al.*, 2016). TXA_2 activation of platelets can be largely prevented by inhibitors of **COX-2** such as **aspirin**, **diclofenac** and **ibuprofen** (Ulrych *et al.*, 2011). However, it is unclear whether COX-2 inhibitors directly inhibit S1P release or platelet aggregation, which eventually leads to the release of S1P. Platelets seem to store S1P in cell membranes as well as in α-granules but not in dense granules (Jonnalagadda *et al.*, 2014). In addition, a series of studies from Whiteheart's laboratory has shown that a soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) complex is required for S1P release in activated platelets (Joshi and Whiteheart, 2017). Selectively, Unc13d mutation causes defective exocytosis in thrombin-induced S1P release (Ren *et al.*, 2010) accompanied by defective aggregation and long tail bleeding times. However, the mechanisms involved in the storage of S1P by resting platelets are unknown. Several studies have reported the involvement of ABC transporters for the export of S1P in platelets. However, the biological functions of these proteins are still not completely understood. Mfsd2b is also highly expressed in platelets, and this transporter is required for S1P release from resting and activated platelets (Vu *et al.*, 2017). In the study by Vu *et al.*, (2017) Mfsd2b was shown to be expressed in cell membranes and intracellular membrane structures. However, the expression pattern of Mfsd2b in resting platelets is not known.

Role of platelet-derived S1P signalling in blood vessels

Although platelets are not the major source of S1P in the circulation during normal conditions, activated platelets release significant amounts of this lipid mediator. Studies by Herzog *et al.*, (2013) showed that S1P released by platelets is important for the maintenance of high endothelial venules (HEV). Activation of the C-type lectin domain family 2 (**CLEC-2**) receptor in platelets results in the release of S1P, which then acts in a paracrine manner to increase VE-cadherin expression in adjacent endothelial cells, thus explaining the function of S1P released by activated platelets in overall vascular integrity. However, the specific S1P deficiency observed in platelets from $SPHK2^{-/-}$ does not show similar phenotypes to those with CLEC-2 deletion. This suggests that activation of CLEC-2 receptors in platelets might have other effects on HEV. Indeed, the specific functions of platelet-stored S1P is still ill-defined.

Role of platelet-derived S1P signalling in thrombus formation

At the site of vascular injury, various mediators are released by activated platelets including S1P. Thrombus formation and stabilization require the persistent activation of recruited platelets to prevent those platelets from dissociating and detaching from the thrombus. A deficiency in SPHK2 results in defective platelet aggregation and arterial thrombosis with unaltered tail bleeding times in mice. Thus, the release of S1P by activated platelets probably activates the autocrine signalling pathway for thrombosis (Urtz *et al.*, 2015). These findings indicate that S1P from platelets is necessary for thrombus formation *in vivo* (Gazit *et al.*, 2016). This S1P signalling function should be explored further to find new inhibitors of SPHK2 that can modulate thrombus formation without interfering with the other functions of platelets. Hence, future studies will no doubt provide additional insights as to the role of platelet-stored S1P in various diseases.

Conclusion and future perspectives

Several sources contribute to the S1P available in the circulatory system. Of significance, erythrocytes and platelets are the major source of S1P in the circulation *via* Mfsd2b (Vu *et al.*, 2017). Nevertheless, endothelial cells also contribute a significant amount of S1P *via* Spns2 (Fukuhara *et al.*, 2012). It is still unclear whether Mfsd2b and Spns2 are the only two exporters that contribute to the S1P in the circulation. In addition, the cellular and molecular origin and transport machinery involved in embryonic S1P have not yet been identified. However, the signalling pathways associated with S1P are being extensively explored as suitable targets for drug development for diverse inflammatory diseases and cancers. The identification of S1P transporters has provided fresh approaches for understanding the role of S1P in various pathological conditions and afforded novel molecular targets for drug development.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b,c,d).

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Conflict of interest

The authors declare no conflicts of interest.

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