Review

Sphingosine signaling and atherogenesis[1]

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ABSTRACT

Sphingosine-1-phosphate (S1P) has diverse biological functions acting inside cells as a second messenger to regulate cell proliferation and survival, and extracellularly, as a ligand for a group of G protein-coupled receptors (GPCRs) named the endothelial differentiation gene (EDG) family. Five closely related GPCRs of EDG family (EDG1, EDG3, EDG5, EDG6, and EDG8) have recently been identified as high-affinity S1P receptors. These receptors are coupled via Gi, Gq, $G_{12/13}$, and Rho. The signaling pathways are linked to vascular cell migration, proliferation, apoptosis, intracellular Ca^{2+} mobilization, and expression of adhesion molecules. The formation of an atherosclerotic lesion occurs through activation of cellular events that include monocyte adhesion to the endothelium and vascular smooth muscle cell (VSMC) migration and proliferation. Thus, S1P signaling may play an important role in the pathogenesis of atherosclerotic vascular disease. This review highlights S1P signalling in vascular cells and its involvement in the formation of atherosclerotic lesions.

INTRODUCTION

Sphingosine-1-phosphate (S1P), converted from sphingosine by sphingosine kinase (SphK) (Fig 1), is a key cell signalling molecule that acts both extracellularly and intracellularly with specific effects on cells of the vessel wall. Serum contains S1P that is mainly released from activated platelets[1], while other blood cells such as erythrocytes, neutrophils, and mononuclear cells release constitutively a small percentage of circulating S1P[2]. Circulating S1P is mainly present in lipoproteins with the rank order high-density lipoprotein (HDL)>low-density lipoprotein (LDL)=very low-density lipoprotein (VLDL)[3]. Thus, lipoproteins appear to be carriers of S1P. LDL that accumulates in atherosclerotic lesions contains S1P and it is possibly produced locally in the atherosclerotic lesions by vascular smooth muscle cells (VSMC) activated by growth factors, which suggests an important role of S1P in atherogenesis.

However, during LDL oxidation, the content of S1P is reduced[4]. LDL, particularly oxidized LDL (Ox-LDL), is closely correlated and HDL is inversely correlated, with the risk of atherosclerosis. What is the relationship between S1P and atherosclerosis? Is it an atherogenic factor or an anti-atherogenic factor? The concentration of S1P in plasma or in serum is much higher than the half-maximal concentration of the sphingolipid needed to stimulate its receptors. Even though the protein content of the lipoprotein fraction contributes to only 4 % of the total protein content of plasma or serum, more than 60 % of S1P is distributed in this

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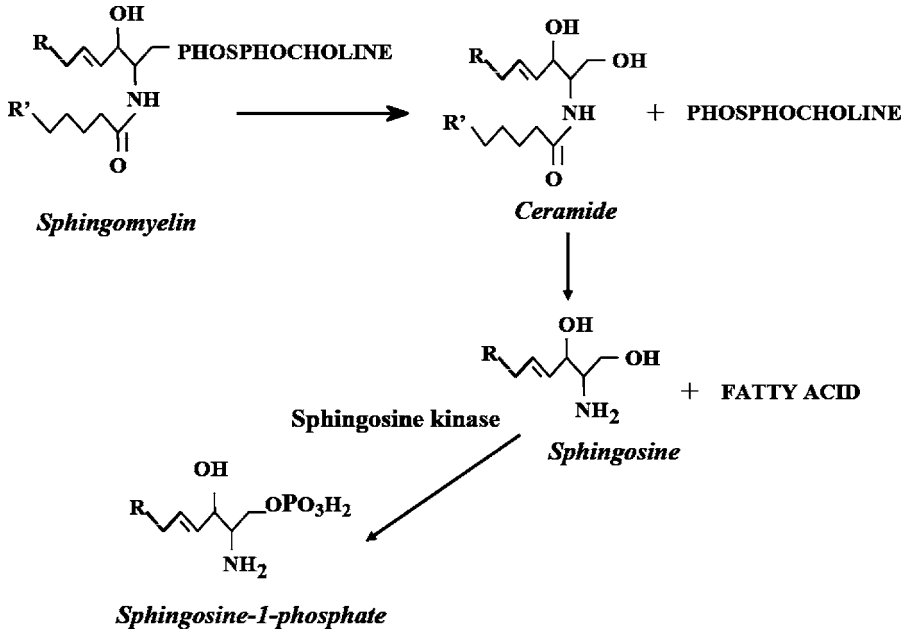

Fig 1. Sphingolipids metabolism.

fraction[4]. Thus, the interaction of S1P with lipoproteins seems to reduce the freedom of S1P and hereby decrease the apparent active concentration of S1P. S1P is shown as an atherogenic factor that results in enhanced VSMC proliferation[5] and migration[6]. Tumour necrosis factor-α (TNF-α) activates SphK and increases intracellular S1P that induces the expression of vascular cell adhesion molecules (VCAM) in endothelial cells[7]. In addition, Ox-LDL induces VSMC proliferation via activation of SphK and generation of intracellular S1P[8]. However, S1P is postulated to be an anti-atherogenic factor based on the finding that HDL-associated S1P is a major component of the HDL-induced cytoprotective action in human endothelial cells[9]. Further investigations are needed to determine whether S1P is athrogenic or anti-atherogenic.

S1P has diverse biological functions. Inside the cells it is a second messenger that regulates calcium mobilization, cell proliferation, and survival[10], while extracellular S1P is a ligand for a novel G-protein coupled receptor (GPCR) family of the endothelial differentiation genes (EDG). EDG elicit signal transduction via Gi, Gq, G12, G13 proteins, and Pho-dependent routes[11,12]. To date 5 members, EDG1/S1P1, EDG5/S1P2, EDG3/S1P3, EDG6/S1P4, and EDG8/S1P5 receptors have been identified[13]. These receptors are highly specific and only bind S1P and dihydro-S1P. S1P1 and S1P5 receptors are coupled to mainly Gi-proteins; the S1P2 receptor can be coupled to all G-proteins; S1P3 is coupled to Gi, Gq, and G12/13; S1P4 activates Gi and G12 in response to S1P. The effector pathways that are regulated by S1P receptors include extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), C-Jun N-terminal kinase (JNK), phospholipase C and D, adenylyl cyclase, inositol 1,4, 5-triphosphate (IP3), phosphoinositide 3 (PI3)-kinase and focal adhesion kinase (FAK)[14-15] (Fig 2). Many external stimuli, particularly growth factors, cytokines, and Ox-LDL activate SphK, the enzyme that forms S1P from sphingosine[8,16,17]. Platelet derived growth factor (PDGF) and serum induce rapid activation of SphK and transient production of S1P in Swiss 3T3 fibroblasts[10]. PDGF, TNF-α, and Ox-LDL enhance SphK activity and increase S1P generation[16,17]. S1P formed intracellularly after PDGF activation of VSMC is apparently not released into the culture medium, but exposed on the cell surface, where it activates the S1P1 receptor in an autocrine or paracrine manner and mediates VSMC migration when stimulated by PDGF. Interestingly, the mitogenic effect of Ox-LDL on VSMC is dependent on the cellular formation of S1P[8]. It is not clear if this action in addition involves the activation of the S1P1 receptor.

SPHINGOSINE KINASE AND VSMC PROLIFERATION

The two sphingosine kinase (SphK) isotypes type 1 and 2, the most important enzymes regulating S1P

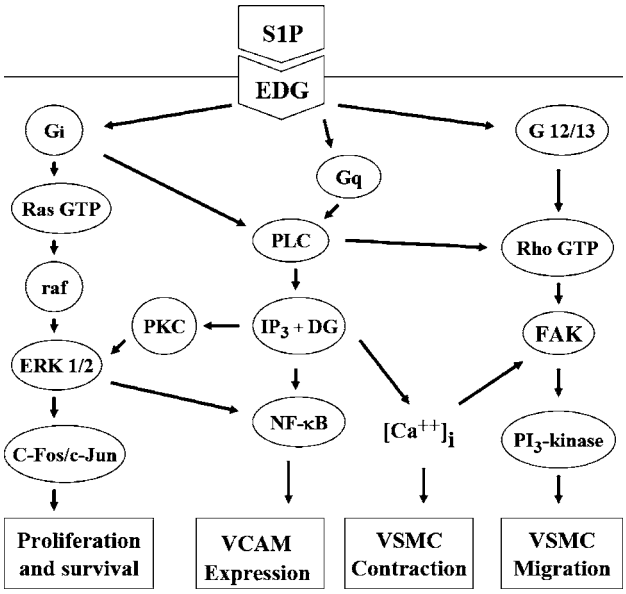

Fig 2. Intracellular signal transduction pathways of S1P receptors in vascular cells. S1P is a ligand for a G-protein coupled receptor (GPCR) family of the endothelial differentiation genes (EDG) that elicit signaling transduction via Gi, Gq, G12, G13 proteins, and Pho-dependent routes. Intracellular pathways of S1P receptors include extracellular signal-regulated kinase (ERK), phospholipase C (PLC), inositol 1,4,5-triphosphate (IP3), phosphoinositide 3 (PI3)-kinase and focal adhesion kinase (FAK). These intracellular transduction pathways of EDG promote cell proliferation, survival, vascular adhesion molecules (VCAM) expression, vascular smooth muscle cells (VSMC) contraction and migration.

levels in eukaryotic cells, have recently been cloned[18]. Although highly similar in amino acid sequence and possessing five conserved domains, SphK type 1 is smaller than type 2 and expressed mainly in the cytosol, while SphK type 2 additionally has several transmembrane regions and a proline-rich SH-3-binding domain[18]. This suggests different cellular functions and regulation mechanisms of the two isotypes. SphK contains Ca^{2+}/calmodulin-binding consensus sequences and several protein kinase phosphorylation sites, eight of them for protein kinase C (PKC)[14]. Interestingly, SphK is stimulated with the PKC activator phorbol 12-myristate 13-acetate (PMA)[19]. In human platelets, S1P is accumulated in and specifically released by thrombin through the activation of PKC[1]. We revealed that SphK, PKC and Gi-protein activities were required for the mitogenic effect of basic fibroblast growth factor (bFGF) in vascular VSMC as based on the blocking effects of *D*-erythro-*N*,*N*-dimethylsphingosine (DMS), staurosporine and pertussis toxin[5]. In agreement with our findings, Kimura *et al*[20] demonstrated that S1P could induce DNA synthesis, cell growth, and cell migration in human aortic endothelial cells and that these responses to S1P were mimicked by dihydrosphingosine-1-phosphate, a S1P receptor agonist, and inhibited by pertussis toxin. These results suggest Gi-protein coupled receptor mediates vascular cell proliferation induced by S1P. Putatively, growth factors, cytokines and thrombin may activate SphK and induce release of S1P via activation of PKC, and subsequently S1P may bind to $S1P_1$ receptor/Gi-protein ultimately resulting in further activation of PKC and the MAPK signaling pathways. On other hand, S1P may play an intracellular role responsible for cell proliferation, because DMS, an inhibitor of SphK, could decrease the intracellular S1P production induced by PDGF and block PDGF-induced cell proliferation[16]. Furthermore, S1P not only stimulates, but also inhibits VSMC migration that depends on the expression pattern of individual S1P receptors[21]. Activation of tyrosine kinase receptor PDGF-β by PDGF stimulates SphK that results in generation of S1P. Furthermore, S1P binds to the $S1P_1$ receptor that activates a small GTPase Rac that leads to cell migration[22]. The activation of $S1P_1$ receptor by S1P promotes angiogenesis and cell migration required for PDGF-directed motility of SMC. This suggests a cross talk between growth factor receptor (PDGF-β) and S1P1 receptor.

SphK is expressed in many cell types including human vascular endothelial cells and VSMC[5,23]. We has recently revealed that the growth factor bFGF can in part be signaling via SphK since DMS blocked the conversion of sphingosine to S1P, inhibited SMC proliferation induced by bFGF and by the exogenous addition of S1P[5]. Further studies have revealed that treatment of human endothelial cells with reconstituted HDL and DMS suppressed the expression of adhesion molecules and apoptosis via inhibition of the SphK activity induced by TNF-α [7]. These data imply a role of S1P in the atherosclerotic process. The signal transduction pathways of S1P and its receptors in vascular cells are very complex and still under investigation.

S1P AND VASCULAR CELL CONTRACTION

S1P induced constriction of cerebral VSMC has a similar time course to activation of Rho-kinase, but this does not occur in aortic VSMC[24]. VSMC from cerebral arteries stimulated by low concentration of S1P

show an increase in intracellular Ca^{2+} via an IP_3-dependent pathway, but only a small increase in intracellular Ca^{2+} was observed after stimulation at higher concentrations of S1P. Subtype-specific S1P receptor antibodies reveals that the expression of S1P3 and S1P2 receptors is 4-fold higher in cerebral VSMC as compared with VSMC from aorta, while S1P1 receptor expression is similar in both[24]. Sphingosine, the precursor of S1P, induces vasoconstriction in coronary arteries that seems to be mediated by the release of cyclooxygenase-sensitive vasoconstrictor prostanoids from the endothelium[25]. In microvessels, SphK modulates vascular tone through activation of RhoA/Rho kinase[26]. S1P also acts as an arteriolar vasodilator in pre-constricted mesenteric arteries in an endothelium-dependent manner. This action occurs via nitric oxide (NO) and PI_3-kinase-modulated signaling pathways[27].

BALANCE OF S1P AND CERAMIDE IN VASCULAR CELL APOPTOSIS

Ceramide (*N*-acylsphingosine), one of the precursors of S1P, is an important apoptotic factor[28], while S1P promotes cell proliferation and survival[29]. The balance between ceramide and S1P has been suggested to determine whether cells survive or die. SphK type 1 induces G12/13-mediated stress fiber formation and promotes growth and survival[30]. Stress and cytokines increase ceramide synthesis while elevated levels of ceramide results in cell apoptosis. The anti-atherosclerotic effect of HDL has been suggested to affect the balance between ceramide and S1P[7]. Many stimuli, particularly growth factors, activate SphK to generate S1P which in turn may suppress ceramide mediated-cell apoptosis. During vascular repair and thrombosis formation after injury to an artery, thrombin activates platelets to release S1P that may promote vascular cell proliferation and platelet aggregation. This may benefit the repairing process but can also contribute to the pathogenesis of atherosclerosis.

SPHK AND VASCULAR ADHESION MOLECULES

The expression of adhesion molecules on the endothelium is an early key event in the formation of the atherosclerotic lesion. Cytokines and growth factors released from platelets, macrophages, T-lymphocytes and vascular cells and Ox-LDL can activate SphK on vascular cells to result in the generation of S1P. The upregulation of adhesion molecules in endothelium and VSMC proliferation induced by activation of the SphK may promote the formation of atherosclerotic lesions. Growth factors, cytokines, and Ox-LDL may stimulate the expression of the S1P receptors on VSMC in atherosclerotic lesions via an inflammatory transcription pathway. SphK is activated by Ox-LDL[8], a key atherogenic factor, in vascular cells. On the other hand, the anti-atherosclerotic effect of HDL occurs via its ability to inhibit of SphK activity beyond the well-known reverse transport of cholesterol and its anti-oxidative property. Studies have demonstrated that TNF-α-induced SphK activity and the expression of adhesion molecules in endothelial cells is inhibited by HDL[7].

In addition, HDL stimulates endothelial cell migration and survival through sphingosine 1-phosphate and its receptors[31].

CONCLUSION

Dysfunction and damage to the endothelium is believed to initiate the process of atherosclerosis[32]. Monocyte adhesion to endothelium, smooth muscle cell migration and proliferation in response to endothelial dysfunction and damage are key steps in the development of atherosclerotic lesions[7,32,33]. A recent study in 308 consecutive patients undergoing coronary angiography reveal that serum S1P is a strong predictor of both the occurrence and severity of coronary stenosis[34]. Thus, S1P released from platelets and damaged vascular cells is implicated in the formation of atherosclerosis, restenosis and vascular replication after injury. However, oxidation of LDL may reduce its S1P content, which implies an anti-atherogenic effect of S1P. One hypothesis is that intracellular S1P (as an atherogenic mediator) and extracellular S1P (as an anti-atherogenic mediator) function in opposite ways in terms of atherogenesis. Since S1P acts as an second messenger that regulates calcium mobilization, cell proliferation and survival, it likely has intracellular receptors influencing calcium signaling, cell proliferation and survival, but this has not been approved yet. Further studies should address to demonstrate the distribution of S1P receptors, determine whether S1P has intracellular receptors and illustrate which subtypes of S1P receptors are involved in atherosclerosis. Different subtypes of S1P receptors may function via different intracellular signal transduction pathways and the distribution of S1P receptors may thus provide new clues for the role of S1P in

atherosclerosis.

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