Sphingosine-1-Phosphate and Platelets in Diseases

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Sphingosine-1-phosphate (S1P) is a versatile signaling lipid involved in the regulation of numerous cellular processes. S1P regulates cellular proliferation, migration, and apoptosis as well as the function of immune cells. S1P is generated from sphingosine (Sph), which derives from the ceramide metabolism. In particular, high concentrations of S1P are present in the blood. This originates mainly from erythrocytes, endothelial cells (ECs), and platelets. While erythrocytes function as a storage pool for circulating S1P, platelets can rapidly generate S1P de novo, store it in large quantities, and release it when the platelet is activated. Platelets can thus provide S1P in a short time when needed or in the case of an injury with subsequent platelet activation and thereby regulate local cellular responses. In addition, platelet-dependently generated and released S1P may also influence long-term immune cell functions in various disease processes, such as inflammation-driven vascular diseases. New pharmacological approaches that target the auto- or paracrine effects of S1P may be therapeutically helpful in the future for pathological processes involving S1P.

sphingosine-1-phosphate platelets immune cells S1P S1P receptors

1. Platelet-Derived S1P

Sphingolipids belong to the class of polar lipid compounds. They are important structural components of the cell membrane and exert various signaling functions, either
intracellularly as second messengers or via membrane-bound receptors. The family of sphingolipids consists of three main types: the ceramides, sphingomyelins (SMs), and glycosphingolipids. Glycosphingolipids are further divided into the cerebrosides and gangliosides. These types differ in the nature of their respective chemical moieties [1]. In contrast to glycerol-based phosphoglycerides, sphingolipids are derived from the unsaturated aminoalcohol sphingosine (Sph) [2]. The phosphoric acid ester of Sph is sphingosine-1-phosphate (S1P), which is found in high concentrations in the lymph fluid and in the blood. The main producers of circulating S1P are erythrocytes, endothelial cells (ECs), immune cells, and platelets. Within organ tissues, S1P is rapidly degraded by the action of S1P lyase (SPL). As a result, there is a steep gradient between high S1P concentrations within the vessels and low S1P concentrations in the tissue [3].

In the event of a blood vessel injury, platelets have the function of covering the wound and stopping blood loss. Various mechanisms are set in motion for this purpose. First, platelets are activated by von Willebrand factor, and they subsequently adhere to the extracellular matrix. Autocrine and paracrine mediators such as adenosine

diphosphate (ADP), thrombin, or epinephrine amplify and sustain the initial platelet response, leading to an activation of integrin glycoprotein IIb/IIIa. Circulating platelets are recruited and form a hemostatic plug [4]. Platelet activation is very complex. Consequently, different mechanisms are involved in activation. Among other things, platelet mitochondria may influence platelet activation and modify the platelet response to stimulation [5]. A lipid that is involved in the autocrine and paracrine activation of platelets is S1P. As described above, S1P is formed from Sph. Results from Tani et al. suggest a limited capacity for the de novo synthesis of sphingolipids within platelets, since platelets barely show serine palmitoyltransferase activity [6]. Platelet Sph, and in turn S1P, is derived from circulating SM through different reactions involving sphingomyelinase (SMase) and CDase, either in plasma or on the platelet plasma membrane [6]. In contrast to erythrocytes, which are devoid of SphK, the SphK activity is proportional to the number of platelets [7]. Here, the lipid is formed by the phosphorylation of Sph through SpK2, which has been reported as the quantitatively dominant isoform in platelets [8][9]. In addition, existing S1P can be dephosphorylated by an ectophosphatase, (possibly lipid phosphate phosphohydrolase, LPP) to Sph, which then can be incorporated into platelets and therefore infiltrated into the cycling pathway of S1P generation in platelets [10]. Since platelets lack the degrading enzyme SPL, platelets store large amounts of the lipid [11] and cause platelets to be the main source of locally elevated S1P levels after activation [12]. S1P is stored either in the platelet plasma membrane or, presumably, within granules. While S1P stored in the plasma membrane is more likely to be the metabolically active pool, the S1P stored in granules seems to function as the main source of secreted S1P after platelet activation and degranulation [13]. It is currently not fully clear whether storage pools or also, in part, as a result of elevated SphK activity upon platelet activation, e.g., by the translocation of SphK to the plasma membrane. This has been suggested recently for SphK1 in fibroblasts [14]. In platelets, SphK2 has been reported as the quantitatively relevant isoform, and the question of whether it is translocated to the plasma membrane upon platelet activation has not yet been resolved. Subsequent to its release, platelet-derived S1P binds in an autocrine manner to its receptors located on platelets or in a paracrine manner to vascular ECs and other blood cells [15].

2. Diabetes Mellitus

Diabetes mellitus describes a group of metabolic diseases that are associated with hyperglycemia. The two main categories are type 1 and type 2 diabetes.

Type 1 diabetes (T1D) is characterized by an autoimmune destruction of insulin-producing beta-cells within the pancreas by CD4+ and CD8+ T cells and by islet-infiltrating macrophages [16]. Pancreatic beta-cells show an imbalance in the enzymatic capacity of S1P formation by SphK1 and SphK2 and degradation by SPL [17]. T1D often includes average or increased HDL cholesterol (HDL-C) values. In patients with T1D, the apoM/S1P complex is shifted towards light HDL particles, which are increased in T1D. While apoM/S1P in dense HDL particles inhibited TNF-α-induced vascular cellular adhesion molecule-1 (VCAM1) expression, light HDL particles had no effect. These effects may foster the development of the increased cardiovascular disease risk associated with T1D evoked through apoM/S1P in dense HDL particles [18]. Conversely, studies also showed that the activation of S1PR1 in the diabetic vascular endothelium prevents monocyte/endothelial interactions and therefore functions in an anti-inflammatory manner [19].

Type 2 diabetes (T2D) is characterized by a relative insulin deficiency caused by pancreatic beta-cell dysfunction and insulin resistance in target organs [20]. Recent studies indicate a possible protective role of the SphK/S1P signaling pathway in T2D, supported by the observation that SphK activation improves the hepatic insulin signaling in obesity and diabetes [21]. Interestingly, the metabolism of S1P appears to modify insulin signaling in peripheral tissue. In particular, an adaptive role of S1P has been proposed to counteract the development of insulin resistance in muscle, adipose tissue, and the liver [22]. S1P promotes proliferation and reduces the apoptosis of pancreatic beta-cells. Specifically, the upregulation of S1PR expression results in a hypoglycemic effect by increasing the number of beta-cells and insulin levels [23]. In addition, physiological concentrations of extracellular S1P inhibit the cytokine-induced apoptosis of beta-cells, again pointing towards a potentially protective function of S1P in T2D [24]. In agreement with these findings, diabetic mice treated with S1P exhibited significant reductions in glucose tolerance, insulin resistance, and the number of apoptotic beta-cells compared with the untreated group [25]. There are also studies suggesting a causal role of S1P metabolism and signaling in insulin resistance in the liver and in adipose tissue [22]. S1P levels in plasma from T2D patients are significantly higher than in plasma from healthy individuals [26]. This might be due to the association of diabetes with platelet hyperreactivity, which is reflected in a relative loss of efficacy of antiplatelet agents [27][28]. Increased platelet sensitivity and the associated increased release of platelet-derived S1P at the vessel wall may also predispose T2D patients to an increased risk of thrombosis and the development of vascular lesions, well-known complications of T2D.

As elaborated above, there is evidence for an important impact of S1P in type 2 diabetes. However, the direct involvement of platelet-derived S1P during diabetes-associated complications, although very likely, has to the knowledge been addressed experimentally only sparsely. Interestingly, the study from Russo et al. [29] indicated that platelets from diabetic patients released less S1P than healthy platelets when mechanically or chemically stimulated in vitro. In addition, the cardioprotective effects of platelets from healthy individuals depend on the platelet's capacity to activate cardiac S1P receptors and the ERK/PI3K/PKC pathways. However, diabetic platelets release less S1P and lose their cardioprotective potential [29].

Metabolic syndrome (MetS) is a cluster of diseases and symptoms that can lead to diabetes. S1P may play multiple roles in the development of these diseases, mainly mediated by S1PR1 and S1PR3. Because S1P has both anti-inflammatory and pro-inflammatory effects and hypertensive versus hypotensive actions, its function remains controversial. In fact, S1P is linked to obesity [30]. Plasma S1P levels are positively correlated with body mass index (BMI), total body fat percentage, and waist circumference [31]. Studies have shown that S1P significantly decreases preadipocyte differentiation into adipocytes as well as the downregulation of adipogenic differentiation markers through multiple pathways [32]. Since inflammatory processes are also involved in MetS pathogenesis, S1P signaling pathways may contribute to the pathogenesis of metabolic diseases. S1P is associated with the upregulation of both pro-inflammatory and anti-inflammatory molecules. One possibility is that S1P increases the expression of pro-inflammatory factors in early stages. These reactions may lead to protective mechanisms and may help to mitigate the onset of obesity [33].

3. Vascular Lesions and Platelet-Derived S1P

Atherosclerosis is a chronic cardiovascular disease caused by lipid deposition at the vessel wall and is characterized by slowly progressing plaque formation and chronic inflammation of the arteries, which becomes clinically manifest when it triggers thrombosis [34]. Lipid deposition caused by endothelial injury, abnormal lipid metabolism, and hemodynamic changes in the susceptible sections of arteries develops into the formation of an atherosclerotic plaque [35]. In this process, ECs become activated and express inflammatory factors, attracting lymphocytes and monocytes to the endothelium. In turn, these adherent immune cells infiltrate the vessel wall, aggravating the inflammatory status of the vessels. Therefore, inflammation plays a central role in the atherosclerotic process [35].

Monocyte recruitment and adhesion to the vascular endothelium are key events in atherosclerosis. Platelets also adhere to ECs and contribute to the recruitment of leukocytes. The interaction between platelets and immune cells is triggered by the platelet production and secretion of inflammatory molecules, leading to activated immune cells being involved in the local vascular inflammation [36][37]. The activation of platelets results in increased local S1P concentrations. In this context, S1P affects lesion progression and thrombus formation in multiple ways. S1P acts synergistically with other factors and potentiates, for example, thrombin-induced tissue factor (TF) expression in ECs. Although it has not been proven directly, since S1P in solution, rather than platelets, was used experimentally, it is very likely that platelet-derived S1P leads to the propagation of thrombus formation at sites of EC injury[38]. However, as described earlier, pro- and anti-atherogenic effects of HDL-associated S1P have been reported. Hence, the influence of S1P on the development of atherosclerosis remains controversial. Depending on the S1PR subtype, the source of S1P (circulating within the plasma or locally released at sites of injury), the S1P concentration, and the affected target cell, S1P appears to exert both pro- and anti-atherogenic effects [39][40]. Recently, a preclinical study investigated the impact of fingolimod in rodent models of stroke, with age or atherosclerosis as comorbidities [41]. The authors found an improved post-ischemic outcome with fingolimod: fingolimod-treated hyperlipidemic mice showed a decreased infarct size but no difference in behavioral performance. They conclude that the effects of fingolimod in stroke are less robust than the existing literature might indicate and may depend on the inflammatory status of the animals [41].

In rabbits, a cholesterol-rich diet resulted in platelet hyperaggregability in response to low doses of agonists as well as in the development of hypercholesterolemic atherosclerosis. Under these conditions, the generation and release of S1P from platelets were significantly increased, pointing towards the involvement of platelet-derived S1P in the development and/or progression of cholesterol-stimulated lesion formation [42]. In addition, studies from Urtz et al. suggested that intrinsic S1P release through platelet Sphk2 controls platelet aggregation and thrombus growth in vivo. In a mouse arterial thrombosis model, they showed severely impaired thrombus stability in Sphk2 null mutants [9]. In contrast, other studies found no effect of the S1P analogue FTY720 on atherosclerosis in ApoE-deficient mice on a regular chow diet [43] or in only moderately hypercholesterolemic LDL-R-deficient mice [44]. Therefore, the effects of S1P in modulating vascular lesion formation may be pronounced and reveal themselves under conditions of elevated cholesterol levels. Further experimental settings in LDL-R-deficient mice demonstrated that FTY720 inhibits atherosclerosis by modulating lymphocyte and macrophage function. FTY720 at a high dose lowered the blood lymphocyte count and decreased the plasma concentrations of proinflammatory cytokines, pointing towards a general and rather unspecific immunosuppressive effect in these studies [45].

4. Multiple Sclerosis

MS is a currently untreatable degenerative neurological disorder associated with increased platelet activation and prothrombotic activity. MS is an inflammatory disease of the central nervous system (CNS) that leads to demyelination and neurodegeneration. The available data indicate the excessive intravascular activation of circulating platelets, implicating coagulation processes and inflammation in the pathophysiology of MS [46][47][48][49].

The proinflammatory activity of platelets is caused by various mechanisms such as the release of inflammatory molecules following platelet activation, as described above. As the lipid mediator S1P is released in bulk from activated platelets and also at a basal level from resting platelets [50][51], S1P is likely to contribute to such inflammatory mechanisms during MS. On the one hand, S1P signaling pathways regulate lymphocyte trafficking, which is a main event in MS. On the other hand, platelet-derived S1P, among others, might contribute to the cellular crosstalk mechanisms of activated platelets, leukocytes, and ECs. There are currently few data on the platelet-associated S1P effect on MS, and the subject needs to be evaluated in more detail.

Studies providing at least some information about platelet-associated S1P functions are experiments that were carried out as part of the studies of available MS therapies. One possible drug therapy for MS is fingolimod. Fingolimod was the first structural analog of sphingosine approved as a medication for the relapsing forms of multiple sclerosis [52][53]. Like Sph, it is phosphorylated by SphK2 to the bioactive form of the drug. Presumably, platelets are the major source of the active fingolimod (FTY720-P). In contrast to bulk S1P secretion, the release is independent of platelet activation [54]. After oral intake and conversion into its bioactive form, fingolimod acts as a modulator of S1PRs (mainly S1PR1), resulting in reduced infiltration and circulation of lymphocytes into the CNS. It has been observed that, after one month of treatment with fingolimod, the level of circulating platelets in both men and women significantly decreased [55]. In murine models of atherosclerosis, FTY720 inhibits atherosclerotic lesion formation and induces lymphopenia [56]. The observation that rats pretreated with FTY720 showed a dose-dependent increase in ADP-induced platelet aggregation compared to controls, however, is contradictory [57]. In humans, a double-blind placebo-controlled study evaluated the effects of fingolimod on platelet function in response to different stimuli ex vivo. Therefore, an increase in light transmission in platelet-rich plasma (PRP) in response to various concentrations of ADP, collagen, epinephrine, and ristocetin was utilized to measure the maximum platelet aggregation. Intriguingly, applied doses of 0.5 and 1.25 mg of fingolimod once daily over a period of one month in healthy volunteers did not affect the platelet function assessment in comparison to a placebo treatment [58]. While this does not point towards a critical impact of fingolimod on platelet functions in treated patients, the possible role of endogenous platelet-derived S1P in MS needs to be further deciphered.

There are also recent data linking the functions of the gut microbiome and alterations in mitochondrial function to MS. The permeability of the gut increases circulating lipopolysaccharides (LPS) through increasing toll-like receptor (TLR) activators. Therefore, nitric oxide synthase and superoxide are increased, which leads to peroxynitrite-driven increases in a-SMase and ceramide, which are associated with a decrease in the gut-microbiome-derived fatty acid butyrate. Butyrate is a histone deacetylase (HDAC) inhibitor that acts to regulate platelet and mitochondrial functions and suppresses the levels and actions of ceramides [59][60]. These circadian and gut-microbiome-derived

changes are important to note, as they impact immune cell as well as platelet functions, with relevance across most medical conditions. As such, the nature of platelet function may be coordinated with variations in immune cells, including from circadian and gut-microbiome-derived products [61].

5. Pharmacological implications and perspective

The importance of platelets goes well beyond their role in stopping acute bleeding. Platelets flow through all organs and provide a link between the blood and the immune system. Thus, platelets support the essential communication functions of immune cells within the immune system. More precisely, platelets affect inflammatory processes on or in the vascular wall, which can occur as part of acute injuries or chronic vascular diseases. A key property of platelets is their ability to form and release inflammatory mediators. S1P is such an inflammatory mediator, or rather a modulator of inflammatory processes, since S1P can trigger both pro-inflammatory and anti-inflammatory responses. Platelet-derived S1P most certainly has a significant impact on direct vascular processes and diseases such as atherosclerosis in which the vasculature has a direct influence. As already discussed in detail, there are unfortunately few studies on the effect of platelet-derived S1P in various diseases. It can be assumed that the S1P released from platelets plays a decisive role in the pathogenesis of diseases with increased platelet reactivity and therefore elevated immune cell involvement. In fact, this needs to be examined more closely in future studies.

Therefore, at present, the immunological properties of blood platelets and their mediators are not the focus of therapeutic approaches. This should be improved or changed in the future. In addition to inhibiting the hemostatic function of platelets to prevent thrombosis, blocking the paracrine release of proinflammatory and proaggregatory mediators such as S1P could lead to an improved therapy for various diseases such as cardiovascular or autoimmune diseases. This may be possible without a fundamental or additional increase in the risk of bleeding, as this is the case to date with the use of conventional inhibitors of platelet function. The development and exploration of new substances that aim to change the immunological functions of blood platelets can be a new therapeutic approach in this regard.

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