Sphingosine-1-phosphate receptors modulate platelet activation and thrombin formation

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Background: The bioactive lipid sphingosine-1-phosphate (S1P) is known as a modulator of various cell functions, including cell proliferation, migration, adhesion and survival. Hence, S1P plays a crucial role in both inflammatory and hematopoietic regulations. It is also known that S1P affects key processes of hemostasis, as platelets store and upon activation release large amounts of the phospholipid. S1P acts through binding to its five G-coupled receptors (S1PR1–5). Accumulating evidence suggests that S1P is involved in mechanisms of atherogenesis linking platelet activation and thrombus formation to inflammatory responses. However, the precise role of S1P and its receptors during platelet activation and hemostasis are still under debate.

Purpose: Until today, the effects of S1P in hemostasis are poorly understood. The aim of this study is to investigate the functions of specific agonists and antagonists for each of the S1PRs 1–5 on platelet aggregation and thrombin generation.

Methods: Blood was obtained from healthy volunteers after written informed consent. For both platelet aggregation studies using light transmission aggregometry (LTA) and thrombin generation measurements quantified by calibrated automated thrombography (CAT), platelet-rich-plasma (PRP) was used. PRP was generated by centrifugation from whole blood. PRP was incubated with either 10 µM S1PR-agonists or -antagonists for 15 minutes. Subsequently, platelets were activated with adenosine diphosphate (ADP) or collagen to evaluate potential synergistic or antagonistic effects.

Results: Platelet aggregation weakens after 2–3 hours. As described in the literature, this is presumably due to activation effects and partial release of platelet granules. Determined by LTA, we found that pre-incubation with the S1PR1-agonist CYM5442 maintains and even elevates ADP- but not collagen-induced platelet aggregation for up to 1.5 h. This effect was reversed by co-incubation with the S1PR1-antagonist Ex26. We only observed this findings for S1PR1. No effect on platelet aggregation was detected when PRP was incubated with S1PR1–5-agonists or -antagonists (10 µM) alone.

In comparison, none of the S1PR-agonists or -antagonists did directly affect thrombin formation in PRP measured by CAT. However, co-incubation with a S1PR4-agonist and ADP (5 µM) shortened the lag time to thrombin formation significantly. Furthermore, co-incubation with collagen (5 µg/mL) and agonists for S1PR1, 3 or 4 did reduce the time-to-peak (ttpeak) of thrombin formation.

Conclusion: Activation of S1PR1 maintains the ADP-induced platelet aggregation. In addition, co-activation of S1PR1, 3 or 4 and collagen enhances and accelerates thrombin generation. Consequently, S1P and its receptors appear to play an important role in different processes of hemostasis. Thus, S1PRs may be promising future therapeutic targets for diseases involving elevated platelet activation such as coronary heart disease or diabetes.

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