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REVIEW

Platelet secretion: From haemostasis to wound healing and beyond

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ABSTRACT

Upon activation, platelets secrete more than 300 active substances from their intracellular granules. Platelet dense granule components, such as ADP and polyphosphates, contribute to haemostasis and coagulation, but also play a role in cancer metastasis. α-Granules contain multiple cytokines, mitogens, pro- and anti-inflammatory factors and other bioactive molecules that are essential regulators in the complex microenvironment of the growing thrombus but also contribute to a number of disease processes. Our understanding of the molecular mechanisms of secretion and the genetic regulation of granule biogenesis still remains incomplete. In this review we summarise our current understanding of the roles of platelet secretion in health and disease, and discuss some of the hypotheses that may explain how platelets may control the release of its many secreted components in a context-specific manner, to allow platelets to play multiple roles in health and disease.

1. Introduction

Platelets have been known to contribute to thrombosis and haemostasis since their first identification by Bizzozero in the 1880s [1]. Recent evidence suggests that their functions extend beyond the immediate environment of the thrombus and platelets have been implicated in a number of other physiological responses aimed at safeguarding the integrity of the vessel. On the other hand, their properties as haemostatic and inflammatory cells can result in disease states under certain conditions. Platelets can 'communicate' with each other and with other cells via a range of bioactive substances secreted from their intracellular granules. In this review, the contribution of platelet secretion to those processes will be discussed.

2. Platelet activation cascade

Under physiological conditions, platelets circulate in close proximity to the vascular walls [2], but are protected from untimely activation by the healthy endothelial monolayer which provides a natural 'barrier' to thrombosis, as well as by the release of inhibitory mediators such as nitric oxide and PGI2 from the intact endothelium. Platelets become activated when the continuity of the endothelial layer is disrupted and the underlying subendothelial matrix is exposed, or if inflammation perturbs the endothelium. Platelet receptors then interact with collagen and von Willebrand Factor among others, which capture the platelets and induce activation signals. The initial events following platelet activation are summarised in Fig. 1.

After the initial 'platelet plug' is formed at the site of injury, engagement of the coagulation cascade leads to fibrin mesh formation that encapsulates and strengthens the thrombus. As well as serving as adhesion sites for coagulation factors (via surface exposure of phosphatidylserine, PS), platelets themselves are an important source of those factors (for example Factors V [3] and XIII [4,5]), and other components that regulate coagulation such as polyphosphates [6,7] and prothrombin [8].

Platelets can also become activated via G-protein coupled receptor (GPCR) signalling downstream from soluble agonists forming at the site of thrombus. Those soluble agonists, and in particular ADP released from dense granules of activated platelets, precipitate a number of positive feedback cascades leading to rapid activation of large numbers of platelets. Ultimately, an orchestrated 'effort' of many other secreted mediators and cells results in restoration of vessel integrity. Arguably, secretion is the most far reaching result of platelet activation, and as such can also account for functions of platelets beyond the immediate environment of the thrombus. Therefore platelet secretion is at the heart of the control of vascular integrity, in health and disease, and is the focus of this review.

3. Secretion in primary haemostasis

3.1. Dense granules

Dense granules contain a range of small molecules [9] such as ADP, ATP, GDP, 5-HT, pyrophosphate, magnesium and calcium. Historically release from dense granules was described as 'fast' and indeed a recent study by Jonnalagadda et al. [10] showed that the release of [^{3}H]-serotonin occurred more rapidly than PF-4 from α-granules or β-

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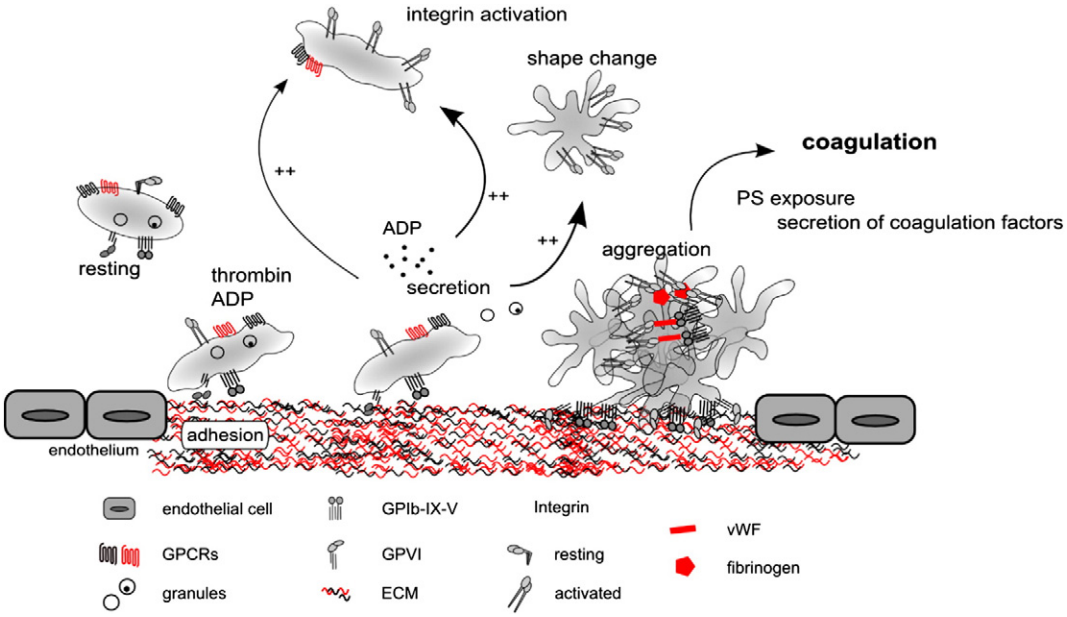

Fig. 1. Schematic of platelet activation cascade leading to the haemostatic plug formation. Platelet adhesion to ECM components via integrin or GPVI receptors or activation with soluble agonists via GPCR receptors leads to platelet activation. One of the hallmarks of platelet activation is secretion of bioactive molecules from dense and α-granules, which can then act to activate further platelets, as well as in an autocrine manner to drive positive feedback cascade. 'Inside-out' signalling initiated by platelet activation also causes activation of integrin αIIbβ3. Platelets also undergo a dramatic shape change, increasing their surface area available for adhesion to ECM and to one another. Activated integrin αIIbβ3 and fibrin contribute to formation of the initial aggregate or platelet plug. Platelets also expose PS providing attachment sites for coagulation factors. The coagulation cascade contributes to the stabilisation of the thrombus.

hexosaminidase from lysosomes, regardless of the agonist used to stimulate platelets [10].

Small molecules released from dense granules together with synthesised thromboxane A_2 act back on circulating platelets and contribute to the positive feedback signalling that sustains platelet aggregation. The central role of the ADP–$P2Y_{12}$ receptor axis in haemostasis is particularly well established. Platelet adhesion on vWF, inside-out activation of integrin $\alpha_{IIb}\beta_3$ and P-selectin expression are all defective in $P2Y_{12}^{-/-}$ mice [11]. Recently, chemically mutagenised mice lacking Munc13-4 (*Unc13D*^{jinx} mice) that show a complete absence of platelet dense granule secretion were generated. Those animals were also found to have significantly reduced aggregation and other markers of platelet activation such as α-granule and lysosome secretion and integrin activation [12], confirming that it is the secreted ADP rather than plasma ADP that contributes to those processes. They also failed to form thrombi in the in vivo and in vitro models of thrombosis [13]. Co-stimulation with exogenous ADP could partially rescue platelet function, underlining the essential role of ADP in driving the positive feedback loop and thus the platelet primary haemostatic response [13]. Interestingly, Munc13-4 deletion did not affect intracranial bleeding following stroke induction in mice, while at the same time they were protected from stroke progression [14]. This apparent paradox hints at different roles for ADP/$P2Y_{12}$ signalling in haemostasis and thrombosis, possibly even in different disease scenarios. The role of ADP emerges as more important and complex than initially thought and may account for some distinguishing features of thrombosis versus haemostasis. ADP/$P2Y_{12}$ mediated signalling also enhances signalling towards procoagulant activity and thrombin activation [15].

3.1.1. ADP and the 'core and shell' model of thrombus structure

Despite many different agonists shown to drive platelet activation in vitro to completion, it is now known that in vivo platelet activation is non-homogeneous, possibly attributable to variations in shear under physiological conditions [16,17]. α-Granule secretion and calcium mobilization have also been shown to be heterogeneous [18]. In a recent study, Stalker et al. suggested a new model of thrombus structure, taking into account that non-uniform activation pattern. They showed that two distinct populations of platelets are present in a growing thrombus in vivo: a 'core' of more stable P-selectin expressing platelets, and a more porous 'shell', containing less activated, P-selectin negative platelets [19]. While the 'core' seemed to be more dependent upon thrombin and local contact-dependent activation, the recruitment of platelets to the 'shell' was shown to be critically dependent upon ADP signalling, with $P2Y_{12}$ inhibition significantly decreasing the size of the 'shell' and not affecting the 'core'. They also show that permeability of the 'core' to plasma-borne molecules is limited, suggesting that efflux of platelet granule contents in that tightly packed region could be similarly limited [19,20].

The most effective anti-thrombotic treatments currently used specifically target the ADP/$P2Y_{12}$ positive feedback cascade, blocking the $P2Y_{12}$ receptors and thus limiting platelet activation and aggregation and the risks of pathological thrombosis, particularly in the management of coronary heart disease [21]. However, the central role of the ADP/$P2Y_{12}$ axis in haemostasis means that abnormal bleeding often occurs in patients treated with potent $P2Y_{12}$ inhibitors such as clopidogrel, prasugrel or ticagrelor [22]. On the other hand, the new core and shell model could account for the ability of the same agents to reduce platelet accumulation without always causing bleeding, as they would not affect the initial platelet adhesion or the size of the 'core' [19]. It is possible that in humans, interindividual variations in platelet activity account for those differences. Increasing our understanding of ADP/$P2Y_{12}$ signalling may help in discovering more selective platelet inhibitors that could specifically limit thrombosis without causing bleeding.

3.1.2. Polyphosphate in coagulation

Dense granule secretion is also important in coagulation. Released calcium for instance is required at several steps of the coagulation cascade and for activation of the prothrombinase pathway. Recently more insight was gained into the role of another dense granule cargo molecule, polyphosphate (polyP). PolyP is a highly anionic linear polymer that is synthesised from ATP and secreted by platelets after activation [23]. Synthetic polyP could restore defective clot formation in platelet-rich plasma from Hermansky–Pudlak patients who lack dense granules [24], suggesting a role in haemostasis. The putative mechanism involves direct polyP binding to Factor XII, thus triggering coagulation by the tissue factor-independent, contact activation pathway [24,25].