

Platelet Purinergic Receptors in Thrombosis and Inflammation

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Abstract

It took approximately 40 years from the seminal identification of adenosine diphosphate (ADP) as the factor R, an agent derived from red blood cells inducing platelet adhesion to glass, to the completion of the repertoire of its receptors on platelets and its importance in haemostasis and thrombosis. ADP, either derived from red blood cells or released by platelets themselves, stimulates platelets via two G protein-coupled receptors, P2Y₁ and P2Y₁₂. In addition, adenosine triphosphate, also contained in the platelet dense granules, activates the P2X₁ cation channel. Each of these receptors plays a specific role during platelet activation and aggregation, with relevance to haemostasis, thrombosis and various inflammatory processes where platelets are involved including chronic responses such as atherosclerosis or acute responses such as sepsis, endotoxaemia or allergic asthma. Finally, platelets also express P2Y₁₄, a receptor activated by released uridine diphosphate glucose. Although devoid of any known role in haemostasis, this receptor seems to play a specific role in neutrophil chemotaxis.

Keywords

- ▶ nucleotides
- ▶ platelets
- ▶ thrombosis
- ▶ inflammation
- ▶ atherosclerosis

Introduction

Sixty years ago now, adenosine diphosphate (ADP) was identified as a factor released from erythrocytes which influenced platelet adhesiveness to glass¹ and induced platelet aggregation.² The crucial role of ADP as a mediator of platelet activation was rapidly recognized in the physiological process of haemostasis and in the development and extension of arterial thrombosis.³

ADP and adenosine triphosphate (ATP) are stored at a very high concentration in platelet dense granules and released upon activation of platelets by strong agonists such as thrombin and/or collagen. Released ADP is an essential secondary agonist, which amplifies most of the platelet responses and contributes to the stabilisation of the thrombus.⁴ Platelet stimulation by ADP leads to a transient increase in free cytoplasmic Ca²⁺ and inhibition of adenylyl cyclase activity. In addition, ATP induces an extremely rapid influx of

Ca²⁺ from the extracellular medium associated to platelet shape change. We know for years now that three different P2 receptors mediate these effects of adenine nucleotides on platelets: two G protein-coupled receptors stimulated by ADP, P2Y₁ and P2Y₁₂, and the P2X₁ cation channel activated by ATP. Each of these receptors plays a specific role during platelet activation and aggregation, with relevance to haemostasis, thrombosis and various inflammatory processes where platelets are involved including chronic responses such as atherosclerosis or acute responses such as sepsis, endotoxaemia or allergic asthma. Platelets also express the P2Y₁₄ receptor activated by uridine diphosphate (UDP) and UDP-glucose but its role, if any, remains elusive.⁵ The role of the P2 receptors in platelet activation, thrombus formation and inflammation has been presented and discussed in many reviews by us and by others that we cannot cite all for reasons of space. In this short overview, we will only briefly highlight several key points.

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The Platelet P2Y₁ Receptor

The P2Y₁ receptor is broadly expressed in many cells and tissues. Its presence and role in platelets were established by the detection of mRNA in megakaryoblastic cell lines and by pharmacological studies using selective P2Y₁ antagonists.⁶ ADP is the preferred natural agonist of the P2Y₁ receptor, while ATP behaves as an antagonist in platelets⁷ or as a poor partial agonist in heterologous transfected or reconstituted systems, depending on the receptor density.⁸ The P2Y₁ receptor is coupled to G_q and Ca²⁺ signalling. The changes in cytosolic Ca²⁺ concentration support the activation of the calcium and diacylglycerol-regulated guanine-nucleotide exchange factor 1 (CalDAG-GEFI), resulting in rapid and reversible activation of the small RAP1 GTPase and integrin α IIb β 3, responsible for platelet shape change and weak and transient aggregation in response to ADP.⁹ Platelets express approximately 150 P2Y₁ receptors per cell,¹⁰ which is very low and probably explains why the signal induced by P2Y₁ activation is weak as compared with the signals of other G_q-coupled receptors. To date, no P2Y₁-deficient patients have been identified.

The Platelet P2Y₁₂ Receptor

The P2Y₁₂ receptor, cloned in 2001,^{11,12} is responsible for completion of the platelet aggregation initiated by the P2Y₁ receptor in response to ADP,¹³ as well as for the role played by ADP in the amplification of aggregation, secretion and stabilisation of platelet aggregates induced by strong agonists such as thrombin, collagen or TxA₂.^{4,14} It is activated by ADP, while ATP and a wide range of its triphosphate analogues behave as antagonists.¹⁵ Platelets express approximately 400 copies of the P2Y₁₂ receptor per cell. Owing its central role in the amplification of platelet responses to any stimulus, it is the molecular target of potent antiplatelet drugs including the thienopyridine compounds clopidogrel and prasugrel, which are prodrugs, and the direct antagonists ticagrelor and cangrelor.^{16,17} The tissue distribution of this receptor was long thought to be restricted to platelets and sub-regions of the brain. Further studies later revealed its expression and functions in microglial cells, vascular smooth muscle cells (VSMCs), dendritic cells (DCs), macrophages and as yet unspecified leukocytes and also bladder smooth muscle cells.^{18–20} In terms of signalling, the P2Y₁₂ receptor activates a G_{ai2} G-protein subtype, responsible for the activation of two phosphoinositide 3-kinase (PI3-K) isoforms (PI3-K p110 β and p110 γ) that regulate α IIb β 3 activation via activation of the serine-threonine protein kinase B/Akt (PKB/Akt) and the small GTPase RAP1 and the downward regulation of the RAP1 RAP-GAP RASA3 inhibitor.^{21,22} G_{ai2} also inhibits platelet adenylyl cyclase, which facilitates platelet activation.

Several patients have been identified with congenital P2Y₁₂ receptor defects associated with quantitative or qualitative abnormalities of the receptor and responsible for deficiencies of ADP-induced platelet activation, leading to mild to severe bleeding diathesis which underscores the importance of this receptor in haemostasis.^{16,23}

The Platelet P2X₁ Receptor

The third member of the platelet P2 receptor panoply is the ligand-gated cation channel P2X₁, responsible for a fast Ca²⁺ entry induced by ATP. Pharmacological studies using specific P2X₁ ligands and P2X₁^{-/-} mice have shown that this receptor triggers transient shape change without causing platelet aggregation in response to ATP²⁴ and participates in collagen- and shear-induced aggregation. No P2X₁-deficient patients have been clearly identified to date. A mutation in the P2X₁ sequence has been reported in a patient with a severe bleeding disorder, but without firm demonstration of a causal link between the mutation and the bleeding tendency.^{25,26}

The Platelet P2 Receptors in Thrombosis

So far, only the P2Y₁₂ receptor is an established target for antithrombotic drugs in clinical use. The P2Y₁ and P2X₁ receptors are also involved in experimental thrombosis and are at a preclinical stage of evaluation as potential targets for new antiplatelet agents.

The Platelet P2Y₁₂ Receptor in Thrombosis

The cornerstone of treatment of ischemic coronary syndromes is the dual antiplatelet therapy using aspirin and P2Y₁₂-targeting drugs.¹⁷ Indeed, the central role of the P2Y₁₂ receptor in platelet activation and the growth and stabilisation of a thrombus makes it a very important molecular target for antithrombotic agents.^{16,18} It is so far the only P2 receptor subtype to be an established target for antiplatelet drugs in clinical use and the major target to treat all kinds of arterial ischemic diseases. Active metabolites of the orally administered thienopyridine prodrugs (clopidogrel and prasugrel) covalently bind to the P2Y₁₂ receptor while the direct-acting P2Y₁₂ antagonists, ticagrelor, which is an oral antagonist, and cangrelor, an intravenous direct antagonist, reversibly bind the receptor and inhibit ADP binding. All these drugs are under clinical use for the treatment and prevention of thrombotic events in acute coronary syndromes. However, one limitation of targeting the P2Y₁₂ receptor, whatever the drug, relates to the bleeding risk, which increases with the degree of inhibition of P2Y₁₂-dependent platelet functions.²⁷ The crystal structure of P2Y₁₂ revealed how this receptor behaves when it binds agonists and antagonists. This should provide valuable insights into the development of improved P2Y₁₂ antagonists.^{28,29}

The Platelet P2Y₁ Receptor in Thrombosis

Although the P2Y₁ receptor plays only a modest role in platelet activation, it plays a central role in experimental arterial thrombosis. This was shown using P2Y₁^{-/-} mice in various models including systemic thromboembolism either induced by infusion of a mixture of collagen and adrenaline^{30,31} or tissue factor,³² or localised thrombosis after ferric chloride- or laser-induced injury of mesenteric arteries.³³ Among selective P2Y₁ receptor antagonists, MRS2500 has so far the highest affinity for P2Y₁ and is able to inhibit thrombosis in treated

animals.³⁴ However, the limited bioavailability of the compound prevents its use in long-term treatment, pointing to the need of developing novel P2Y₁ receptor antagonists with an improved pharmacokinetic profile. The resolution of the X-ray crystal structure of the P2Y₁ receptor³⁵ should open up new possibilities in this field. Of note, inhibition of the P2Y₁ receptor results in a moderate prolongation of the bleeding time in mice, which is a definite safety advantage over inhibition of the P2Y₁₂ receptor. However, so far, no candidate drug has really emerged from any pharmaceutical company involved in antithrombotic drug development.

The Platelet P2X₁ Receptor in Thrombosis

The generation of P2X₁^{-/-} mice helped to reveal the important role of this receptor in arterial thrombosis. P2X₁^{-/-} mice display resistance to systemic thromboembolism induced by injection of a mixture of collagen and adrenaline and to localised arterial thrombosis triggered by laser-induced injury of mesenteric arteries.²⁴ These mice display also resistance to tissue factor-induced systemic thromboembolism, i.e., in a thrombin-dependent system (unpublished data). Interestingly, P2X₁^{-/-} mice have no increase in their bleeding time compared with wild-type animals, indicating that they display normal haemostasis.²⁴ This receptor is therefore a potential new target for safe antiplatelet agents. However, due to the limited bioavailability and selectivity of currently existing P2X₁ antagonists, the development of novel selective P2X₁ antagonists is warranted for the preclinical evaluation of this receptor as a target for novel antiplatelet agents.

The Platelet P2 Receptors in Inflammation

Platelets play an important role in modulating inflammatory responses through the release of inflammatory mediators or

compounds with trophic activity and exposure of P-selectin, CD40 and CD40 ligand (CD40L). These molecules allow interaction of platelets with leukocytes and their subsequent activation with the release of a range of inflammatory cytokines and exposure of tissue factor.³⁶ Therefore, in addition to acting as antithrombotic drugs, antagonists and inhibitors of the platelet P2 receptors have anti-inflammatory effects, which might be relevant to various diseases such as atherosclerosis, restenosis, endotoxaemia and sepsis, or allergic asthma. In addition, besides platelets, P2Y₁, P2Y₁₂ and P2X₁ receptors, expressed by cells of the immune system and by vascular cells, are also directly involved in the modulation of inflammation and in immune responses during thrombus formation, independently of platelet-related processes³⁷ (→Table 1).

The P2Y₁₂ Receptor

Studies in mice or patients receiving clopidogrel have highlighted the contribution of the platelet P2Y₁₂ receptor to P-selectin and CD40L exposure, formation of platelet-leukocyte aggregates, tissue factor exposure and release of various inflammatory mediators (tumour necrosis factor- α , C-reactive protein).³⁸ These inflammatory events might be relevant for the role played by the platelet P2Y₁₂ receptor in restenosis,³⁹ atherosclerosis^{40,41} and transplant atherosclerosis. An additional contribution from the P2Y₁₂ receptor of VSMCs and leukocytes, including DCs, possibly through P2Y₁₂-dependent migration properties has been suggested.^{42,43} In the context of allergic asthma, in which platelet activation is required for the recruitment of inflammatory cells to the lungs and remodeling of the airway wall, divergent results have been reported concerning the role of the platelet P2Y₁₂ receptor. The pro-asthmatic action of leukotriene LTE₄ in mice could require the P2Y₁₂ receptor through a mechanism yet to be identified.⁴⁴ Further evidence of the involvement of the P2Y₁₂ platelet

Table 1 Role of the P2Y₁, P2Y₁₂, P2X₁ and P2Y₁₄ receptors in inflammatory processes and related diseases

Inflammatory disease		Cell type	Reference
Atherosclerosis	P2Y ₁₂	Platelets	Li et al (2012) ⁴⁰ ; West et al (2014) ⁴¹
		VSMCs	Li et al (2012) ⁴⁰ ; West et al (2014) ⁴¹
	P2Y ₁	Endothelial cells	Hechler et al (2008) ⁵¹ ; Zerr et al (2011) ⁵⁰
Restenosis	P2Y ₁₂	Platelets	Evans et al (2009) ³¹
Transplant atherosclerosis	P2Y ₁₂	VSMCs	Abele et al (2009) ⁴² ; Harada et al (2011) ⁴³
		Leukocytes	Abele et al (2009) ⁴² ; Harada et al (2011) ⁴³
Intimal hyperplasia	P2Y ₁	Platelets	Liu et al (2015) ⁵²
		Macrophages	Liu et al (2015) ⁵² ; Marques-da-Silva et al (2011) ⁵³
Endotoxaemia/sepsis	P2Y ₁₂	Platelets	Hagiwara et al (2011) ⁴⁷ ; Liverani et al (2016) ⁴⁸
	P2X ₁	Neutrophils	Lecut et al (2012) ⁵⁶ ; Maître et al (2015) ⁵⁷
	P2Y ₁₄	Platelets	Sesma et al (2016) ⁵⁹ ; Amison et al (2017) ⁶⁰
Transfusion-related acute lung injury	P2X ₁	Monocytes/macrophages	El Mdawar et al (2019) ⁵⁸
Asthma	P2Y ₁₂	Platelets	Paruchuri et al (2009) ⁴⁴ ; Cattaneo (2015) ⁴⁵
	P2Y ₁	Platelets	Amison et al (2015) ⁴⁶

Abbreviation: VSMCs, vascular smooth muscle cells.

receptor in asthma is provided by clinical observations of a large family-based asthma cohort, in which P2Y₁₂ receptor variants were associated with altered lung function, while prasugrel tended to decrease the bronchial hyper-reactivity to mannitol in patients with allergic asthma.⁴⁵ In contrast, Amison et al reported that the platelet P2Y₁₂ receptor is dispensable in allergic inflammation in ovalbumin-challenged mice.⁴⁶ The platelet P2Y₁₂ receptor contributes also to the deleterious pro-inflammatory role of platelets in enhancing the pulmonary neutrophil infiltration and exacerbating tissue damage during lipopolysaccharide (LPS)-induced endotoxemia.⁴⁷ Concerning the potential role of the platelet P2Y₁₂ receptor in sepsis, here also, contradictory results have been reported. On the one hand, Liverani et al reported a central role of P2Y₁₂,⁴⁸ while on the other hand our group reported that inhibition of the P2Y₁₂ receptor with clopidogrel or platelet P2Y₁₂ receptor deficiency (unpublished data) does not protect mice from septic shock. Further studies are required to clarify all these discrepancies, most probably related to differences in models, administered drugs and/or the use of receptor-deficient mice, to elucidate the contribution of the P2Y₁₂ receptor in various settings. The perspective could be to extend the use of P2Y₁₂ targeting drugs beyond their current indications.

The P2Y₁ Receptor

The platelet P2Y₁ receptor contributes to platelet P-selectin exposure and the formation of platelet-leukocyte conjugates resulting in leukocyte activation and tissue factor exposure.⁴⁹ These P2Y₁ receptor-mediated events might be relevant for the platelet-dependent recruitment of leukocytes to the lung tissue in the case of inflammation of the airways in allergic mice,⁴⁶ although the contribution of the P2Y₁ receptors from other cell types such as leukocytes or endothelial cells cannot be excluded. Indeed, in an experimental model of acute vascular inflammation, the endothelial P2Y₁ receptor plays an important role in the upregulation of adhesion molecules (P-selectin, VCAM-1, ICAM-1 and the recruitment of leukocytes at the vascular wall).⁵⁰ The endothelial P2Y₁ receptor also contributes to development of atherosclerosis, a chronic inflammatory process, in ApoE^{-/-} mice.⁵¹ The P2Y₁ receptor has also been shown to be present on VSMCs, where it contributes to their proliferation and migration in vitro. This could be relevant for the intimal hyperplasia observed in a vein graft model in mice.⁵² In this case, a contribution from the P2Y₁ receptors present on platelets and macrophages cannot be excluded. Macrophages would indeed appear to be required, as their depletion abrogated hyperplasia,⁵² while data indicated a role of the P2Y₁ receptor in the phagocytic and migration activity of macrophages.⁵³ Altogether, these pieces of evidence suggest that the P2Y₁ receptor could represent an attractive and original target for drugs with multiple sites of action, to treat atherothrombosis and, possibly, other inflammatory diseases. Again, the main problem is the lack of good drug candidates to further evaluate this receptor as a potential target for new drug candidates.

The P2X₁ Receptor

The platelet P2X₁ receptor, along with the neutrophil P2X₁ receptor, may contribute to thrombus formation in a context of inflammation as highlighted in a specific model of neutrophil-dependent thrombosis of cremasteric arterioles triggered by laser injury. The neutrophil P2X₁ receptor appeared essential for their recruitment at the site of vessel injury and subsequent fibrin production and thrombus formation.⁵⁴ Very recently, a role of the platelet P2X₁ receptor in the enhancement of FcγRIIIa-induced Ca²⁺ increases and functional responses has been reported, which may be relevant for in vivo platelet-dependent immune responses.⁵⁵ The P2X₁ receptor from neutrophils plays also an important part in facilitating the neutrophil chemotaxis induced by various chemoattractants, possibly by favouring contraction and retraction of the trailing uropod.⁵⁶ Accordingly, the P2X₁ receptor from neutrophil, or from monocyte/macrophage, displays an important role in inflammatory diseases leading to organ damage, such as endotoxemia⁵⁷ or transfusion-related acute lung injury (TRALI).⁵⁸ Thus, similarly to P2Y₁, the P2X₁ receptor could constitute an attractive target for new therapeutics not only in thrombosis but also in inflammatory processes. Here also, the lack of potent and selective compounds with favourable bioavailability hampers the further evaluation of the potential of this receptor as a valuable target for new drugs.

The P2Y₁₄ Receptor

As already mentioned, platelets express the P2Y₁₄ receptor but its function in platelet physiology is unknown and no modulation of thrombosis has been detected using P2Y₁₄^{-/-} mice in various models of arterial thrombosis (unpublished data). However, using P2Y₁₄ antagonists, separate studies have reported a role of this receptor in leukocyte recruitment in vitro and in vivo. Pulmonary neutrophil recruitment induced by intranasal LPS administration and LPS-induced thrombocytopenia were inhibited in mice administered with a P2Y₁₄ antagonist.^{59,60} In addition, the stimulation of platelets with selective P2Y₁₄ agonists (UDP-glucose, MRS2690) resulted in significant platelet-dependent neutrophil chemotaxis toward macrophage-derived chemokine (CCL22), in vitro. Similarly, the P2Y₁ receptor seems to play a specific role in platelet-dependent neutrophil chemotaxis.⁶⁰

Conclusions

Each of the platelet receptors for extracellular adenine nucleotides plays a specific role in platelet functions and arterial thrombosis. Each of these receptors also contributes to the development of various processes related to acute or chronic inflammation. The respective contributions of the platelet receptors and those of other cell types are not yet well unravelled. Undoubtedly, the generation of mice with a specific tissue deletion of these P2 receptor subtypes will help to define their specific functions under various pathological conditions. In addition, the development of novel P2X₁ and

P2Y₁ receptor antagonists with an improved pharmacokinetic profile should clearly open up new possibilities in this field. The perspective is that these P2 receptors could also represent potential therapeutic targets for the treatment of inflammatory diseases.

Disclosures

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

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