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Atherosclerosis, platelets and thrombosis in acute ischaemic heart disease

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Abstract

Atherosclerosis is the underlying reason for nearly all causes of coronary artery disease and peripheral arterial disease and many cases of stroke. Atherosclerosis is a systemic inflammatory process characterised by the accumulation of lipids and macrophages/lymphocytes within the intima of large arteries. The deposition of these blood borne materials and the subsequent thickening of the wall often significantly compromise the residual lumen leading to ischaemic events distal to the arterial stenosis. However, these initial fatty streak lesions may also evolve into vulnerable plaques susceptible to rupture or erosion. Plaque disruption initiates both platelet adhesion and aggregation on the exposed vascular surface and the activation of the clotting cascade leading to the so-called atherothrombotic process. Yet, platelets have also been shown to be transporters of regulatory molecules (micro-RNA), to drive the inflammatory response and mediate atherosclerosis progression. Here we discuss our current understanding of the pathophysiological mechanisms involved in atherogenesis – from fatty streaks to complex and vulnerable atheromas – and highlight the molecular machinery used by platelets to regulate the atherogenic process, thrombosis and its clinical implications.

Keywords

Atherosclerosis, platelets, thrombosis, vulnerable atherosclerotic plaque

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Atherosclerosis

Atherosclerotic lesions result from a complex interplay between circulating factors and various cell types in the vessel wall, triggered by chronic and repeated exposure to several systemic and local injurious stimuli. A high level of plasma lipids, particularly low-density lipoproteins (LDL), is a major cause of vascular damage. Apart from epidemiological evidence for the pro-atherogenic role of lipoproteins, mechanistic studies suggest that they play a role in relevant features for initiation and progression of lesions, such as endothelial dysfunction, intimal disorganisation and thickening. In advanced atheromatous plaques, high extra- and intracellular lipid deposits are associated with a high risk of vulnerability to rupture, causing thrombosis and its clinical complications.[1,2]

Inititial changes in the pathogenesis of atherosclerosis

LDL infiltration, retention and modification. Sustained high plasma levels of LDL cholesterol is thought to be the major determinant for the entry and retention of LDL particles within the subendothelial layer.[3] Yet, other features such as lipoprotein size, cholesterol enrichment, endothelial permeability and endothelial cell-derived biosynthetic activity (i.e. synthesis of the basement membrane and extracellular matrix) also affect LDL entrance and retention.[4] Once LDL enters the intimal space, several specific regions of the apoB-fraction interact with extracellular proteoglycans, especially with those that contain side chains of chondroitin sulphate such as versican or biglycan. LDL retention within the intimal layer may also occur, although to a lesser extent, via lipoprotein association with other matrix molecules (such as lipoprotein lipase, sphingomyelinase, and phospholipase A_2),

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collagen and/or elastin.[3] Once sequestered in this intimal microenvironment, LDL particles become susceptible to modifications including aggregation/fusion, oxidation (via lipoxygenase, myeloperoxidase, free radicals, etc), enzymatic cleavage (via proteolytic, lipolytic and hydrolytic enzymes) and incorporation in immune complexes rendering LDL particles pro-atherogenic.[5–7]

LDLs drive leukocyte recruitment, transmigration and differentiation. Modified LDL particles induce endothelial secretion of chemotactic substances and the expression of adhesion receptors, including integrins and selectins, which favour leukocyte (monocyte and lymphocyte) recruitment, adhesion and transmigration into the arterial wall. Transmigration of monocytes preferably occurs in areas where the subendothelial layer is enriched with modified LDL particles and takes place mainly through the junctions between endothelial cells.[8] Junction adhesion molecule (JAM)-A and -C have been shown to be involved in the control of vascular permeability and leukocyte transmigration across endothelial-cell surfaces.[9] Interestingly, recent evidence supports that high LDL-cholesterol levels selectively recruit distinct monocyte and T-cell subsets into the atherosclerotic lesion.[10,11] Once monocytes reach the intimal space, colony–stimulating factor induces monocytes to phenotypically transform into macrophages and express scavenger receptors, which uptake many of the cholesterol molecules and cholesterol esters contained in modified LDL particles, becoming foam cells – a characteristic cell constituent of atherosclerotic lesions.[12] Scavenger receptor class A (SRA)-I and SRA-II, CD36, LOX-1, or CXCL16 are involved in oxidised LDL internalisation,[13] whereas we have demonstrated that LRP-1 (low-density lipoprotein receptor related protein-1) is mainly involved in the internalisation of aggregated LDLs, in a process regulated by SREBP1 and SREBP2.[14] Additionally, LRP5 – a receptor that links Wnt signalling and migration of mononuclear cells – is also upregulated by agLDL.[15] Once converted, macrophage-derived foam cells release cytokines, growth factors, metalloproteinases (MMP), reactive oxygen species (ROS) and tissue factor[16] perpetuating the inflammatory response, inducing vascular remodelling and increasing plaque susceptibility to rupture and subsequent thrombus formation.

Vascular remodelling. As atherosclerosis evolves, the presence of LDL and atherogenic cytokines stimulates vascular smooth muscle cells (VSMCs) to alter extracellular matrix (ECM) composition leading to vascular remodelling.[7,17] Under physiological conditions, VSMCs in the media are known to produce most of the main components of the ECM found in the arterial intima (proteoglycans, collagen and elastin) as well as a large number of enzymes responsible for the equilibrium between ECM synthesis (lysyl oxidase) and degradation (MMP, plasminogen activators). However, under the effect of atherogenic stimuli, VSMCs undergo phenotypic changes switching from a non-proliferative contractile phenotype (typical in healthy arteries) into an actively proliferative cell (synthetic phenotype) with the capacity to migrate and increase ECM synthesis. In fact, migration of VSMCs from the vascular media to the vascular intima is a key process in intimal thickening and vascular remodelling. Circulating bone marrow progenitor cells and progenitor cells present in the vessel adventitia may also be a potential source of VSMCs in the intima.[18] Once in the intimal layer, VSMC express a variety of receptors for cholesterol uptake thereby participating in the early lipid accumulation process in the atherosclerotic plaque. These include different members of the LDL-receptor family (LDL-R, LRP, VLDL-R) and the scavenger receptor family (CD36, type I and type II scavenger receptors, CXCL16).[11] In our group, we have reported that in the presence of proteoglycan-induced LDL aggregates (agLDL), VSMCs over-express receptors such as LRP-1, which not only facilitates LDL internalisation and the subsequent transformation of VSMC into foam cells, but also acts as a receptor to many other ligands and participates in signalling processes.[6,14,19,20] Lipid-rich VSMCs show significantly lower migration and repair capacity[21,22] rendering plaques less able to be populated by VSMCs and therefore, more susceptible to rupture. Indeed, whereas VSMCs account for 90–95% of the cell component in initial lesions, this proportion decreases to 50% in advanced atherosclerotic lesions making those plaques more vulnerable to rupture. Indeed, unstable plaques contain a substantial lipid core, little collagen and a small number of VSMCs. We have recently shown, by proteomic approaches, that atherogenic concentrations of LDL particles affect the expression and phenotypic profile of different cytoskeleton and ER-stress proteins of the VSMCs involved in migration and survival thereby mediating the instability and vulnerability of plaques in advanced stages.[22,23]

From fatty streaks to vulnerable plaques

Even in the presence of extensive coronary atherosclerosis, rarely more than a few plaques appear to be at risk of rupture at any given moment. Yet, when ruptured, these plaques precipitate approximately 75% of all fatal coronary thrombi. The risk of suffering a thrombotic complication depends more on the biochemical and cell composition of the lesions rather than their stenotic severity. Pathological studies performed on patients dying from cardiovascular events have shown the existence of an acute thrombus anchored on the disrupted areas of atherosclerotic lesions in the majority of the patients. The same evidence has allowed to associate certain plaque features with plaque vulnerability. Indeed, autopsy studies,[24] atherectomy specimens of coronary origin,[25] endarterectomy specimens of carotid origin[26] and intravascular imaging with optical coherence tomography[27] have provided information about ruptured plaques.

However, all these techniques have the same limitation: they provide data on the structure and components of ruptured plaques and only by extrapolation do we learn about the features of rupture-prone plaques. Undoubtedly, a useful animal model, in which the mechanisms leading to spontaneous plaque rupture could be studied prospectively, would overcome some of these problems, but such a model is not yet available.[28,29] Nonetheless, the human anatomopathological studies have revealed, so far, that the main features characterising plaques as 'vulnerable' include: (a) a large necrotic lipid core; (b) a thin fibrous cap; (c) increased inflammation in the fibrous cap; (d) reduced collagen and VSMCs amount; and (e) neovascularisation.[30,31]

Vulnerable plaque 'phenotype'

The lipid-rich core. The formation of a lipid-rich core is the essential mechanism in the development of the rupture-prone plaque. It has been suggested that the lipid-rich core is the result of smaller pools of accumulated lipid in the intima combined to a larger lipid pool, which becomes acellular due to apoptosis and necrosis of VSMC and macrophage foam cells.[32–34] Therefore, the lipid-rich atheromatous core is hypocellular, totally devoid of supporting collagen and presents high-free cholesterol content in the centre with a low-free to esterified cholesterol ratio at the edges, possibly because of macrophage breakdown and active inflammation.[35] The lipid core also contains pro-thrombotic oxidised lipids and is impregnated with TF derived from macrophage- and VSMC-derived foam cells making it highly thrombogenic when exposed to flowing blood.[36] Studies conducted to evaluate the relative thrombogenicity of the various components of atherosclerotic plaques have demonstrated that the lipid-rich nucleus is up to six times more thrombogenic than all other components.[37] Moreover, inhibition of TF by local administration of TF- pathway inhibitor (TFPI) effectively reduces arterial thrombosis in atherosclerotic lesions.[36] Besides, LDL-laden foam cells have also shown to release TF increasing the susceptibility of the plaque to thrombus formation. In this regard, we have reported that the interaction between LRP-1 and LDL aggregates is one of the mechanisms that induce VSMC TF expression and the release of microparticles enriched in active TF to the ECM.[38,39]

Thin fibrous cap. The fibrous cap is the connective tissue layer covering the lipid-rich core. It consists of VSMCs and the ECM they synthesise (mainly collagen and proteoglycans). The cap also contains inflammatory cells, predominantly macrophage foam cells.[40] Vulnerable plaques tend to have thin fibrous caps and the integrity of this fibrous cap depends, at least in part, on the constituents of the ECM.[41] Therefore, the balance between collagen synthesis by VSMCs (synthetic phenotype) and breakdown of collagen fibrils by MMPs, collagenases, membrane-type MMPs, gelatinases and stromelysins plays a key role in fibrous cap stability. Several lines of evidence suggest that inflammatory cytokines are responsible for this balance since these inflammatory mediators not only induce endothelial cells, macrophages and VSMC apoptosis but markedly enhance MMPs' expression and activity in these cells.[42,43] Moreover, death of these cells may cause the continuous release of certain MMPs that may be particularly active in destabilising plaques and thus predispose them to rupture.[44,45] In fact, quantification of certain MMPs and their inhibitors in blood has been correlated with the degree of atherogenesis in humans.[46] VSMCs apoptosis may also be involved in plaque destabilisation by decreasing the number of collagen-synthesising cells within the atherosclerotic lesion.[47]

Inflammation. The core of the rupture-prone lipid-rich plaque is essentially hypocellular with little inflammation. In contrast to the plaque core, the ruptured fibrous caps have been found to be heavily inflamed (26% and 17% macrophage density in the coronary artery and aorta, respectively).[24,48] Accordingly, it is not diffuse inflammation that characterises ruptured plaques but the heavy inflammation of the fibrous cap, specifically, at the shoulders.

Calcification. Calcium deposits in the vascular wall occur through all the atherogenic processes, initially as small aggregates, and later as large nodules. Arterial calcification occurs in two distinct forms involving either the atherosclerotic intima or the tunica media. The coronary artery calcium score detected by computed tomography has been proposed to provide prognostic information beyond that provided by traditional risk factor scoring.[49] As such, clinical observations suggest that culprit plaques in ACS are less calcified and the individual calcifications are smaller compared to culprit plaques in stable angina.[50–52]

Neovascularisation. Plaque angiogenesis may have an important role in the development of severe atherosclerosis. Vasa vasorum angiogenesis provides nutrients to the developing and expanding intima and, therefore, may prevent cellular death and contribute to plaque growth and stabilisation in early lesions. However, in more advanced plaques, inflammatory cell infiltration and concomitant production of numerous pro-angiogenic cytokines may be responsible for induction of uncontrolled neointimal microvessel proliferation resulting in production of immature and fragile neovessels that may contribute to development of an unstable haemorrhagic rupture-prone environment.[53–55] In fact, in rupture-prone and ruptured plaques, the microvessel density is two- to four-fold higher than in stable plaques both in carotid and coronary arteries.[56,57] In line with these observations, we have reported – from coronary atherosclerotic lesions excised from patients' hearts – that the highest neovessel content is associated with the most-advanced stage plaques and, in turn, is linked with the highest rate of thrombotic episodes.[58] Moreover, using laser dissection microscopy, we have deciphered novel angiogenic factors that may contribute to plaque vascularisation and vulnerability.[59–61]

Other features. During plaque development, remodelling of the artery takes place and the flow-limiting effect of the growing plaque in the arterial intima may be attenuated (expansive or positive remodelling) or accentuated (constrictive or negative remodelling) by reactive changes in the underlying vessel wall. Human studies using intravascular ultrasound have shown that outward arterial expansion caused by positive remodelling is more common at culprit lesion sites in unstable angina, whereas inward or negative remodelling is more common in stable angina.[62] Moreover, such positive remodelling has been considered a potential surrogate marker of plaque vulnerability.[63,64] Such observations have also been advocated by different computer models that have shown that larger lumens create greater circumferential stress on the fibrous caps, thereby increasing their likelihood of rupture. Consequently, the level of circumferential stress is higher in plaques with mild stenosis – as compared with severe stenosis – due to the larger lumen, partly explaining the fact that most cases of acute coronary syndrome (ACS) occur in plaques with mild to moderate stenosis. In addition, plaques causing severe stenosis tend to have a higher fibrous and lower lipid content than those producing less severe lesions resulting in them being less prone to rupture. However, as detailed below, plaque vulnerability varies throughout the vascular bed. Finally, haemodynamic forces, including bloodflow, and shear- and flexion-stress may also contribute to disrupting the vulnerable plaque.[65–67]

Differences in plaque vulnerability throughout the vascular bed

Coronary artery vulnerable plaques. Retrospective analysis of serial angiograms, as well as prospective serial angiographic observations, have suggested that coronary occlusion and myocardial infarction most frequently occur in sites that have diameter narrowing of less than 70% (often less than 50%).[67] This concept is supported by the demonstration of a mild residual stenosis on angiography after thrombolytic therapy for an acute myocardial infarction.[68] However, it is important to take into account that less severe stenotic plaques are 5–10 times more common than severely stenotic plaques.[68] Furthermore, severely stenotic plaques are more likely to stimulate collateral circulation to the post-stenotic segment; thus, subsequent plaque rupture and thrombosis at such sites may be clinically silent because of the protective effect of collateral recruitment.[69,70] However, these mild stenotic plaques usually present a large lipid-rich core that, after disruption, exposes the thrombogenic gruel to the flowing blood causing about 70–80% of the coronary thrombus formation.[71]

Carotidartery vulnerable plaques. In contrast to coronary plaques, the vulnerable plaques in carotid arteries are severely stenotic and appear to be ulcerated and disrupted.[72–74] The vulnerable carotid plaques are not necessarily lipid-rich but rather heterogeneous, and they are very stenotic; their rupture or dissection probably relates to the impact of blood during systole against the resistance that they offer by being stenotic.

Aorticvulnerable plaques. Autopsy and transoesophageal echocardiography studies have shown that parameters such as luminal irregularities, plaque composition and non-calcified plaques in the aorta that are greater than 4 mm in thickness are strong predictors of future aortic vascular events.[75,76]

Thrombosis

Role of platelets in atherogenesis

Reservoir of atherosclerotic enhancers. Platelets do not adhere or activate to the intact, non-activated endothelium. However, inflammatory events such as those observed in the early stages of atherosclerosis lead to endothelial activation which, in turn, may stimulate platelet attachment.[77] Hence, endothelial disruption is not an absolute prerequisite to allow platelet activation and attachment to the arterial wall.[78] Although the mechanisms that lead to platelet–endothelial interaction remain to be fully described, it has been postulated that platelet activation may be attributed to: (a) reduction in the mechanisms implicated in maintaining endothelial antithrombotic properties (Figure 1); (b) reactive oxygen species (ROS) generated by atherosclerotic risk factors (in fact, the presence of hypertension,[79] hypercholesterolemia,[80] cigarette smoking[81] and diabetes[82] correlates with a higher number of circulating activated platelets); and (c) an increase in prothrombotic and pro-inflammatory mediators in the circulation or immobilised on the endothelium.[83] Activated endothelium allows platelets to roll on even under high shear rates. Platelet rolling, primarily mediated by P-selectin, is followed by firm adhesion mediated by integrin binding. Thus, platelet P-selectin, expressed upon activation, seems to be essential to allow platelet–endothelium adhesion.[84–86] Indeed, the absence of P-selectin has been shown to protect against the development of atherosclerotic lesions in both low density lipoprotein (LDL)-receptor and apoE$^{-/-}$ knock-out mice, especially in the early stages of lesion development.[77] Platelet attachment to intact but activated/dysfunctional endothelium may also be initiated by interaction of GPIbα and αIIbβ3 (GPIIb/IIIa) with endothelial P-selectin and von Willebrand factor (VWF). Indeed, there is an increased synthesis and (sub)endothelial presence of VWF in atherogenesis, with functional consequences for platelet deposition on the vessel wall.[87] Therefore, blockade of platelet adhesion using either GPIbα or αIIbβ3 antagonists has been shown to decrease platelet adhesion, leukocyte recruitment and lesion size.[88]

Activated platelets, in addition to selectin and integrin expression, release several mediators retained within their granules that result in cell adhesion, survival and proliferation, coagulation and proteolysis, and synthesise chemokines and proinflammatory cytokines all of which

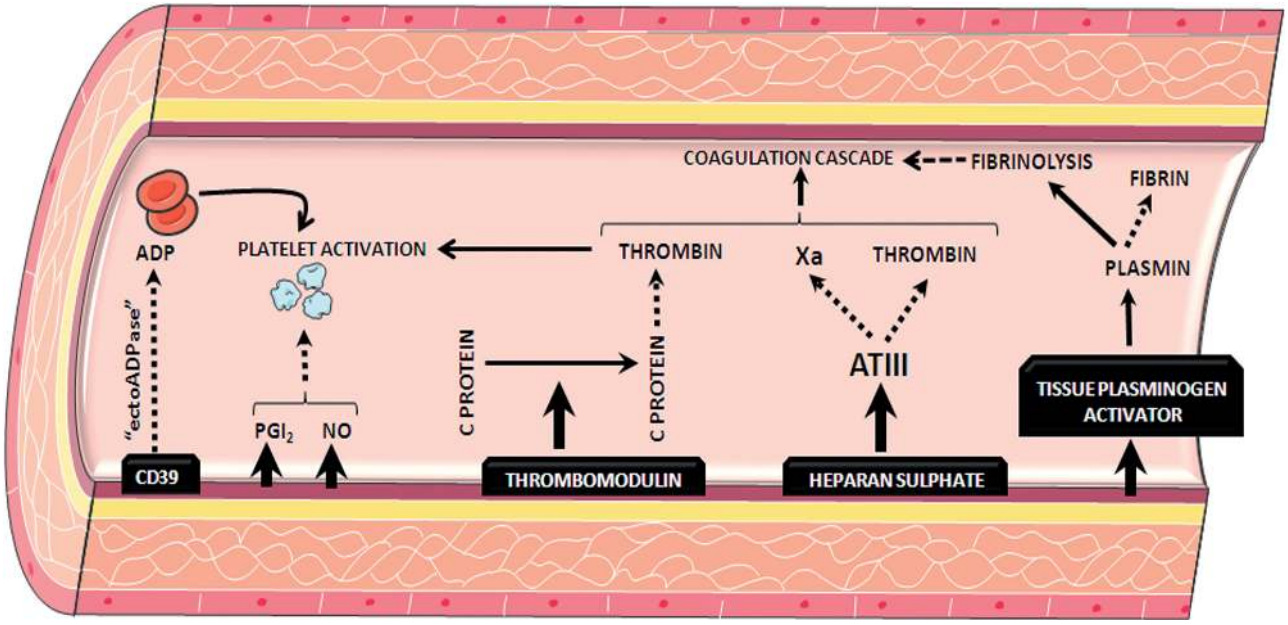

Figure 1. Antithrombotic properties of the healthy vascular endothelium.
PGI_2, prostacyclin; NO, nitric oxide; ATIII, antithrombin III; ADP, adenosine diphosphate

accelerate and enhance the inflammatory process promoting plaque development (Figure 2).

Platelets also contain high amounts of micro-RNAs (miRNAs) – small RNA molecules that modulate protein expression by degrading mRNA or repressing translation. Several reports have documented the role of platelet miRNAs in haematopoiesis, including differentiation and lineage commitment to megakaryocytes.[89,90] In addition, certain miRNA levels in platelets have been found to associate with reactivity to specific agonists and to pathological states. Although their clinical relevance is still under investigation, miRNAs have been suggested as potential biomarkers for platelet reactivity and vascular thrombosis as well as potential delivery vehicles for miRNAs, either as a physiological response to vessel injury or as potential therapeutic approach.[91]

Bridge between atherosclerosis and inflammation. A vast amount of platelet-related secretory molecules mediate the interaction between leukocytes and the endothelium in the early stages of atherosclerosis. Indeed, studies performed during recent years bring consistent evidence that platelets, besides driving thrombus formation on plaque rupture, play a key role in the inflammatory response. For instance, platelet delivery and deposition of RANTES and platelet factor (PF)-4 to the monocyte and endothelium surface, respectively, induces activation of monocyte-related integrins and eventually promotes macrophage infiltration in the vascular wall.[92] Moreover, both activated platelets and endothelial cells actively secrete pro-inflammatory cytokines such as CD40L and IL-1β, which further stimulate the endothelium and promote the activation of endothelial nuclear factor-B (NFKB).[93] Activation of NFKB, in turn, triggers the transduction and translation of key genes such as MCP-1,$\alpha_v\beta_3$, ICAM-1 and VCAM-1 – crucial for monocyte attachment and transmigration.[94] On the other hand, platelet–leukocyte interactions also occur via P-selectin/P-selectin glycoprotein (PSGL)-1 or integrin Mac-1/GPIb and/or fibrinogen-αIIbβ3 binding.[95–97] Such interactions facilitate firm leukocyte adhesion to endothelial-adhered platelets or directly to the endothelium supporting plaque formation.[16] Leukocytes, however, are not the only cells that are recruited by platelets into a vascular lesion. Platelets have recently been shown to contribute to progenitor cell recruitment for vascular regeneration. Platelets store an abundant amount of stromal derived factor-1 (SDF-1; a potent chemokine for progenitor cells) in their granules that supports the adhesion of progenitor cells to either the endothelium or thrombus surface. In addition, platelets are able to regulate progenitor cell differentiation into foam cells or endothelial cells depending on the conditions.[11] In summary, platelets adherent to collagen or to endothelial cells may serve as a bridging mechanism directing inflammatory cells and circulating progenitor cells to sites of atherosclerosis.[98]

Finally, platelets may also contribute to atherogenesis by mediating cholesterol uptake in the vascular wall. Free-cholesterol retention in cells and tissues can not only originate from endocytosed cholesterol esters that are hydrolysed in phagolysosomes but also directly from free cholesterol of cell membranes. Membranes of circulating cells, including activated platelets and probably dead leucocytes, can release free cholesterol.[99,100] It has been shown that focal

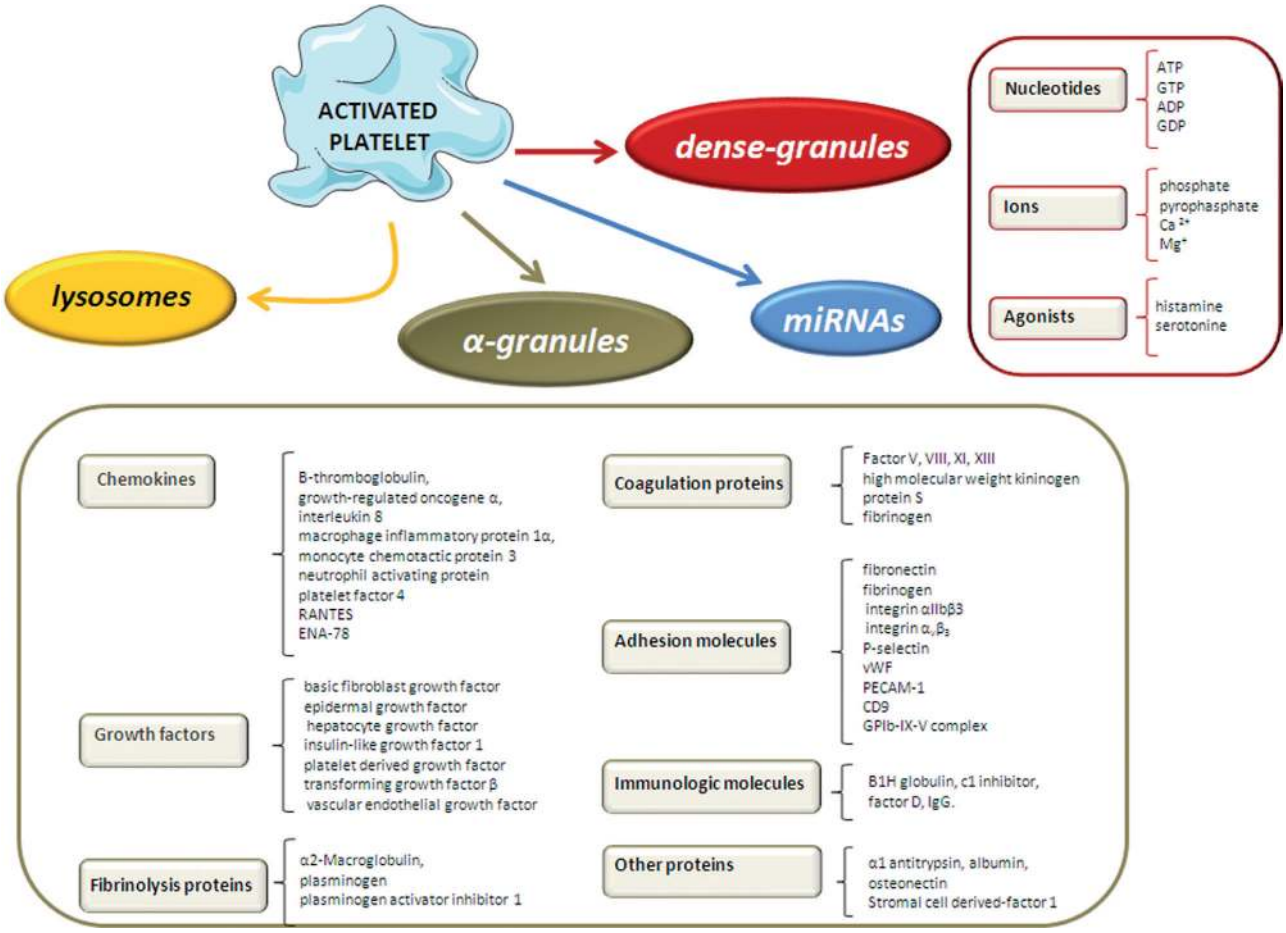

Figure 2. Molecules released from activated platelets.
miRNA, micro-RNAs; PECAM-1, platelet endothelial cellular adhesion molecule-type 1; vWF, von Willebrand factor; RANTES, regulated on activation normal T-cell expressed and secreted; ENA-78, epithelial cell-derived neutrophil-activating peptide 78; ADP, adenosine diphosphate; ATP: adenosine triphosphate; GDT, guanosine diphosphate; GTP, guanosine triphosphate.

intraplaque microhaemorrhages initiate platelet and erythrocyte phagocytosis, leading to iron deposition, macrophage activation, ceroid production and foam-cell formation.[101] Interestingly, the cholesterol content of erythrocyte membranes exceeds that of all other cells in the body, with lipids constituting 40% of their weight.[102]

Role of platelets in thrombosis

Platelet activation/adhesion and aggregation. Although platelets attach to intact endothelium actively participating in atherosclerosis progression, platelets play a key role in thrombus formation on erosion or rupture of an atherosclerotic plaque.[103] The exposure of the thrombogenic substrates to circulating platelets challenges platelet recruitment to the injured vessel wall in a well-coordinated – both in time and place – series of events: platelet 'arrest' onto the exposed subendothelium; recruitment and activation of additional platelets through the local release of major platelet agonists; and stabilisation of the platelet aggregates.[1,11,104,105] Hence, thrombus formation at the site of plaque rupture initiates with platelet interactions with the ECM components exposed to blood including fibrillar collagen and/or non-collagenic adhesion proteins such as VWF, fibronectin, and laminin (Figure 3). The rheological conditions largely influence these adhesive interactions. Thus, while at low shear rate, platelet adhesion to the vessel wall primarily involves binding to fibrilar collagen, fibronectin and laminin, under conditions of elevated shear stress, platelet tethering to the damaged subendothelium is critically dependent on their interaction with subendothelial-bound VWF. Platelets tethered to VWF roll along the vessel wall in the direction of the blood flow until other receptors provide stable attachments. In contrast, platelet binding to collagen via GPVI receptor induces the activation of other platelet-adhesion receptors, such as integrins αIIbβ3 and α2β1 (GPIa/IIa) that act in concert promoting subsequent firm, irreversible and stable platelet adhesion to the damaged surface.[106] Firm adhesion of platelets to collagen then provides the stimulus for platelet activation, shape change and exocytosis of the aforementioned granules constituents, which, in combination with several circulating

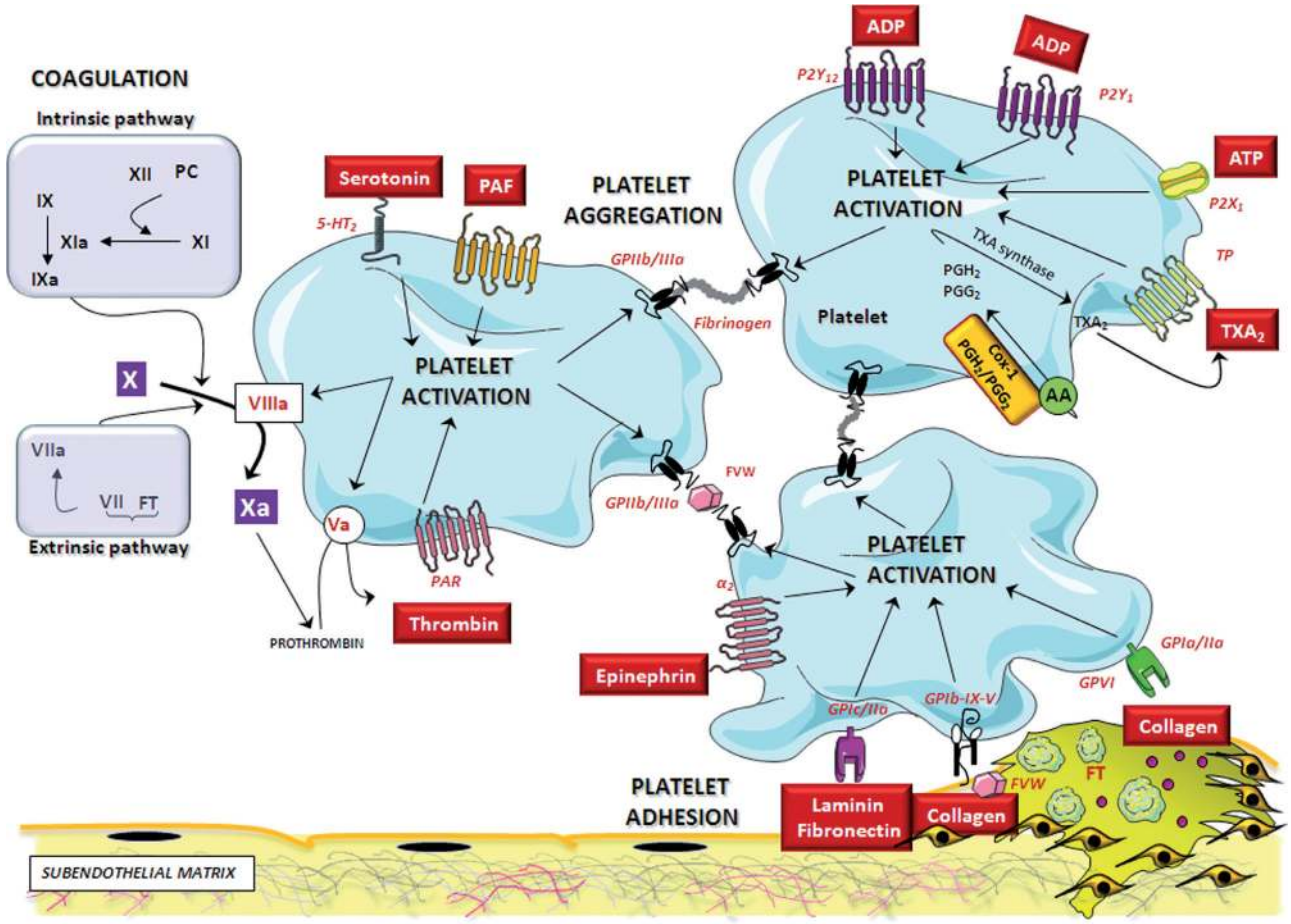

Figure 3. Diagram of platelet, coagulation, and vessel-related mechanisms involved in platelet adhesion, activation and further aggregation.

PAF, platelet activating factor; VWF, Von Willebrand Factor; TXA2, thromboxane A2; AA, arachidonic acid; PG, prostaglandin; ATP, adenosine triphosphate; ADP, adenosine diphosphate; TF, tissue factor; PAR, protease-activated–receptor; PC, protein C; TP, thromboxane receptors; PG, prostaglandin.

agonists at the site of lesion, perpetuate and enhance the thrombotic process (Figures 2 and 3). Platelet activation and granule release therefore play a crucial role in both atherogenesis and acute atherothrombosis.

Besides delivering many thrombotic agonists into the circulation, the degranulation process also alters the composition of the platelet membrane, resulting in surface expression of P-selectin and the generation of lipid-derived mediators such as thromboxaneA_2 (TXA_2). interactions.[107] TXA_2 released to the circulation binds to thromboxane (TP)-receptors largely distributed in platelets, circulating inflammatory cells, the vascular wall and atherosclerotic plaques thereby enhancing platelet activation, vasoconstriction and promoting plaque progression. Finally, activated platelets participate in a positive feedback loop that amplifies and perpetuates the platelet response to the given original stimulus, causing a change in the conformation of both the ligand-binding extracellular region and the cytoplasmic tails of αIIbβ3 thus expressing a high affinity-binding site for fibrinogen and VWF allowing stable bridges between platelets – a process commonly referred to as platelet aggregation (Figure 3).[108]

Cross-talk between platelets and the coagulation cascade. One of the early events after vascular disruption, and complementary to platelet activation, is the activation of the coagulation cascade (Figure 4). Strong evidence supports that TF expressed by foam cells, is the principal non-fibrillar thrombogenic factor in the plaque's lipid-rich core, which by binding clotting FVII/VIIa promotes local thrombin generation by initiating the extrinsic pathway of the coagulation cascade.[109,110] However, in addition to TF, both dysfunctional endothelium and activated platelets also play an important role in further promoting the coagulation cascade and the subsequent production of fibrin. Indeed, endothelium switch from an anticoagulant to a procoagulant phenotype and releases tissue plasminogen inhibitor (tPA) inhibitor. On the

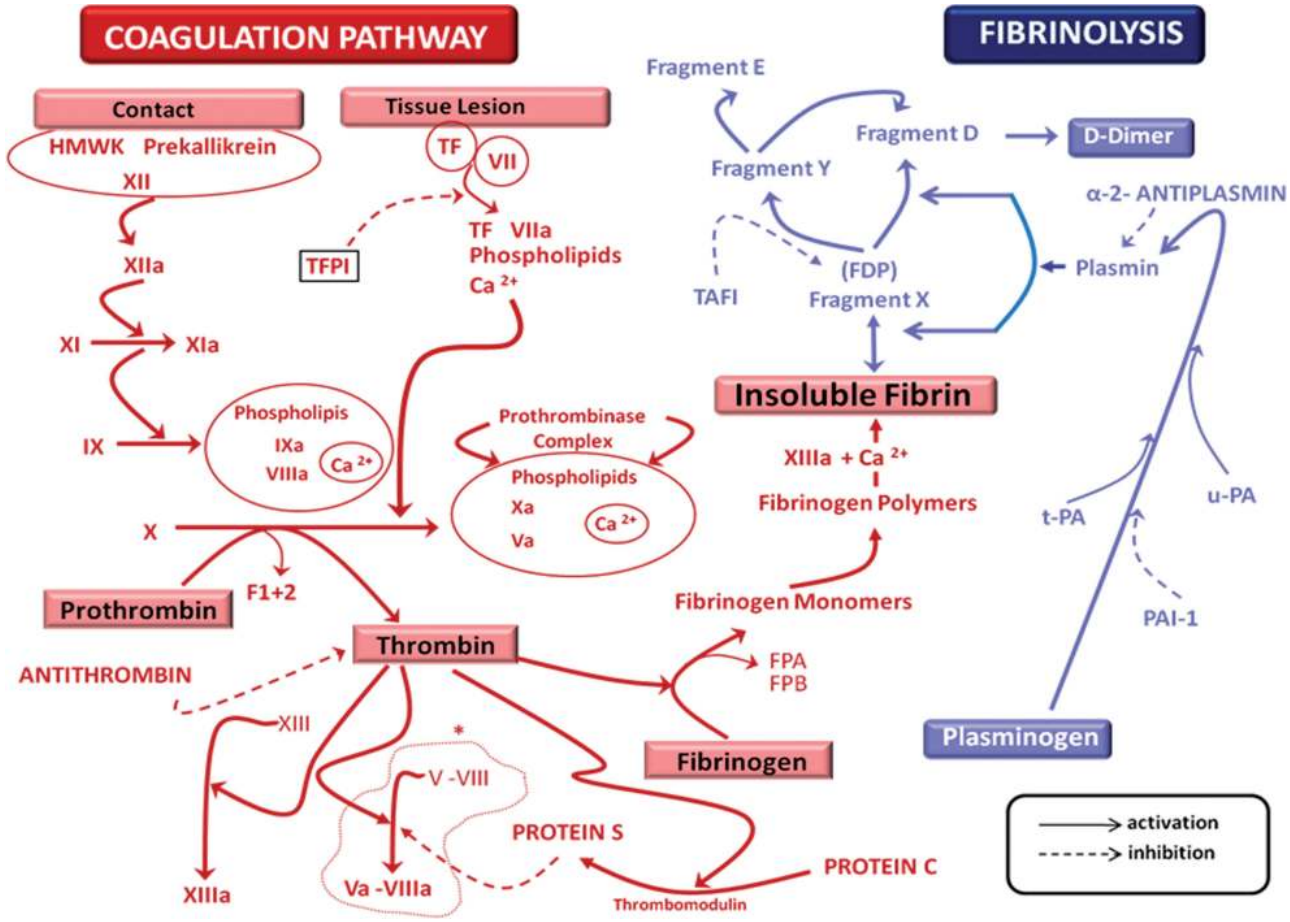

Figure 4. Coagulation and fibrinolytic pathways.
HMWK, high molecular weight kininogen; TF, tissue factor; TFPI, tissue factor pathway inhibitor; FPA, fibrinopeptide A; FPB, fibrinopeptide B; FDP, fibrin degradation products; t-PA, tissue plasminogen activator; u-PA, urokinase plasminogen activator; PAI-1, plaminogen activator inhibitor type-1.

other hand, activated platelets expose phospholipids in the outer surface of the plasma membrane that allow binding of several coagulation factors. Indeed, coagulation proteins usually circulate in plasma as inactive zymogens that are activated in the platelet surface (Figure 3). Nevertheless, both types of coagulation cascade activation leads to thrombin formation resulting in conversion of fibrinogen to fibrin monomers, which cross-link to stabilise the platelet-rich thrombus and, ultimately, form a solid clot (Figure 4).

Physiological pathways involved in clot dissolution. Fibrinolysis is the enzymatic process leading to fibrin clot solubilisation by plasmin originating from fibrin-bound plasminogen (Figure 4). Proteolysis of fibrin by plasmin induces generation of fibrin degradation products (FDP). The most specific stabilised FDP are D-dimers. Elevated plasma levels of D-dimers are a marker for increased thrombin formation and fibrin degradation turnover. Plasminogen is synthesised by hepatocytes and has a high affinity for fibrin through peptidic loops called 'kringles'. The principal plasminogen activator is tissue-plasminogen activator (t-PA), which also exhibits two kringle loops with a high affinity for fibrin. T-PA is synthesised mainly by endothelial cells, and is secreted locally after stimulation of the endothelium by histamine, adrenalin, thrombin, FXa and hypoxia. The second plasminogen activator is urokinase-plasminogen activator (u-PA), which is synthesised by numerous cell types including fibroblasts, epithelial cells and the placenta, and plays a minor role in physiological fibrinolysis. The native form of u-PA is pro-urokinase, a one-chain protein that is turned into a two-chain protein by plasmin or the contact factors (FXII, prekallikrein and HMWK).

Under physiological conditions, thrombin may play a pivotal role in maintaining the complex balance of initial prothrombotic events and subsequent endogenous anticoagulant and thrombolytic pathways. Thrombin, generated at the site of injury, binds to thrombomodulin – an endothelial surface membrane protein – initiating activation of

protein C, which in turn and in the presence of protein S inactivates factors Va and VIIIa (Figure 4).

Local and systemic regulation of thrombus growth: role of platelet-derived microparticles

A wide range of factors has been identified in prospective epidemiological studies to have a systemic effect on blood thrombogenicity. Certainly, there is increasing evidence of a close relationship between the traditional cardiovascular risk factors such as diabetes mellitus, hypertension and hyperlipidaemia and increased thrombogenicity, which is characterised by hypercoagulability, hypofibrinolysis or increased platelet reactivity.[111] Conversely, improvements of these cardiovascular risk factors have been associated with a lower prothrombic tendency.[112] In recent years, several reports have suggested that the role of platelets in atherosclerosis and its thrombotic complications may be mediated, in part, by local secretion of platelet-derived microparticles (PMps; microvesicular platelets formed during the platelet activation process).[113] Indeed, high concentrations of circulating PMps have been reported in patients with atherosclerosis, acute vascular syndromes and/or diabetes mellitus, suggesting a potential correlation between the quantity of microparticles and the clinical severity of atherosclerotic disease.

Platelets are probably the main source of MPs in the vascular compartment whereas the contribution of other vascular cells to the release of MPs varies in accordance with the pathophysiological context and the extent of cellular damage. The role of PMps in the in vivo thrombus formation is being investigated, PMps possess procoagulant properties that lead to thrombin generation. Such procoagulant activity relies on the exposure of membrane-anionic phospholipids that enable the assembly of coagulation complexes at the MP surface, and on the eventual formation of thrombin. Nevertheless, plaque microparticles are more thrombogenic than their circulating counterparts, possibly because plaques contain highly thrombogenic SMC-derived microparticles with higher thrombin-generating potential than circulating microparticles, mainly derived from platelets but not from SMC.[114]

PMps have also been described as intervening in inhibition of fibrinolysis by promoting PAI-1 activation.[115] PMps surface also present an array of platelet-derived adhesion and chemokine receptors, such as P-selectin, αIIbβ3, GPIbα, and PF4-receptor that induce monocytes- and endothelium-cytokine production, and an increase in leukocyte aggregation and recruitment via P-selectin/PSGL-1 dependent interactions.[116] PMps may also adhere to activated subendothelium where they enhance the adhesion of leukocytes via intercellular adhesion molecule-1 (ICAM-1) upregulation and enhance the inflammatory environment through the production of interleukins (IL-1, IL-6 and IL-8). Moreover, Mause et al[117] interestingly suggested that circulating PMps may even serve as a transfer module system for the platelet-derived chemokine RANTES on activated early atherosclerotic endothelium. Thus, elevated levels of PMps may not solely reflect an epiphenomenon of platelet activation but rather be regarded as an active transcellular delivery system for proinflammatory mediators and platelet receptors that overall contribute to thrombus growth and propagation.

Therapeutic implications in atherothrombosis

Atherosclerosis prevention is mainly focused on the management of so-called 'cardiovascular risk factors'. Indeed, abundant studies have reported on the effect of healthy lifestyle habits such as exercise,[118] body weight,[119] Mediterranean diet,[120] light-to-moderate alcohol consumption,[120] smoking[121] and stress[122] on limiting not only atherosclerosis progression but also reducing blood thrombogenicity. As to prevention and treatment of thrombosis-related complications, progression in understanding the processes of platelet activation/aggregation and the activation of the coagulation cascade has led to the widespread use of antiplatelet[104,123] (Figure 5) and anticoagulant[124] (Figure 6) agents in cardiovascular disease.

Conclusion

Atherosclerosis is a diffuse pathological process that involves structural changes in the intima and media of arterial vessels mainly driven by cholesterol accumulation, inflammatory cell infiltration and VSMC migration. Although an atherosclerotic plaque may remain clinically silent, it is prone to disruption, leading to local platelet activation, aggregation and the subsequent atherothrombotic episode. Moreover, platelet activation has also been shown to play a crucial role in driving atherosclerosis progression. Over the past few years it has been shown that the atherosclerotic plaque composition, rather than the degree of arterial stenosis, can be the determinant of rupture promoting the subsequent interplay with circulating blood components. Despite the established safety and effectiveness of several antithrombotic therapies, there is still a large scope for improvement. Indeed, new insights at the cellular-proteomic level will help the understanding of platelet pathology in the course of atherosclerosis and unveil molecular interactions prevalent in thrombosis. Undoubtedly, these advances will serve for the development of more accurate, safe and powerful strategies of pharmacological intervention for selectively inhibiting the pathways most relevant to the atherothrombotic disease process.

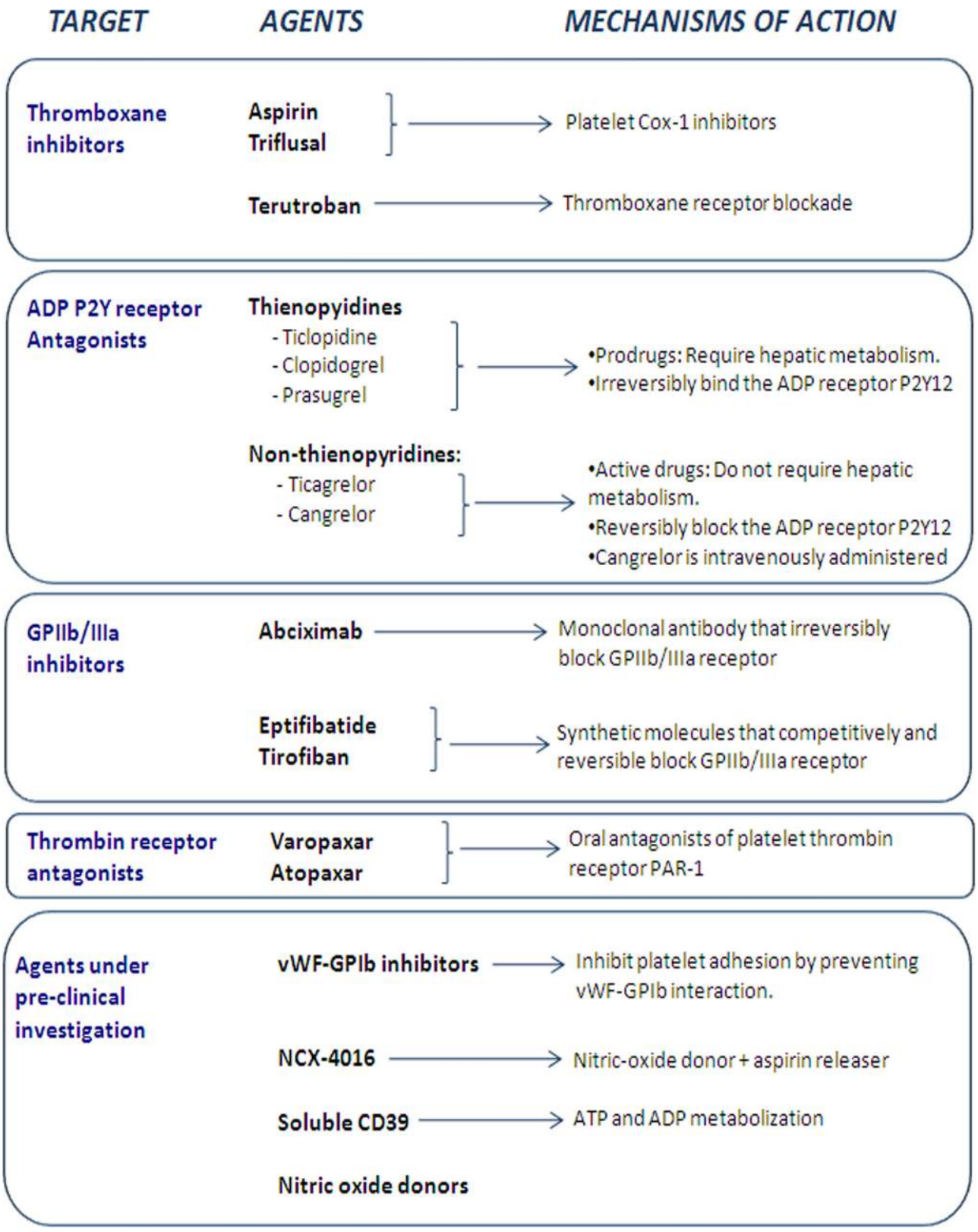

Figure 5. Antiplatelet agents currently used in the clinical setting, under clinical testing and under development.

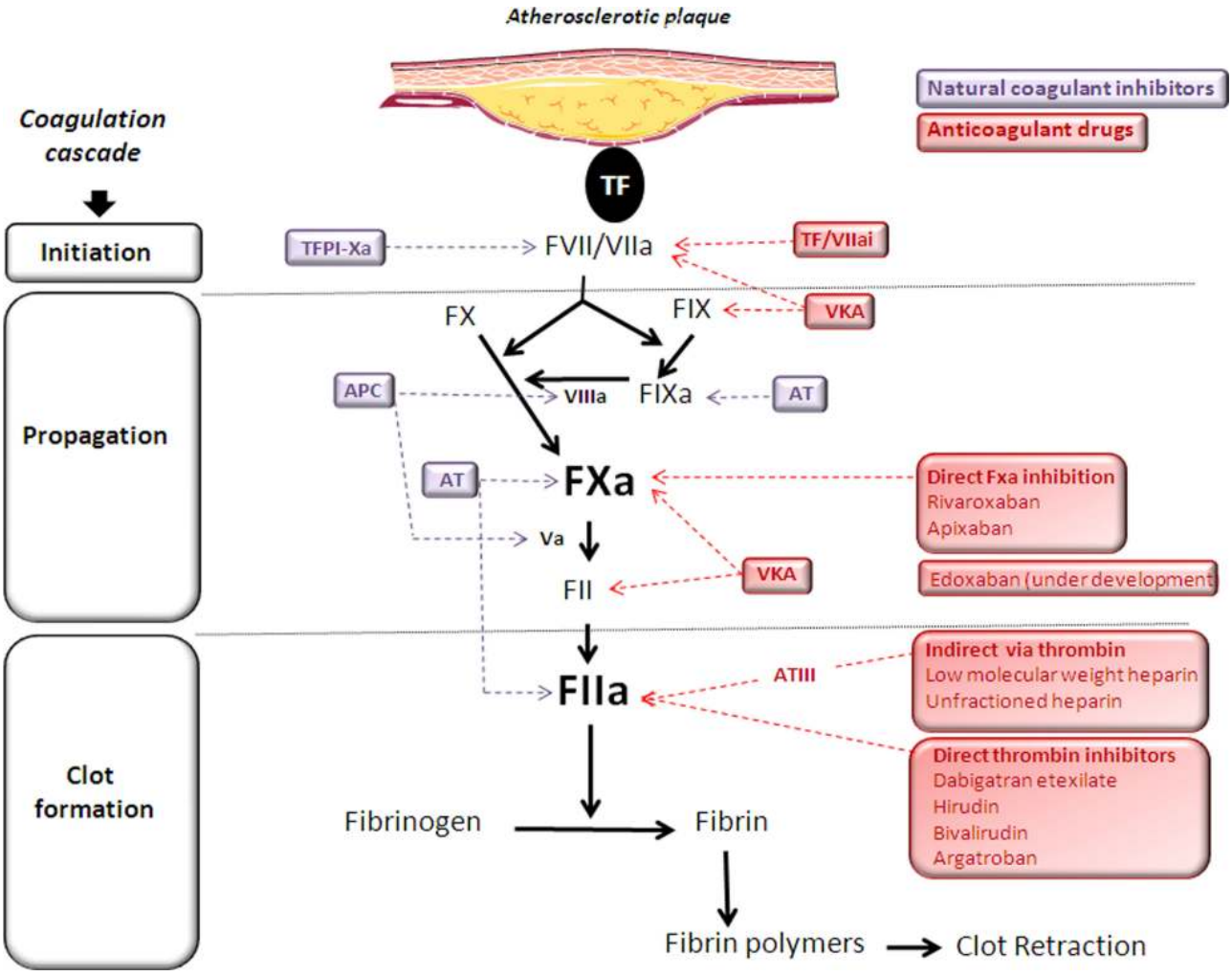

Figure 6. Natural inhibitors of coagulation and anticoagulant drugs.
TF, tissue factor; VKA, vitamin K antagonists; AT, antithrombin; APC, activated protein C; TFPI, tissue factor pathway inhibitor type-1.

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Conflicts of interest

None declared.

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