

Platelet activation as a trigger factor for inflammation and atherosclerosis

La activación plaquetaria como factor desencadenante de la inflamación y la aterosclerosis

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Abstract

Platelets, in addition to participating in atherosclerosis, play a very active role in the immune response of this disease since they have the ability to interact with various inflammatory cells, in addition to secreting cytokines, chemokines, growth factors, etc. The functions of platelets go beyond their interaction with the endothelium, as they participate in creating an inflammatory environment, which contributes to the loss of homeostasis. On the other hand, platelet-derived microparticles induce the activation of other platelets, of endothelial cells and in recruiting leukocytes. For all the above, platelets and the inflammatory environment can be considered as possible therapeutic targets to prevent the development of atherosclerosis and the events associated with it.

Key words: Atherosclerosis. Inflammation. Platelets.

Resumen

Las plaquetas, además de participar en la aterosclerosis, desempeñan un papel muy activo en la respuesta inmunitaria de esta enfermedad, ya que tienen la capacidad de interactuar con diversas células inflamatorias, además de secretar citocinas, quimiocinas, factores de crecimiento, etc. Las funciones de las plaquetas van más allá de su interacción con el endotelio, pues participan en crear un ambiente inflamatorio, lo que contribuye a la pérdida de la homeostasis. Por otra parte, las micropartículas derivadas de plaquetas inducen la activación de otras plaquetas y de células endoteliales, y el reclutamiento de leucocitos. Por todo lo anterior, las plaquetas y el ambiente inflamatorio pueden considerarse como posibles blancos terapéuticos para evitar el desarrollo de la aterosclerosis y los eventos asociados a esta.

Palabras clave: Aterosclerosis. Inflamación. Plaquetas.

Introduction

Atherosclerosis is classified as a chronic, progressive, inflammatory vascular disease with high immune

participation¹. This disease is characterized by an accumulation of lipids, especially the fraction known as low-density lipoprotein (LDL), beneath the intima layer of blood vessels, thus becoming a powerful stimulator of immune response in vascular endothelial cells².

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Although the best known and most widely studied risk factor for the development of atherosclerosis is obesity, it is not the only one, since intestinal microbiota has been described to be able to generate lipids that develop atherosclerotic plaques³. The role played by platelets in atherosclerosis, especially in its development, is not yet fully understood. Plaque rupture and subsequent exposure of extracellular matrix components allow platelets to activate and trigger thrombus formation, which is common at advanced stages of this pathology; therefore, platelets are not mere hemostasis and thrombosis mediators, but are a key factor in the characteristic inflammatory process of this disease¹.

Low-density lipoprotein and its relationship with atherosclerosis

LDL is considered one of the main triggers of atherosclerosis. This macromolecular complex made up of lipids and proteins participates in lipid metabolism. In vitro studies have shown that, regardless of its concentration, native LDL does not accumulate in macrophages; first, it must be oxidized in order to be recognized by the scavenger receptors of said macrophages to then be internalized by them. LDL oxidation can take place inside or outside the intima layer of blood vessels⁴. In blood circulation, LDL can interact with free radicals, which leads to its oxidation, and if this goes on for a long time, leads to the formation of cholesteryl ester hydroperoxides, which have been proposed as oxidative stress biomarkers. Through transcytosis, LDL can pass to the endothelial intimal layer and be oxidized by various mechanisms:

- Oxidation mediated by endothelial cells, smooth muscle cells or macrophages, among others.
- Oxidation by peroxynitrite, which comes from nitric oxide (NO) that has been inactivated by the superoxide anion.
- Oxidation by proteoglycans, which are abundant in the extracellular matrix, especially chondroitin sulfate, which can bind to LDL apolipoprotein B100.
- Oxidation by metal ions, such as iron or copper, which react with enzymes such as myeloperoxidase and lipoxygenase⁵.
- Oxidation by pH, which has been described as slightly acidic in the center of atherosclerotic lesions, which favors for endothelial cells to carry out anaerobic glycolysis, which in turn generates

lactic acid and thus causes extracellular space acidification.

- LDL non-enzymatic glycation oxidation in apolipoprotein B, particularly in the lysine amino acid.

These processes favor LDL internalization in macrophages and their polarization towards foam cells, which are key to the development of atherosclerosis^{4,6}.

Currently, LDL concentrations (≥ 132 mg/dL) are used as a coronary heart disease risk marker⁷. Fatty acids processed (oxidized) in the liver are transported by very low-density lipoproteins, also referred to as triglyceride-rich lipoproteins, which in turn exchange these triglycerides with high-density lipoproteins (HDL) and LDL, which causes both lipoproteins to become rich in triglycerides and then lose them in the liver by the liver enzyme lipoprotein lipase. This makes LDL smaller, denser and more atherogenic, while HDL, having also become smaller, loses its protective capacity and is also catabolized faster in the kidney, which makes for its concentration to drop. This entails that, even when adequate LDL concentrations are maintained, there is an increase in pro-atherogenic risk, caused by the size and loss of content of these lipoproteins, and on the other hand, triglyceride concentrations increase in an inversely proportional relationship to LDL particles diameter⁷⁻¹⁰.

Platelet adhesion to the endothelium during atherosclerosis

Vascular tissue is of utmost importance in the human being and is more than a layer of cells that line vessels; it is considered a metabolically active organ that performs various functions depending on the received stimulus¹¹. Circulating platelets not only participate in atherosclerotic plaque rupture, but also potentiate and modulate inflammatory reactions. Consequently, platelets can adhere to the wall of damaged blood vessels or to activated or dysfunctional endothelium, and thus promote the recruitment of cells involved in innate and adaptive immunity^{1,12}. The endothelium normally maintains an antithrombotic state due to the release of various substances, such as NO, prostacyclin and cyclooxygenase 2 (COX-2); however, an active endothelium changes its state to pro-thrombotic by increasing the release of adenosine diphosphate (ADP) and von Willebrand factor, and increasing tissue factor (TF) expression, as well as of various adhesion molecules, such as intercellular adhesion molecules (ICAM), vascular cell adhesion molecules (VCAM) and monocyte chemotactic proteins 1

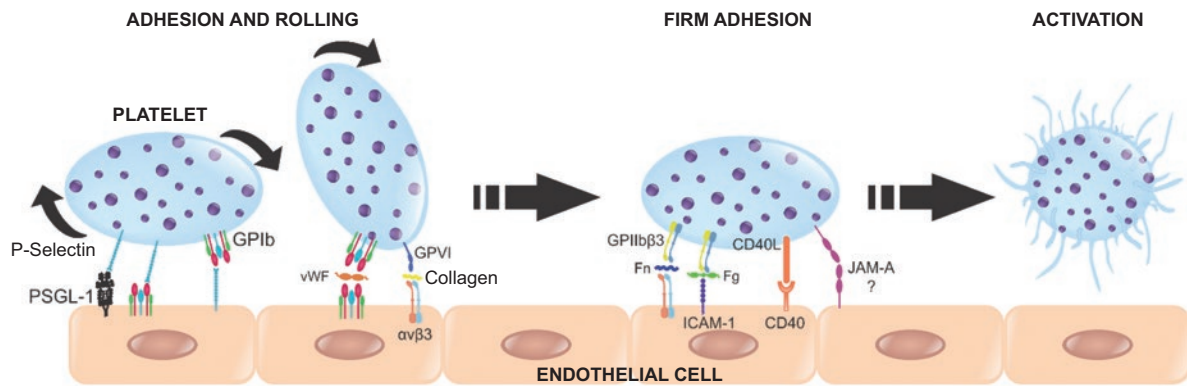


Figure 1. Platelet activation. Platelet activation is a multistage process that comprises their approach, adhesion, rolling and firm adhesion to the endothelium. During this process, platelets interact with endothelial cells. The first interaction is through platelet P-selectin and endothelial cell PSGL-1, followed by endothelial cell P-selectin and platelet GPIb interaction. Rolling requires GPIb binding to GPIb in platelets and endothelial cells, mediated by von Willebrand factor. Platelet GPVI integrin interacts with endothelial cell $\alpha v \beta 3$ integrin, and in turn triggers GPIIb/IIIa integrin activation in platelets, which interacts with endothelial cell $\alpha v \beta 3$ through fibrinogen. In addition, endothelial cell ICAM-1, through fibrinogen, binds to platelet GPIIb/IIIa integrin. Finally, platelet CD40L binds to endothelial cell CD40, thus promoting platelet firm adhesion and subsequent activation. Fg: fibrinogen; Fn: fibronectin; PSGL-1: P-selectin ligand 1; vWF: von Willebrand factor.

(MCP-1), among others^{12,13}. Native or oxidized LDL deposition on the intimal layer of arteries promotes endothelium activation, which causes for circulating platelets to bind and activate¹⁴. Endothelium-independently activated platelets can bind to it; for example, circulating oxidized LDL (oxLDL) is known to bind to platelet scavenger receptors (CD36), which activate NADPH oxidase isoform 2 (NOX-2) and thus promote their activation and consequent binding to the endothelium¹⁵.

P-selectin is an adhesion molecule that is essential for the binding of platelets to the endothelium through P-selectin ligand (PSGL-1) or with von Willebrand factor receptor glycoprotein Ib (GPIb) present in the endothelium; in turn, platelets, through the same GPIb receptor, bind to endothelial P-selectin, allowing their adhesion and rolling on the endothelial layer. Under high shear conditions, activated platelets can also adhere and roll using the same mechanism¹³. Collagen receptor GPVI, which is located on the platelet surface, has been observed to bind to subendothelial matrix exposed collagen or to soluble collagen, and this in turn is recognized by endothelial cell $\alpha v \beta 3$ integrin, which allows platelets to adhere and roll on the endothelium¹³. In addition, the GPIb receptor that recognizes von Willebrand factor binds to its receptor expressed in endothelial cells, which also allows platelets to roll on the endothelium (Fig. 1)^{13,16}.

Moreover, the above-described events trigger integrins $\alpha IIb \beta 3$ and $\alpha IIb \beta 1$ activation, which generates platelet aggregation¹⁷. Integrin $\alpha IIb \beta 3$ is capable of

binding to ICAM-1-bound fibrinogen and $\alpha v \beta 3$ -bound fibronectin in endothelial cells, thus contributing to stable adhesion¹⁸. $\alpha IIb \beta 1$ can bind to collagen that is recognized by integrin $\alpha v \beta 3$ in endothelial cells, which cooperates to stable adhesion, thus allowing platelets to activate (Fig. 1)^{13,19}.

Another protein related to platelet adhesion and activation is C-type lectin-like receptor 2, which activates signaling pathways involving Src and PI3K, which have also been observed to be activated by the aforementioned receptors (GPIb and GPVI)²⁰.

Another molecule that participates in platelet adhesion to the endothelium is CD40 ligand (CD40L or CD154), which, due to platelet activation, forms trimeric complexes that bind to its CD40 receptor on endothelial cells, and it has even been described to be able to bind to other platelets $\alpha IIb \beta 3$ integrin, which allows the formation of aggregates. Platelet CD40L has also been observed to be fragmented and solubilized to form soluble CD40L (sCD40L or sCD154), thus promoting activation and aggregation of other platelets and endothelial cell activation, which increase the expression of adhesion molecules and the release of interleukins and chemokines, generating platelet adhesion as well²¹ (Fig. 1).

Platelet junctional adhesion molecule A (JAM-A) was thought to bind to another JAM-A molecule in endothelial cells, and that this generated platelet activation, but platelet JAM-A binding to another platelet JAM-A has been observed to generate integrin $\alpha IIb \beta 3$ inactivation, which inhibits platelet aggregation²⁰.

All these described interactions are just a few of those that can occur during platelet-platelet and platelet-endothelium adhesion, the consequence of which is platelet activation in order to promote thrombus formation or exert their immune function.

Platelet activation mechanisms

Von Willebrand factor binds to GPIIb α , which is part of platelet GPIIb-IX-V receptor complex, thus inducing members of the Src kinase family to phosphorylate GPIIb α ITAM motifs, which in turn attracts SYK (another member of the Src family), which phosphorylates LAT²². Phosphorylated LAT recruits PI3K, which induces PLC γ 2 and PKC activation, which in turn trigger the release of calcium from endoplasmic reticulum²³. Calcium increase induces Rap-1b activation, and activated Rap-1b attracts talin, which causes the activation of several integrins and, consequently, platelet aggregation²⁴. The next activation stage, which we may call “amplification”, is dependent on platelet granule-derived agonists, such as ADP, thromboxane A₂ (TxA₂), thrombin, prostacyclin, serotonin or adrenaline, to name a few²³. These agonists activate G-protein-coupled receptors, such as P2Y₁ and P2Y₁₂ (ADP receptors), PAR1 and PAR4 (thrombin receptors), TP (TxA₂ receptor), 5-hydroxytryptamine (serotonin receptor) and EP3 (prostaglandin E₂ receptor)²⁵. These receptors allow PLC β activation, which, like PLC β 2, activates PKC by promoting intracellular calcium increase and Rho activation, which results in platelet activation and a consequent secretion of granules, which increases platelet aggregation^{23,26}.

Endothelium as a pro-atherosclerotic organ

The endothelium is considered a metabolically active organ (autocrine and paracrine) that plays a critical role in vascular tone physiological regulation, adhesion, cell migration and resistance to thrombosis, among others, all this depending on the stimulus it receives. These functions are carried out through the release or expression of adhesion molecules, growth factors, antioxidant compounds and vasodilator or vasoconstrictor agents. For atherosclerosis to be established, it is necessary for the endothelium to be activated and become dysfunctional. Activation refers to the ability of endothelial cells to perform new functions without evidence of cell damage or dysfunction. Moreover, endothelial dysfunction arises when

adaptive responses are maintained in an excessive, sustained and inappropriate form, which alters vascular homeostasis, thus predisposing the vascular wall to vasoconstriction, leukocyte adhesion, platelet activation, oxidative stress, thrombosis, coagulation or inflammation, and favoring the establishment of various pathologies^{12,27,28}.

In cells, NO is generally synthesized from L-arginine by a group of enzymes called nitric oxide synthases (NOS); in endothelial cells, this process is carried out by endothelial nitric oxide synthase (eNOS). In atherosclerosis, increased and sustained LDL concentrations in contact with reactive oxygen species (ROS), especially O₂, allow its oxidation. Hyperlipidemia causes endothelial cell NADPH oxidase upregulation, which in turn generates more ROS²⁷. Increase in this O₂ cannot be inactivated by superoxide dismutase (SOD) and reacts with NO, giving rise to the formation of peroxynitrite, which in turn is capable of destabilizing eNOS. On the other hand, asymmetric dimethyl arginine (ADMA) competes with L-arginine for the eNOS binding site, and inhibits it²⁹. oxLDL also increases the synthesis of caveolin-1, an integral membrane protein that inhibits eNOS as well³⁰. eNOS inhibition results in an increase in ROS (especially O₂) and a decrease in NO, which favors platelet activation and aggregation (Fig. 2)³⁰. When oxidative stress is generated, it induces a deviation from the normal redox state towards an oxidative environment. The redox state is the chemical environment concerning the number of available reductor equivalents, with one of the main ones being the NADH/NAD⁺ system³¹⁻³³.

Platelet adhesion to the endothelium promotes chemokine CCL5 (RANTES) and soluble CD40L release by platelets, as well as CD40L expression, which leads to platelet binding to endothelial cells, which in turn induces an increase in the expression of adhesion molecules and cytokines and chemokines release by endothelial cells²¹. Similarly, interleukin (IL) 1 β release by platelets induces endothelial cells to release IL-6, IL-8, ICAM and MCP-1, among others, which also contributes to their interaction with inflammatory cells (Fig. 2)³⁴.

At atherosclerosis more advanced stages, the inflammatory process involving innate and adaptive immunity cells, such as neutrophils, macrophages, dendritic cells and lymphocytes, among others, generates a cytokine-rich environment, which promotes endothelial activation and dysfunction, and platelet activation and binding to the endothelium³⁴. In addition, oxidative stress has been observed to amplify

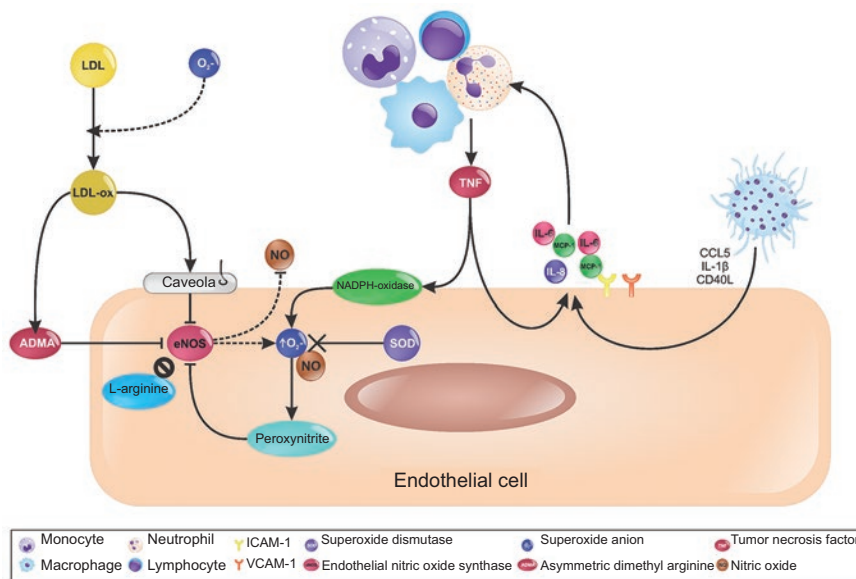


Figure 2. Endothelial dysfunction in atherosclerosis. Superoxide anion (O_2^-) increase interacts with LDL, favoring its oxidation. oxLDL promotes an increase in blood ADMA; this ADMA is an eNOS competitive inhibitor, which entails a decrease in NO production by endothelial cells. On the other hand, oxLDL favors caveolae synthesis in endothelial cells, also inhibiting eNOS. In addition, NADPH-oxidase positive stimulation by TNF secreted by inflammatory cells leads to O_2^- anion overproduction, which prevents the SOD enzyme from inactivating said O_2^- and interacting with NO within endothelial cells, which leads to the formation of peroxynitrites that inhibit eNOS. In conjunction with the above, platelets, through the secretion of CCL5, IL-1 β and CD40L, can increase the expression of adhesion molecules and the secretion of cytokines by endothelial cells, which act by promoting the recruitment of inflammatory system cells. ADMA: asymmetric dimethyl arginine; eNOS: endothelial nitric oxide synthase; LDL: low-density lipoprotein; NO: nitric oxide; SOD: superoxide dismutase; TNF: tumor necrosis factor.

the inflammatory response by endothelial cells that recruit monocytes, lymphocytes and neutrophils, which secrete cytokines such as tumor necrosis factor alpha (TNF- α), which, in endothelial cells, can positively regulate NADPH oxidase and negatively regulate eNOS, thereby promoting both endothelial dysfunction and cytokines, chemokines and adhesion molecules increased expression²⁷.

Advanced atherosclerotic lesions express an important component, called urokinase-type plasminogen activator (uPA), which is a serine protease involved in the conversion of plasminogen into plasmin and in vascular remodeling³⁵. This uPA is expressed by endothelial cells present in these lesions, as well as by macrophages³⁶. uPA can be cleaved into several biologically active fragments: amino-terminal fragment, kringle domain and carboxy-terminal fragment, each one with unique properties³⁷. For example, the amino-terminal fragment, by binding to urokinase-type plasminogen activator receptor (u-PA), is capable of inducing vascular smooth muscle cells migration³⁶, which triggers the activation of various signaling pathways involved in cell migration and proliferation, such as MAPK and JAK-STAT³⁸. On the other hand, uPA from endothelial cells in the area of lesion and foam cells can cleave, in addition to

plasminogen, other extracellular matrix proteins, degrade fibrin and activate metalloproteinases (MMP), including MMP-9³⁹. uPA also promotes plasmin formation, which activates MMP-9, which in turn degrades the extracellular matrix, causing for atherosclerotic plaque to become vulnerable⁴⁰⁻⁴². For all the above reasons, it is proposed that the uPA system mediates atherosclerosis progression⁴³.

Platelet and leukocyte interaction

Just as platelet adhesion to the endothelium is mediated by P-selectin, leukocyte adhesion to the endothelium and platelet-leukocyte aggregates formation is carried out by P-selectin interaction with its ligand (PSGL-1)¹². However, polymorphonuclear cells, especially neutrophils, by secreting CCL5, and the latter by binding to its CCR1 and CCR5 receptors, have been observed to allow them to enter arterial intimal layer and release their content, such as azurocidin, which can act on the endothelium by promoting an increase in the expression of adhesion molecules such as ICAM-1, VCAM and E-selectin, cathepsin G and CAMP (LL37), which promote the attraction of monocytes towards it⁴⁴. Neutrophils have been observed to be

able form platelet-neutrophil aggregates, and these respond to oxLDL by increasing their capacity for transmigration towards the intimal layer⁴⁵.

Platelet factor 4, also called CXCL4, is one of the most abundant chemokines present in platelet alpha granules. This CXCL4, together with CCL5, has been reported to promote monocyte adhesion to the endothelium⁴⁴. CXCL4 promotes macrophage polarization towards a phenotype called M4, which is characterized for having low phagocytic capacity and high expression of cholesterol transporters⁴⁶.

PSGL-1/P-selectin and CD40/CD40L participate in the formation of monocyte-platelet aggregates. This interaction causes nuclear factor kappa B (NF- κ B) activation in monocytes, which in turn induces an increase in the expression of inflammatory proteins, such as MCP-1, TNF- α , IL-8 and prostaglandin E2 (PGE2)⁴⁷. The formation of monocyte-platelet aggregates is considered a platelet activation marker with even higher predictive value than the presence of inflammatory cytokines⁴⁸.

When platelets bind to the endothelium via CD40L/CD40, an increase of adhesion molecules, such as ICAM-1 and VCAM-1, is promoted in endothelial cells, as well as chemokine CCL2 release, which elicits the recruitment of lymphocytes. Lymphocyte binding to platelets via PSGL1/P-selectin triggers integrin α L activation in the lymphocyte, causing its adhesion to endothelial cells via ICAM-1^{49,50}. Platelets also promote T-helper lymphocytes adhesion to P-selectin- and CD40L-dependent sub-endothelial matrix. It has also been documented that, when platelets bind to B lymphocytes via CD40L/CD40, they promote both their differentiation and immunoglobulin class switching⁴⁹.

In addition, the interaction of dendritic cells with platelets has been described to contribute to the development of atherosclerosis; for example, in vitro evidence proposes that platelets can recruit dendritic cells via JAM-C/Mac-1 (macrophage antigen-1) interaction, which promotes platelet phagocytosis and dendritic cell subsequent death, thereby inducing an inflammatory environment⁵¹. Platelets can also interact with dendritic cells via the CD40L/CD40 couple, which promotes dendritic cells maturation and differentiation, in addition to stimulating the release of cytokines and chemokines by them^{14,47}.

Platelet-derived microparticles

Most cells release extracellular vesicles deriving from their membrane; these vesicles can contain different

biomolecules, such as proteins, lipids, carbohydrates or nucleic acids, among others, and have an impact on adjacent or distant cells. Extracellular vesicles were originally observed by Chargaff and West in 1946 as platelet-derived pro-coagulant particles; later, in 1967, they were referred to by Wolf as platelet dust⁵². These vesicles carry membrane fragments of the cell they were released from, and are classified according to their function and cellular origin (tolerosomes, prostatosomes, cardiosomes, vexosomes, etc.) or based upon their size (40-120 nm, exosomes; 100-1000 nm, ectosomes, microparticles or microvesicles; 500-2000 nm, apoptotic bodies). Platelet-derived microvesicles or microparticles are the most abundant in human blood and the most widely studied⁵³. These microparticles, under physiological conditions, play a role in the maintenance of homeostasis, but under certain stimuli, such as cancer, cardiovascular conditions, infection or inflammation, among many others, their number increases and there is a dysregulation of various systems, among them lymphatic and coagulation systems⁵⁴. These platelet-derived microparticles can contain caspases (3 and 9), transcription factors (NF- κ B, STAT, SOX), ligands (CD40L, CXCL4), cytokines (IL-1 β), chemokines (CCL5, CXCL7, CXCL4), growth factors (vascular endothelial growth factor [VEGF], platelet-derived growth factor [PDGF]), nucleic acids (miRNA, mRNA), lipid mediators (TxA₂, arachidonic acid), enzymes (COX-1, heparanase) and mitochondrial residues⁵⁵. These microparticles can also be pro-coagulant because their membrane is asymmetric in terms of the content of phospholipids, such as phosphatidylcholine and phosphatidylserine⁵⁵. Exposed phosphatidylserine in microparticles provides a negatively charged surface, which is required by the extrinsic prothrombinase and tenase complexes formed as part of the coagulation process. As already known, coagulation starts with TF exposure and its subsequent binding to factor VII to form the extrinsic tenase complex (TF-VIIa), which acts on factor X, activating it. This Xa factor binds to factor Va in the presence of Ca⁺² to form the prothrombinase complex (FVa-FXa). In turn, factor X is also activated by intrinsic tenase complex (FVIIIa-FIXa) in the presence of Ca⁺², and said factor Xa also participates in the generation of the prothrombinase complex^{55,56}. These same platelet-derived microparticles also secrete plasminogen activator inhibitor (PAI-1), which inhibits the fibrinolytic system; this is especially important in atherosclerosis advanced stages, when the plaque becomes susceptible to rupture, which triggers the coagulation process (Fig. 3)⁵⁷.

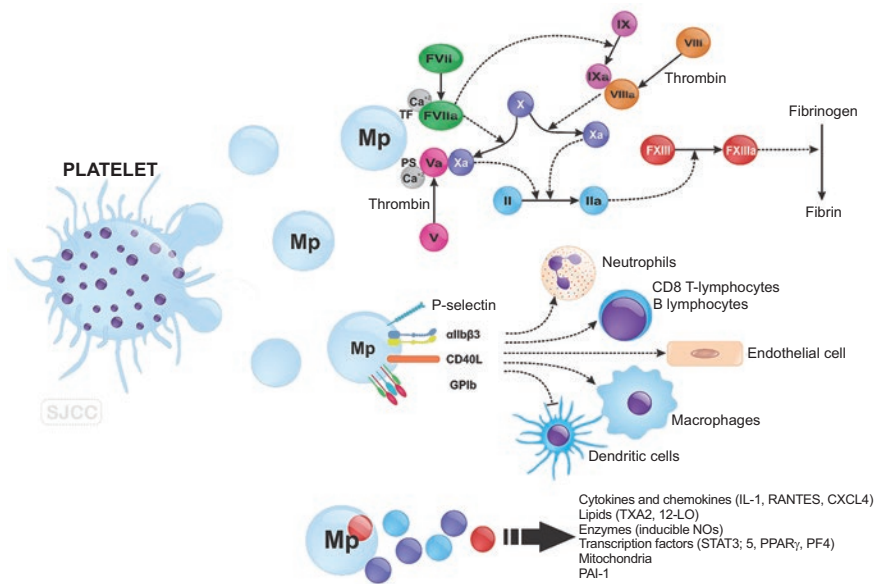


Figure 3. Platelet-derived microparticles possess anionic phospholipids that allow the assembly of the coagulation initiation complex (TF + FVIIa + Ca^{+2}), in addition to the prothrombinase complex (FVa + FXa + Ca^{+2}), which leads to an increase in thrombosis. Microparticles also contain integrins, ligands and adhesion molecules that allow aggregates to be formed with leukocytes. These microparticle and leukocyte aggregates interact with dendritic cells and epithelial cells. On the other hand, these microparticles contain cytokines and chemokines, other lipid-derived molecules (TxA_2 and 12-LO), enzymes and growth factors, among others, which favors the inflammatory process. 12-LO: 12-lipoxygenase; MP: microparticles; PS: phosphatidylserine; TF: tissue factor; TxA_2 : thromboxane A₂.

Due to their caspase content, platelet-derived microparticles promote endothelial cell and leukocyte apoptosis, and this favors, in thrombotic plaques, an increase of microparticles deriving both from platelets and leukocytes and endothelial cells, which accumulate and potentiate thrombus formation and the occurrence of inflammatory events⁵⁸.

Platelet-derived microparticles can recruit leukocytes, since they possess integrins such as $\alpha\text{IIb}\beta_3$, GPIb, GPIX (CD42a) and GPIIb (CD36), and adhesion molecules such as P-selectin and PECAM-1, JAM-A, etc. This generates leukocyte adhesion to the microparticles, thus forming microparticle-leukocyte aggregates⁵⁵. Given that these microparticles harbor cytokines and chemokines, such as IL-1 β , RANTES and CXCL4, they recruit leukocytes into endothelial cells, which promotes inflammation and atherosclerosis. In addition, microparticles contain platelet activating factor, which allows neutrophils and macrophages activation (Fig. 3)⁵⁵. Moreover, oxLDL can interact with the CD36 receptor on platelets, thus generating their activation and subsequent generation of microparticles⁵⁹.

Therapeutic approaches

Currently, statins are first-line drugs used for the treatment of plasma lipid high concentrations, and

even in primary prevention of coronary heart disease. Statins act by inhibiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is the first and main enzyme in cholesterol biosynthesis; this way, the generation of cholesterol precursors in the liver is prevented, and a reduction of LDL-cholesterol (considered one of the main cardiovascular risk factors) is achieved, since reducing this enzyme increases the number of LDL receptors in the liver (Fig. 4)⁶⁰. On the other hand, statins have a rather broad pleiotropic effect, since they can have direct antithrombotic effects; for example, simvastatin prevents oxLDL interaction with monocytes and at the same time decreases oxLDL-induced TF expression without causing a change in cholesterol concentrations⁶¹. In turn, various statins have been implicated in TF expression downregulation, thrombomodulin generation increase and factor V inhibition, among other actions, which prevents platelet activation and aggregation. In addition, atorvastatin has been shown to be able to directly inhibit platelet activation through COX-1 activation downregulation⁶². Other pathways involved in platelet activation inhibition include oxidative stress reduction through NOX2 and TxA_2 downregulation and NO release; this, on one hand, prevents activation and therefore the release of cytokines and chemokines, such as CXCL4 and CXCL7, and on the

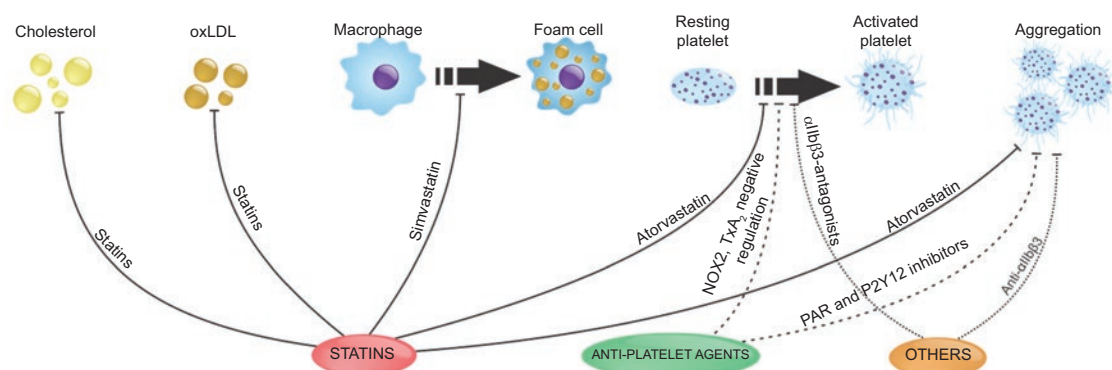


Figure 4. Atherosclerosis treatment. Statins inhibit oxLDL interaction with macrophages, which prevents foam cell formation, and also block platelet activation and aggregation. Antiplatelet drugs act by inhibiting platelet activation and aggregation by blocking various ADP, thrombin and TxA_2 receptors; in addition, some are capable of inhibiting soluble CD40L and IL-6 release, which further enhances their antiplatelet effect. Monoclonal antibodies, such as $\alpha\text{IIb}\beta_3$ integrins inhibitors, have been used. oxLDL: oxidized low-density lipoprotein; TxA_2 : thromboxane A₂.

other, oxidative stress reduction decreases LDL capacity for being oxidized^{62,63}. In conjunction with the above, statins can inhibit the platelet amplification phase by inhibiting thrombin receptor (PAR-4) activation⁶⁴. Moreover, statins (rosuvastatin) have been shown to be able to decrease mean platelet volume (MPV), which is regarded as a possible platelet activation determining marker, given that circulating resting platelets are discoid, but when activated, they switch shape, since they develop filopodia and lamellipodia that generate an increase in their surface area, which is reflected in a higher mean platelet volume⁶⁵.

Other drugs that are widely used for the treatment of coronary heart disease are antiplatelet agents, among which three stand out: clopidogrel, prasugrel and ticagrelor. All three are platelet ADP receptor (P2Y₁₂) inhibitors, but the latter two have been shown to be more potent and faster-acting than clopidogrel (Fig. 4)⁶⁶. Clopidogrel is used as standard treatment in combination with aspirin as an anti-aggregant therapy to reduce ischemic events in patients with atherothrombotic disease; clopidogrel, as already mentioned, is an ADP receptor inhibitor, while aspirin is an irreversible COX-1 inhibitor, which results in a decrease in the production of prostaglandins and TxA_2 , and thereby in a reduction of platelet activation and aggregation⁶⁷.

Thrombin PAR-1 receptor antagonists have also been developed, such as vorapaxar and atopaxar; the latter, in addition to inhibiting said receptor in murine models, has been shown to decrease the release of pro-inflammatory molecules, such as soluble CD40L and IL-6, and P-selectin expression. In humans, it was

also shown to be capable of inhibiting both the release of CD40L and the increase in adhesion molecules expression in endothelial cells⁶⁸.

Ticagrelor has a unique and different mode of action from the above agents: it does not require metabolic activation for antiplatelet activity (unlike clopidogrel and prasugrel, which have a delayed onset of action because they require metabolic activation) and reversibly binds to P2Y₁₂, which allows faster platelet function recovery (unlike clopidogrel, which irreversibly binds to P2Y₁₂)⁶⁹.

According to the above, antiplatelet agents effects are based on blocking one or another receptor for platelet agonists; however, as previously mentioned, the signaling pathways each receptor activates downstream in some cases converge between each other, while in others they do not, and this is what drives to the use of dual therapies to try to cover the largest amount of proteins related to these pathways and thus obtaining the expected effect. In turn, it is necessary pointing out that these drugs have permanent effects, which might generate some adverse effects for certain patients. For example, dual therapy with aspirin and clopidogrel has the limitation that clopidogrel is an irreversible antiplatelet agent, which can cause a significant risk of hemorrhage in patients who require surgery and have not discontinued treatment for 5-7 days. Another effect is platelet inhibition variability with clopidogrel, which explains why some patients, even with sustained doses of this drug, exhibit high platelet reactivity⁶⁷. Due to the above, new versions of these P2Y₁₂ (ADP receptor) blockers have emerged, such as cangrelor, which is a direct and reversible

inhibitor of this receptor, and elinogrel, which also acts directly on P2Y₁₂ and is a competitive and reversible inhibitor of this receptor that appears to have fewer side effects than clopidogrel⁷⁰. Due to the complexity of platelet physiology, various attempts have been made to improve platelet inhibition, and blocking certain integrins, such as α IIb β 3, has been attempted with monoclonal antibodies. Abciximab is a chimeric mouse/human antibody that is able to inhibit platelet aggregation by directly acting on these integrins⁷¹. Eptifibatide and tirofiban are also antiplatelet agents, given that they are α IIb β 3 antagonists. The former is a cyclic heptapeptide of less than 1 kDa, and a potent inhibitor of fibrinogen binding to platelets. The latter is a short-acting, low molecular weight peptide, which in humans binds reversibly, and apparently with low affinity, to α IIb β 3⁷². The latter drugs have not been standardized for human use, since they do not reach the desired effect in most patients, and have therefore been relegated.

Conclusion

Atherosclerosis is a disease with thrombotic and inflammatory characteristics. The study of atherosclerosis in humans is limited and it is generally obtained from autopsy dissections or measurements of some molecule increase in blood in response to a stimulus. Platelets, which most times are only known for their participation in thrombosis, play an important role both in early and advanced stages of atherosclerosis, when there is a rupture and subsequent exposure of the sub-endothelial matrix. The role of platelets is not limited to thrombosis or hemostasis, but they have an active participation in the inflammatory process, since they activate cells such as monocytes, neutrophils, lymphocytes and dendritic cells, among others, which favor an inflammatory microenvironment that helps atherosclerosis progression. Platelets have the capability to form aggregates and secrete cytokines and chemokines that act on both inflammatory cells and the endothelium, promoting for it to change to a pro-inflammatory and pro-atherosclerotic state. These processes involve various signaling pathways that trigger the activation of adhesion molecules, integrins and G-protein-coupled receptors. Said pathways have contributed to the development of pharmacological therapies, but their complexity has precluded the development of more effective drugs. Anti-inflammatory treatment for this condition is only partially represented by statins. This opens the possibility for

trying to inhibit or control the inflammatory state with new treatment strategies, in addition to the development of new atherosclerosis-selective anti-inflammatory drugs.

Conflict of interests

The authors of this article declare that they have no conflicts of interest.

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Ethical disclosure

Protection of people and animals. The authors declare that no experiments were performed on humans or animals for this research.

Confidentiality of data. The authors declare that no patient data appear in this article.

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