Platelets and lipoproteins: how much do we know about their interactions?

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Introduction

The fact that platelets and lipoproteins play a key role in the development of atherosclerosis has become more and more established recently.1,2

The formation of vascular lesions is characterized by invasion and migration of vascular inflammatory cells and expression of potential mediators within the vessel wall, and also thrombosis, low density lipoprotein (LDL), especially its modified forms (e.g. oxidized LDL) and other risk factors accompany this process.3,4 Since platelets are directly involved in the development of atherosclerotic lesions, the interaction of platelets with other plasma constituents especially with lipoproteins and apoproteins has become of special interest.5

This review clarifies the influence of lipoproteins upon activation and metabolic behavior of platelets.

Differences in the lipoprotein composition between normal and familial hypercholesterolemic subjects have been shown and platelet composition and functions have been found to change in hyperlipidemia.5,6 Platelets from type II hypercholesterolemic patients indicate an increased sensitivity to aggregating agents such as epinephrine, ADP and collagen. Enhanced levels of platelet derived growth factor, platelet factor 4 as well as higher levels of thromboxane A2 and malondialdehyde have been reported in hypercholesterolemic patients.10 At the same time, it has been observed that arachidonic acid mobilization from phospholipids and fibrinogen binding to platelets are enhanced but membrane fluidity is decreased in hyperlipidemia.11

Similar results were obtained by other groups, indicating an increased aggregability of platelets from type II hypercholesterolemia. However, administration of lipid lowering drugs with hypercholesterolemic patients reverse platelet hyper-reactivity.12

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Recently, in our study we have suggested that in hyperlipidemia, platelets are in their active form in circulation and increased platelet lipid peroxidation, apoptosis, platelet-leukocyte aggregate formation and platelet aggregation altogether accompany this process. We also pointed that increasing platelet MDA level thus increasing oxidative stress triggers platelet apoptosis and activation in hyperlipidemia.\(^{13}\)

The effect of low density lipoproteins on platelet function has been provided by in vitro and in vivo studies.\(^{1,6,7,14}\) The investigators have found that LDL, VLDL and especially ox-LDL, which all contain apoprotein B-100, are atherogenic lipoproteins and increase platelet activation.\(^{15}\) Additionally, LDL and VLDL sensitize platelets to agonists if these lipoproteins are added to PRP or they are interacted with isolated platelets.\(^{16}\) The same effect was observed when LDL was added to whole blood and platelet function was measured with filtragometry.\(^{17}\)

Agonists such as thrombin reinforce the effects of LDL ve VLDL on platelet activation. While platelet membrane fluidity has negative correlation with VLDL-cholesterol levels, it has positive correlation with HDL cholesterol levels.

Low density lipoprotein has been shown to act as an independent agonist on platelets at high concentrations and to enhance the action of other well known strong promoters of platelet aggregation at lower concentrations.\(^{16}\)

A more detailed investigation revealed that atherogenic triglyceride-rich lipoproteins such as VLDL increases in diabetic dyslipidemia, enhances platelet thrombaxane A2 production and causes increased collagen-mediated platelet aggregation.\(^{18}\) In vitro studies have suggested that differences of LDL particle size may effect the development or progression of atherosclerosis. Since susceptibility to copper-induced oxidation, free radical generation and high affinity to arterial wall proteoglycans were reported to be greater for small dense LDL (s-LDL), investigators emphasized that s-LDL is atherogenic and more significant risk factor for vascular diseases than LDL-cholesterol.\(^{19}\)

Recently we have found that s-LDL particles affect ox-LDL levels and platelet functions including platelet activation and apoptosis response (unpublished data).

LDL, as an independent agonist, induces the increase of cytosolic Ca\(^{2+}\) and inositol phosphate derivatives at 10 mg/dl concentration, and also the shape change and aggregation of platelets at physiological concentrations.\(^{20}\)

Previous findings have shown that LDL also causes degradation of platelet dense granules, thus it leads to secondary aggregation of platelets and increased superoxide anion production by platelets.\(^{21,22}\) Since hypercholesterolemic platelets have lower sensitivity to prostacyclin, it can be stated that LDL impairs the inhibitory effects of prostacyclin on the aggregation of platelets.\(^{23}\)

Besides these effects, LDL enhances the induction of mitogen-activated protein kinase (p38 MAPK) which is the initial event in LDL-induced cellular signaling in platelets. In addition to this, LDL inhibits the Na+/H+antiport activity in platelets by stimulating p38 MAPK.\(^{24}\) It has been shown that LDL increases GpIIb/IIIa and P-selectin (\(_{-}\)granule membrane protein of platelets) expression in platelets.\(^{25}\) Similar results were obtained from our previous studies. We found that GpIIb/IIIa, GpIIIa and P-selectin receptor numbers increased significantly in hypercholesterolemic patients using flow cytometric method.\(^{26}\)

In vitro studies, after preincubation of platelets with LDL, dose dependent increase has been found in fibrinogen molecules per platelet.\(^{27}\)

Native LDL contains a great amount of easily oxidized unsaturated fatty acid and LDL undergoes some kind of modification with copper, iron or chemical acetylation or glycation. Besides, LDL apoproteins, especially apoB100 are degraded and free radicals play a major role in this process. Thus, modified LDL is recognized by scavenger receptors which is distinct from LDL receptors. Modified form of LDL is taken up more rapidly by the monocyte/macrophages, it doesn’t undergo to feedback regulation and generates the lipid droplet rich foam cell.\(^{28}\) On the other side, ox-LDL induces monocyte adhesion to endothelium, migration, proliferation of smooth muscle cells, prothrombinase complex activity on platelet membrane, injures cells (necrotic and apoptotic pathways), interferes endothelium-mediated relaxation and promotes procoagulant properties of vascular cells.\(^{29,30}\) Cu\(^{2+}\) ox-LDL enhanced tumor necrosis factor-induced expression of vascular cellular adhesion molecule-1. Minimally modified-LDL increased the intracellular stores of P-selectin without changing the level of the surface expression, whereas
Cu2+ ox-LDL caused redistribution of intracellular P-selectin to the cell surfaces.30

It has been reported that Ox-LDL binds to scavenger receptors such as CD36, LOX-1, SR-BI in macrophage and other cells.31

In fact, it has been demonstrated that ox-LDL reduces platelet and macrophage nitric oxide synthase (NOS) expression thus, the decrease in NO further increases platelet activation and cellular production of O2 radical. These events lead induction of adhesion and aggregation and finally facilitate platelet dependent arterial thrombosis.32,33 Several results indicated that Lp(a) also is oxidized in a more extent than LDL and induces p-selectin expressions.34

Several observation shows glycated modified LDL triggers apoptosis in endothelial cells.35 Recently in our study, we determined that purified ox-LDL and native LDL enhanced platelet activation, platelet lipid peroxidation and apoptotic process, whereas HDL had reverse effects on platelet P-selectin expressions and lipid peroxidation status.36

In fact, contrary to LDL, HDL has antiatherogenic effect on platelet functions and also inhibits fibrinogen binding to monocytes and prevents development of atherosclerosis.37 HDL protects LDL against oxidative modification. ApoA-I, a key constituent of HDL, serves as a cholesterol scavenger in a process called reverse cholesterol transport. Since NO synthase is well-recognized as a regulatory mechanism for platelet hemostasis, apo E-rich HDL markedly elevates platelet NO synthase activity and intraplatelet levels of cGMP thus, inhibits platelet aggregation.38

Lipoproteins affect platelets via specific binding receptors on the platelets. However, there have been certain opinions concerning these binding regions. There are LDL and ox-LDL receptors on the platelet surface which are different from the classical receptors found on other cell types.39

The current studies reported that altered platelet LDL binding sites might be responsible for platelet reactivity in hypercholesterolemic patients.3 Curtis and Plow proposed that LDL was found to be a poor inhibitor of 125I-HDL binding to platelets, whereas HDL was found to be an effective inhibitor of 125I-LDL binding.39 In a recent study of ours, we first pointed out that HDL binds to platelets via Apo A-I in hypercholesterolemic subjects and GpIIb/IIIa mediated to this binding.39 In another study, GpIIb/IIIa complex has been proposed to be the platelet receptor for HDL3 which is associated with phospholipase D activation and diacylglycerol formation.41 On the other hand, in a flowcytometric study held by Tetik S. et al, it was shown that FITC-conjugated HDL does not bind to purified GpIIb/IIIa complex whereas binds to a receptor other than fibrinogen receptor in isolated platelets.42 Nofer et al clarified that, HDL has antiatherogenic effect on platelet membrane through ATP dependent transporter (ABCA 1).37

Barre et al indicated that Lp(a) inhibits platelet aggregation by displaying fibrinogen from its receptor and decreased platelet aggregation.43 It was reported that gold-labeled LDL enhances fibrinogen binding to platelets. Additionally, LDL sensitizes platelets via binding apo B100 to receptors on platelets and via transfer of lipids to platelet membrane.44 There are evidences regarding the effects of LDL on platelets via outside-in signalling through GpIIb/IIIa receptors.39 Tetik S et al showed that purified GpIIb/IIIa caused inhibition of LDL-FITC binding to isolated platelets and GpIIb/IIIa is a platelet LDL receptor 44. Furthermore, several studies demonstrated that GpIIb/IIIa acts as a receptor for ox-LDL in platelets, this way might be the first step in platelet activation by plasma lipoproteins.45 These findings agree with our previous observations. We have shown that isolated LDL and ox-LDL increase fibrinogen binding to platelets depending on the dose, whereas both of them inhibit (100%) the binding of GpIIb/IIIa antibody to platelets. As for HDL, it reverses the effect of LDL and ox-LDL on fibrinogen binding. According to our findings, GpIIb/IIIa is a binding region on the platelet surface for LDL and ox-LDL, but the binding domain of these lipoproteins on GpIIb/IIIa is different from the domain of fibrinogen (unpublished data).

There are also inconsistent results about ox-LDL binding sites. Therefore it was suggested that mildly oxidized LDL (mox-LDL) stimulates two signal transduction pathways in platelets through activation of LPA (lysophosphatidic acid) receptor independent from GpIIb/IIIa. These pathways are the Scr family kinase mediated stimulation of protein tyrosine phosphorylation and stimulation of Ca2+ influx.46

Pedreno et al. clarified that platelet LDL receptor recognizes both ox-LDL and native LDL with the same affinity but these sites are independent from GpIIb/IIIa.47 In another study held by the same group,
binding of native and ox-LDL to platelet G-protein coupled receptors were detected.\(^{46}\)

In a study carried out with radio-iodinated modified LDL, Wolf et al reported that binding of oxidatively modified LDL (thus apo B100) to the platelet surface is not essential for platelet stimulation whereas these platelet stimulating effect may be related biologically active substances which had formed in the lipid phase during the oxidation process.\(^{47}\)

It was suggested that thrombospondin 1 receptor (GpIV,CD36) plays a role in uptake of oxidized lipoproteins by macrophages and platelets during arterial atherogenesis, at the same time this receptor has a key role in VLDL binding to platelets.\(^{10}\)

Interestingly, Lp(a) also binds to platelet membrane which is GpIIb domain, RGD sequence in Lp(a) does not interfere its binding.\(^{51}\)

As its known, platelets and umbilical vein endothelial cells are activated and adhesion of platelets to endothelial layer are enhanced in hyperlipidemia where GpIIb/IIIa, von willebrand factor (VWF), intracellular adhesion molecule 1 (ICAM-1) and P-selectin mediate to this pathway. Therefore, binding sites of these adhesion ligands and effects on platelet-endothelium interaction are quite important. In recent years, there have been various studies regarding platelet-endothelium adhesion regions where lipoproteins take place in these interactions and therapeutic targets are being focused on these binding sites to prevent the development of premature atherosclerosis.\(^{51}\)

We are just beginning to learn something about modified platelet function whether it is related with lipoproteins or age related disease or structural changes in vascular wall or acquired thrombic features or interactions among each other and research on these matters will be still of great interest.

References


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