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Review

Low-density lipoprotein and its effect on human blood platelets

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Abstract. Events leading to hyperactivity of human blood platelets are accompanied by an enhanced risk of atherosclerosis and arterial thrombosis. Lipoprotein disorders affect platelet functions, and hypersensitive platelets are observed in various stages of hyperlipidemia. Low-density lipoprotein (LDL), a circulating complex of lipids and proteins that is increased in hypercholesterolemia, enhances platelet function and increases sensitivity of platelets to several naturally occurring agonists. LDL sensitizes platelets via binding of apoB-100 to a receptor on the platelet membrane and via transfer of lipids to the platelet membrane. The receptor that mediates binding of LDL to the platelet and initiates subsequent intracellular signaling cascades has not yet been identified. Modification of native LDL generates a platelet-activating particle, and this interaction might contribute to the development of the atherosclerotic plaque. Lysophosphatidic acid is formed upon mild oxidation of LDL and is responsible for subsequent platelet activation induced by the modified LDL particle. Thus, LDL changes the functions of platelets via a broad spectrum of interactions.

Key words. Thrombocyte-LDL interaction; aggregation; native and modified LDL; receptor; signaling pathway.

Introduction

Lipoprotein-related disorders are caused by abnormalities in the synthesis or processing of plasma lipoprotein particles. Cells may bind low-density lipoprotein (LDL) particles, endocytose and transfer them to lysosomes where the receptor releases the particle. Subsequently, the receptor is recycled back to the cell surface. Components of LDL particles are hydrolyzed, and free cholesterol is used for cell function and stability or deposited intracellularly as cholesterol ester. After sufficient cell loading with cholesterol, the rate of LDL uptake becomes downregulated, which is apparent as a lower number of cell surface receptors. This process is mediated by receptors that belong to the family of the LDL receptors. The LDL-receptor family consists of a growing number of members that share structural similarity and function in endocytosis [1].

The classical LDL receptor detected on fibroblasts is also termed the apolipoprotein (apo) B/E receptor. Defects in receptor-ligand interaction correlate with high plasma LDL concentrations and an increased risk for cardiovascular disease [2]. An example of a defect in the LDL pathway is the R3500Q mutation in the apoB-100 protein, which impairs the binding of LDL to the receptor in pa-

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tients with familial defective apoB-100 [3]. An example of a defect in LDL processing is a mutation in the LDL-receptor gene in patients with familial hypercholesterolemia, again resulting in diminished LDL binding.

Human blood platelets play an important role in the pathogenesis of atherosclerosis and acute coronary syndromes. The increased hypersensitivity and hyperaggregability of platelets from hypercholesterolemic patients detected in vivo and in vitro suggest that high levels of atherogenic lipoproteins, e.g. LDL, may alter platelet function [4–6]. Indeed, LDL sensitizes platelets in vitro to a variety of stimulating agents such as ADP, collagen and thrombin [7–15].

Binding of native LDL to platelets

Binding of cholesterol-rich LDL to the membrane is the first step in the initiation of alterations in platelets that make them more responsive to activating agents [5]. The question whether this step involves a specific receptor or merely includes perturbations of the membrane bilayer is still unanswered. Binding studies using ^{125}I-labeled LDL revealed the presence of specific and saturable binding sites on nonstimulated platelets, ranging from 1000 to 8000 sites per platelet (table 1) [16–21]. The fact that the binding capacity, i.e. K_d and B_{max} values for LDL to the platelets, was similar to that reported for LDL binding to other cells suggested the presence of similar binding proteins and receptors for LDL on platelets [5]. However, reports on how different lipoprotein classes interfered with each other were controversial, with some reports showing inhibition of LDL binding by antiatherogenic high-density lipoprotein (HDL) and vice versa [20, 21], while others showed minimal interference [8, 16]. Curtiss and Plow [21] reported competition between HDL and LDL but suggested the presence of two binding sites for lipoproteins, an HDL binding site that interacts poorly with LDL and an LDL binding site that reacts with or is altered by HDL binding. The capacity of HDL to inhibit LDL binding was not mediated by apoE. Koller et al. [20] also proposed distinct binding sites for LDL and HDL, as the nature of inhibition seemed not to be simple competition based on analysis of binding kinetics. However, after ligand blotting, they concluded that LDL and HDL bound to a single class of lipoprotein-binding proteins in the platelet membrane [22]. The lipoprotein binding sites showed higher affinity for HDL than LDL, and the lipoproteins interfered with binding of each other in a noncompetitive manner.

Table 1. Binding properties of native LDL to human blood platelets.

K_D (mol/l)	B_{max} molecules/platelet	Reference
$1.6 \pm 0.5 \times 10^{-8}$	1470 ± 640	20
$4.0 \pm 1.8 \times 10^{-8}$	7075 ± 4800	21
2.0×10^{-8}	1965 ± 177	19
$5.0 \pm 0.9 \times 10^{-8}$	1348 ± 126	17
$6.0 \pm 2.1 \times 10^{-8}$	2940 ± 860	18

Means ± SD are given.

LDL binding to platelets was independent of divalent ions, as EDTA treatment did not alter LDL binding [21]. This characteristic, however, differs from the binding characteristics of the classical LDL receptor through which LDL is removed from the circulation via holoparticle uptake [2]. Furthermore, an antibody directed against the ligand binding domain of the classical LDL receptor did not alter the binding of LDL to platelets [17]. Taken together, these findings support the concept that LDL binding sites on platelets differ from the apoB/E receptors on nucleated cells. Studies of LDL-platelet interaction after enzymatic treatment revealed a different pattern of proteolytic susceptibility between the 'platelet LDL receptor' and the classical LDL receptor; protease treatment abolished LDL-binding to fibroblasts but not to platelets [23]. Hence, a platelet receptor that is resistant to proteolytic digestion might be involved in LDL binding to platelets. Pedreno et al. [24] reported that LDL binds to a receptor which is coupled to a pertussis toxin-sensitive G protein. Ligand-binding assays demonstrated that high levels of LDL (1.5 g/l) downregulate the number of binding sites, suggesting a conformational change of the receptor or even the possiblity of internalization of receptors. This downregulation was reversible and time- and dose-dependent, and inhibited by protein kinase C inhibitors.

Attempts to identify candidate LDL-binding proteins by ligand-blotting experiments revealed that LDL binds to platelet membrane proteins with molecular masses between 115 and 156 kDa [19, 22]. Purification and immunochemical characterization identified these proteins as glycoprotein IIb (CD41) and glycoprotein IIIa (CD61). Both are constituents of the glycoprotein IIb-IIIa complex, also known as integrin αIIbβ3, which is the receptor for fibrinogen and a few other adhesive proteins [25]. Immunoelectron microscopy revealed colocalization of gold-labeled LDL with fibrinogen on platelets [26], confirming the concept that the fibrinogen receptor could also act as a possible LDL receptor on the platelets. These observations appear in agreement with findings that LDL increases the exposure of fibrinogen binding sites on platelets [7, 26] and that platelets from familial hypercholesterolemia patients – compared with platelets from normolipidemic subjects – bind more fibrinogen [27].

In divergence from the concept that integrin αIIbβ3 serves as an LDL receptor on platelets, Hackeng et al. [28] showed that specific antibodies directed against integrin αIIbβ3 failed to inhibit LDL binding to the

platelets. Furthermore, similar binding characteristics were found with platelets from controls and patients suffering from type I and II Glanzmann's thrombastenia, who are deficient in integrin αIIbβ3. Together, these observations argue against a role for integrin αIIbβ3 as the putative LDL receptor on the platelets [28, 29]. Also the fact that a single platelet contains about 50,000 surface-exposed copies of this integrin, which is about 10-fold more than the number of LDL receptors identified in ligand binding studies appears in conflict with such a role. Although these studies propose that αIIbβ3 might not act as an LDL receptor on the platelets, they did demonstrate that this integrin contributes to LDL-induced platelet sensitization via ligand-induced signal generation in a process known as outside-in signaling [30].

Recently, Riddell et al. [30] demonstrated that platelets and the megakaryocytic cell lines MEG-01 and HEL express LRP8, a new member of the LDL-receptor family. This receptor differs from the LRP receptor, which is absent in platelets [31]. Platelet LRP8 is a variant of the previously identified LDL-receptor family member apoER2 that is predominantly expressed in the brain. The extracellular domain of apoER2 contains seven to eight ligand-binding domains of which domains 4–6 are absent in platelet LRP8. LDL-receptor family members function in receptor-mediated endocytosis and signal transduction. ApoER2 is known to bind apoE, but not the major apolipoprotein of LDL, apoB-100. The cytoplasmic tail of apoER2 contains only a single NPXY motif, which is known as an endocytosis motif. However, compared with other members of the LDL-receptor family, the rate of endocytosis mediated by apoER2 is low, suggesting that this domain plays a role in intracellular signal transduction [30, 32]. The fact that antibodies directed against the binding domain of apoB-100 for the apoB/E-receptor, the so-called B site, impaired binding of LDL to the platelets [33] supports the hypothesis that a member of the LDL-receptor family mediates binding of LDL to the platelet via specific domains in the apolipoprotein moiety. This hypothesis is confirmed by the fact that the LDL-receptor binding site for apoB-100 induced platelet signal transduction via the platelet-LDL binding site. This might point towards the presence of a yet unidentified member of the LDL receptor on the outer leaflet of human platelets. Alternatively, the platelet LRP8 receptor might be capable of binding apoB-100 and be responsible for LDL-induced platelet sensitization.

After prolonged platelet-LDL contact, the signaling pathway induced by LDL binding to the platelet LDL-receptor becomes desensitized [33]. This is in agreement with the previously reported reversible downregulation of binding sites for LDL and the observation that the LDL signaling pathway via a pertussis toxin-sensitive G-protein-coupled receptor becomes desensitized [24].

Lipid transfer between LDL and platelets

Apart from apoB-100, LDL consists of a lipid portion containing free and esterified cholesterol, as well as triglycerides and phospholipids. Each of these constituents has the potential to change the composition of platelet membranes, thereby altering platelet dynamics and function. The combined pools of sphingomyelin, phosphatidylcholine and phosphatidylethanolamine comprise more than two-thirds of the total phospholipid content of lipoproteins. A similar composition is found in platelets [34]. Phospholipids play a role in signal transduction events in platelets. Cytosolic phospholipase A_2 cleaves fatty acids from phospholipids, e.g. phosphatidylethanolamine and phosphatidylcholine, leading to generation of bioactive eicosanoids. Thus, for proper signal transduction, phospholipids have to be generated. This can be accomplished by resynthesis or remodeling of phospholipids at the level of the cell membrane or by incorporation of phospholipids from circulating lipoproteins. Thus, a second mechanism by which LDL alters platelet functions is by changing the composition of phospholipids, ether-phospholipids in particular, which are preferential targets for dietary ω-3 polyunsaturated fatty acids [35–37]. Platelets trap diacyl-, alkylacyl- and alkenylacyl-glycerophospholipids from vesicles by simple diffusion [38], and a similar mechanism might occur when lipoproteins act as lipid donors. Engelmann et al. [39] have shown that LDL rapidly donates sphingomyelin, phosphatidylcholine and phosphatidylethanolamine to platelets [39]. Platelet agonists increase the specific transfer of ethanolamine phospholipids from LDL to the platelet membrane via a mechanism that is partially prevented by inhibitors of protein kinase C [40]. Furthermore, LDL directly contributes to the formation of platelet eicosanoids by supplying both phospholipid-bound or free arachidonic acid [41, 42].

Signaling by LDL

The underlying mechanisms of enhanced platelet activation by LDL are multifactorial, and inhibition of the Na^+/H^+ antiport that regulates the cytosolic pH and activation of the phosphatidyl inositol cycle by LDL have been addressed [43–45]. Enhanced activation of protein kinase C, phosphorylation of the protein kinase C substrate pleckstrin (47 kDa), Ca^{2+} influx and mobilization from the dense tubular system and subsequent stimulation of phospholipase A_2 by LDL have been reported [43, 44, 46–48].

Hackeng et al. [49] reported that a first step in platelet sensitization by LDL is the release of arachidonic acid via activation of p38 mitogen-activated protein kinase ($p38^{MAPK}$) and subsequent activation of cytosolic phospholipase A_2. $P38^{MAPK}$ is a member of the family of pro-

line directed serine/threonine kinases, which is activated by the simultaneous phosphorylation of Thr^{180} and Tyr^{182} (fig. 1) [50]. This activation was not inhibited by a wide variety of inhibitors of platelet signaling, including Ca^{2+} mobilization, phospholipase C activation, thromboxane A_2 formation and extracellular signal-regulated kinase 1/2 activation, indicating that $p38^{MAPK}$ is upstream of several platelet signal transduction pathways and therefore an early step in the signaling cascade initiated by LDL. Inhibition of protein kinase C slightly reduced LDL-induced $p38^{MAPK}$ phosphorylation, suggesting a modulating role for this kinase. The LDL-induced $p38^{MAPK}$ phosphorylation is under control of cyclic AMP (cAMP), as addition of agents that raise intracellular cAMP levels (prostaglandin I_2 and dibutyryl cAMP) prevented LDL-induced $p38^{MAPK}$ phosphorylation. The transduction of signals generated by LDL into the cell was mediated neither by integrin $\alpha 2\beta 1$ nor by FcgammaRIIa, which are both known to signal towards $p38^{MAPK}$ after receptor activation. Candidate platelet LDL receptors such as integrin αIIbβ3, CD36 (platelet glycoprotein IV) and CD68 (gp110) were not implicated in LDL-induced $p38^{MAPK}$ activation either.

P125FAK is a focal adhesion kinase implicated in signaling pathways mediated by integrins, G-protein-coupled receptors, tyrosine kinase receptors, and the v-Src and v-Crk oncoproteins. Phosphorylated FAK is the docking site for signaling proteins such as the p85 regulatory subunit of phosphoinositol 3-kinase and phospholipase Cγ [51, 52]. LDL induced p125FAK phosphorylation in a dose- and time-dependent manner independent of integrin αIIbβ3, since platelets from patients with Glanzmann's thrombastenia showed the same phosphorylation pattern of p125FAK as did control platelets [53]. Similar to $p38^{MAPK}$, LDL signaling to p125FAK was independent of integrin $\alpha 2\beta 1$, the FcgammaRIIa receptor and the lysophosphatidic acid (LPA) receptor, and not affected by inhibitors of cyclooxygenase-1, protein kinase C, extracellular signal-regulated kinase 1/2 or $p38^{MAPK}$. Furthermore, precipitation of activated small GTPases revealed that LDL activated Rap1 and Ral but not Ras [54].

In summary, the mechanism by which LDL affects platelets is likely to be based on two processes. First, there is binding and activation of an LDL receptor on the platelets that appears to differ from the classical LDL receptor on nucleated cells. Second, there is exchange of lipids between LDL particles and the plasma membrane. Both processes appear to alter signal transduction cascades in the platelet, resulting in an increased sensitivity to platelet-activating agents.

Lipid-lowering treatment

Drugs that inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase – the rate-limiting enzyme in endogenous

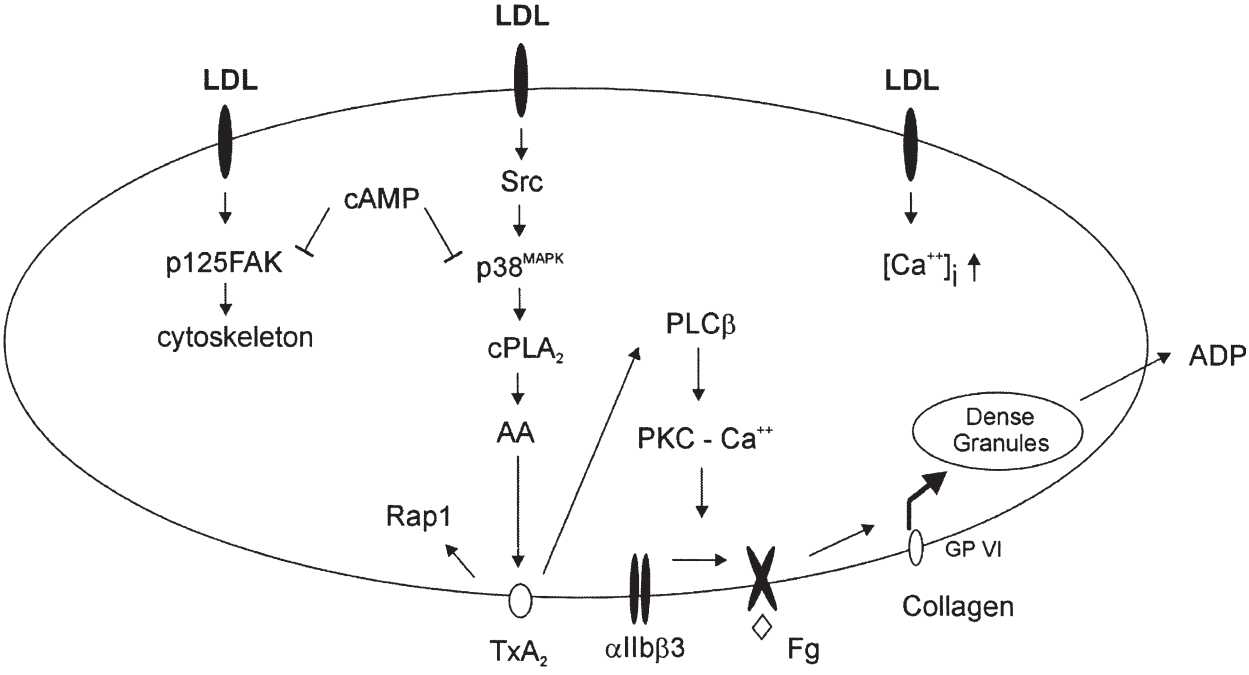

Figure 1. Signaling pathways in human platelets induced by LDL. LDL interacts with its receptor on the platelet membrane and initiates phosphorylation of focal adhesion kinase (FAK). A second pathway is mediated by $p38^{MAPK}$, which results in enhanced fibrinogen binding to integrin aIIbβ3 via phospholipase A_2 (PLA_2), arachidonic acid (AA), thromboxane A_2 (TxA_2), phospholipase Cβ (PLCβ) and protein kinase C (PKC). In addition, LDL induces an increase in intracellular Ca^{++}. These signals lead to exposure of αIIbβ3 and fibrinogen (Fg) binding and secretion via a mechanism enhanced by outside-in signaling via αIIbβ3.

cholesterol biosynthesis pathway – have proven useful in secondary prevention in patients with myocardial infarction or coronary heart disease [55]. In agreement with this observation, platelet reactivity and arachidonic acid metabolism in hyperlipoproteinemia are sensitive to lipid-lowering intervention [56, 57]. Apart from reducing platelet aggregation via lowering of platelelet cholesterol, lovastatin, simvastatin and fluvastatin reduced platelet aggregation via a second effect of the drug binding on the platelets, since inhibition did not correlate with the change in platelet cholesterol. A third possibility is that statins alter the LDL particle thereby interfering with LDL binding to platelets [58–60]. Fluvastatin also decreased the membrane activation markers P-selectin (CD62P) and CD63, reflecting reduced platelet activity in type II hypercholesterolemic patients ex vivo [61]. Schrör et al. [62] reported that reduction of total and LDL serum cholesterol by simvastatin normalized platelet function in patients who suffered from familial hypercholesterolemia. Platelets from untreated patients who suffered from familial hypercholesterolemia showed enhanced aggregation responses, secretion of ATP and release of thromboxane after stimulation by collagen and ADP [6]. Simvastatin treatment significantly decreased these responses to the range found in normocholesterolemic subjects [62, 63]. After 8 and 12 weeks of intake of pravastatin, ADP-induced platelet aggregation, thromboxane B_2 and expression of P-selectin were reduced [64]. CD40L, which is upregulated in hypercholesterolemia and expressed on activated platelets, is also downregulated by extensive pravastatin and cerivastatin treatment [65]. CD40L is a member of the tumor necrosis factor family of ligands and appears to be an $\alpha_{IIb}\beta_3$ ligand and necessary for stability of arterial thrombi [66]. Pravastatin treatment increased the activity of the Na+/K+ pump in hypercholesterolemic patients via cholesterol depletion of the platelet membrane [67]. Platelet-procoagulant activity was reduced by administration of simvastatin, atorvastatin, cerivastatin and prolonged treatment with pravastatin and fluvastatin. However, there was no correlation with LDL-cholesterol changes, which might indicate that there was a direct effect on the platelets [68]. Duration and dose of statin administation appeared critical for the influence on platelets, as short-term pravastatin therapy (4 weeks) had no effect on thromboxane B_2 production or even enhanced platelet aggregability ex vivo and increased plasma serotonin levels [69, 70]. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors may prevent signaling via the Ras and Rho pathways in platelets that are dependent on isoprenylation, thereby preventing signaling via these pathways. In platelets, Ras is activated by the G-protein-receptor agonist thrombin, the glycoprotein VI agonist convulxin and the cytokine receptor Mpl agonist thrombopoietin [71]. In many cell types Ras activation starts signaling to the $p42^{MAPK}$ ERK2 and further signal transduction to the nucleus, but in the a-nucleated platelet the role of Ras is still unknown [72]. Possibly, active, GTP-bound Ras contributes to the regulation of glycoprotein IIb-IIIa (integrin $\alpha_{IIb}\beta_3$), which is the binding site for fibrinogen and mediates platelet aggregation. A second small GTPase that might be affected by cholesterol-lowering therapy is Rho, which signals to myosin light chain phosphatase, cytoskeletal rearrangements and platelet shape change [73]. At present it is uncertain whether these platelet functions are disturbed when cholesterol synthesis is attenuated by administration of statins.

In summary, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors lower plasma cholesterol levels. Current evidence indicates that this is not the only explanation for a reduction in platelet function, and other mechanisms are likely to contribute to this inhibition.

Platelet interaction with modified LDL

Modification of LDL and subsequent uptake by scavenger receptors on monocyte-derived macrophages results in lipid-laden foam cells and is thought to initiate and accelerate the development of an arterial lesion. A characteristic of scavenger receptors is that the binding of modified LDL does not trigger downregulation of receptor expression and that uptake proceeds without downregulation until the cells transform to foam cells. The first example of a modified LDL particle that bound to scavenger receptors on macrophages was acetylated LDL, a form of LDL that is not observed under physiological conditions. Acetylated LDL was taken up by macrophages without inducing downregulating receptor numbers, indicating that the binding was independent of the LDL receptor [74]. There are several modifications of LDL such as glycation and aggregation of LDL particles that can enhance uptake by macrophages in vitro and in vivo, but most studies have focused on oxidation of LDL. Several scavenger receptor subtypes have been identified, such as scavenger receptor class A (AI and AIII) and class B (B-I and CD36). Scavenger receptors on macrophages bind acetylated LDL and different forms of oxidized LDL (ox-LDL). Platelets contain an ox-LDL receptor that does not bind acetylated LDL [75]. CD36 has been identified as a scavenger receptor on platelets. It consists of two membrane-spanning domains with both the N- and C-terminal domains located at the cytosolic side [76]. Monocyte-derived macrophages from CD36-deficient patients showed decreased binding and uptake of ox-LDL, illustrating that CD36 functions in the binding of ox-LDL. Antibodies directed against the ox-LDL-binding domain of CD36 (155–183) reduced ox-LDL binding to platelets, thereby identifying CD36 as an ox-LDL receptor on platelets [75].

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) – a novel ox-LDL receptor – has also been recently identified on platelets and megakaryocytic cell lines [77]. LOX-1 is a type-II membrane protein that belongs to the C-type lectin family and is translocated to the membrane upon thrombin stimulation by fusion of α granule membranes with the plasma membrane. Analysis of binding proteins for ox-LDL revealed that CD36 and LOX-1 served as major binding proteins in platelets. As resting platelets exposed little LOX-1, CD36 might be the ox-LDL receptor on resting platelets. In contrast, LOX-1 might be the dominant receptor involved in ox-LDL binding to activated platelets [77].

During oxidation of LDL, heterogeneous modification occurs as diverse radical-mediated chemical changes occur both in the lipid and the apoB-100 moiety. Hydroperoxides, primary products of lipid peroxidation, oxysterols as well as a variety of secondary products are generated. Many of these are aldehyde-containing products, e. g. malondialdehyde and hydroxynonenals, which modify apoB-100 side chains that may alter the binding properties of LDL and thereby the reactivity of platelets [78, 79]. In addition, oxidation of apoB-100 leads to modification of amino acid side chains, cleavage of amide linkages and cross-linking between amino acids.

In vivo, different reactive oxygen and nitrogen species (such as superoxide anion, hydrogen peroxide, hydroxyl radical, hypochlorous acid, peroxinitrite), lipid hydroperoxides and the products of enzymatic pathways, including lipoxygenases and myeloperoxidase, contribute to the development of atherosclerosis. Oxygen-free radicals that are generated by platelets may enhance or initiate platelet aggregation and secretion [80, 81]. The involvement of lipid peroxidation in platelet signaling is illustrated by the peroxidation of arachidonate by cyclooxygenase 1, leading to endoperoxide and thromboxane formation [82, 83]. In particular, the effect of isoprostanes, eicosanoids that are nonenzymatic products of peroxidation of arachidonyl-containing phospholipids catalyzed by free radicals, have been shown to enhance platelet aggregation and secretion [84, 85].

In in vitro studies, copper-oxidized LDL (ox-LDL) is frequently used as an experimental tool to study the effect of oxidation of LDL on platelet functions [(reviewed in [5]) [86–90]. Ox-LDL inhibited the plasma membrane Ca^{++}-ATPase, resulting in an increase in cytoplasmic Ca^{++} and increased platelet sensitivity to agonists [87]. Furthermore, incubation of platelets with ox-LDL induced shape change and pseudopodia formation [91]. Ox-LDL activated platelets and accelerated adhesion. This raises the possibility that oxidation of LDL contributes to thrombosis and arteriosclerosis [91]. However, in other studies ox-LDL failed to affect platelet function [92]. During oxidation of LDL, both the lipid and the protein moiety undergo chemical changes via radical-mediated reactions. During the modification, chemically active lipid and apolipoprotein degradation products are formed in ox-LDL. This extensive damage of LDL inflicted by copper-induced oxidation leads to an unreproducable pattern of platelet–ox-LDL interactions. In particular, oxidized derivates of cholesterol generated by different modes of oxidation, such as specific enzymatic oxidation, chemical oxidation, autooxidation and lipid peroxidation, may act either as inhibitors or potentiators of platelet aggregation [93, 94]. The degree of oxidation as well as consumption of antioxidants has not always been characterized by appropriate analytical techniques.

Mildly oxidized or minimally modified LDL (mox-LDL) enhanced platelet aggregability and release reaction to a greater extent than heavily ox-LDL [89, 92]. Weidtmann et al. [92] reported that activation of platelets by mox-LDL occurred through a phospholipase A_2 and cyclooxygenase-dependent pathway. Native LDL contains minimal amounts of LPA, and upon mild oxidation the LPA content increases eight-fold [95]. In vivo, mild oxidation of LDL is caused by radical formation by activated macrophages and endothelial cells. LPA is present in extracellular lipid deposits and foam cells of human atherosclerotic lesions where modified lipoproteins may accumulate and is therefore considered a biologically relevant component. LPA is exposed on the surface of mildly oxidized LDL particles and interacts with LPA receptors on the platelet membrane, thereby initiating platelet activation. Platelets contain the LPA receptors LPA_1, LPA_2 and LPA_3 [96]. LPA receptors are seven transmembrane receptors coupled to the G proteins G_i, which inhibits adenylyle cyclase and thereby formation of cAMP; G_{13}, which signals to the small GTPase Rho and regulates platelet shape change and G_q, an activator of phospholipase $C_{\beta II}$, which signals to Ca^{2+} mobilization and protein kinase C (reviewed in [96]). Antagonists of the LPA receptor prevented platelet activation induced by mox-LDL [95]. However, LPA was not involved in sensitization of platelets induced by native LDL, as the LPA receptor blocker *N*-palmitoyl-L-serine-phosphoric acid failed to affect LDL-enhanced fibrinogen binding to platelets [97]. LPA induced platelet activation and induced phosphatidylinositol-4-phosphate formation via phosphatidylinositol-4-kinase activation, through processes that were independent of protein kinase C and thromboxane A_2 [98, 99]. Retzer et al. [73] reported that mox-LDL induced platelet shape change via the GTPase Rho and Rho-kinase-dependent phosphorylation of myosin light chain and via moesin, which are both steps required for platelet shape change (fig. 2). Low concentration of mox-LDL did not change the intracellular Ca^{2+} concentration during shape change. This observation makes it unlikely that the calcium/calmodulin-dependent activation of myosin light-chain kinase is involved during shape change. However, Maschberger et al. [100] reported that

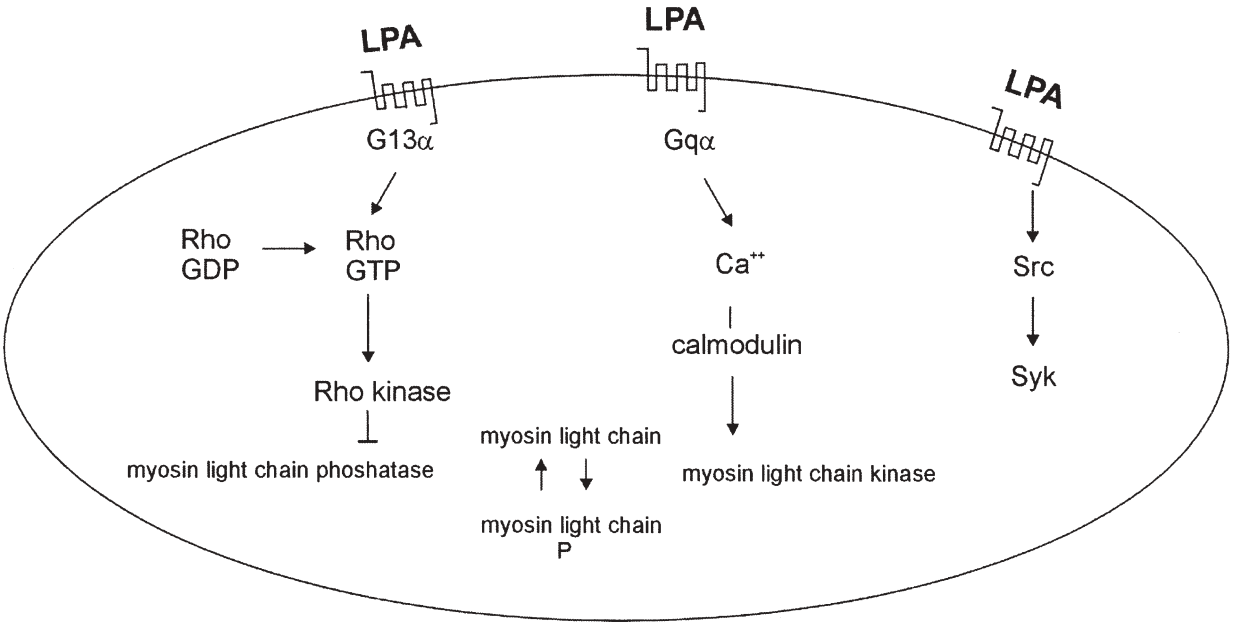

Figure 2. Signaling pathways involved in platelet shape change induced by moxLDL. The platelet-activating component of moxLDL, lysophosphatidic acid (LPA) binds to its receptor, which initiates signaling via G_{13} and G_q signaling. Signaling via G_{13} leads to activation of Rho kinase and inhibits myosin light-chain phosphatase. Signaling via G_q leads to an increase in Ca^{++} and together with calmodulin to activation of myosin light-chain kinase. The result of both routes is phosphorylation of myosin light chain, actin-myosin interaction and cytoskeleton reorganization during shape change. The third route involves activation of Src and Syk (modified from [72]).

mox-LDL stimulated two additional signal transduction pathways in human platelets: Src family kinase-mediated stimulation of protein tyrosine phosphorylation and Syk induced by low concentrations of mox-LDL, as well as stimulation of the Ca^{2+} influx induced by higher concentrations. Activation of platelets by mox-LDL was inhibited by LPA-receptor antagonists as well as lovastatin, which could have implications in the prevention and therapy of cardiovascular disease [100, 101].

Platelet aggregation was enhanced to a greater extent by LDL from diabetic patients than by LDL from healthy donors, suggesting that glycation may act as an agent that alters platelet function [102]. However, both the reactivity of platelets to various aggregating agents and the production of thromboxane B_2 were similar for the various LDL preparations, although their degree of glycosylation varied according to the concentration of glucose in the incubation media [102]. Phospholipids react directly with glucose to form advanced glycosylation end products that initiate lipid oxidation [103]. In vitro glycated LDL caused a significant increase in platelet aggregation and enhancement of thromboxane B_2 synthesis, increase of intracellular calcium concentrations and inhibition of Na^+/K^+-ATPase activity [102, 104, 105]. Hence, glycation induces compositional and structural changes in LDL. Glycated LDL may interact with platelets differently and may play a role in the vascular complications of diabetes.

Another highly reactive oxidant generated enzymatically via the myeloperoxidase-H_2O_2-halide system of activated phagocytes is hypochlorous acid/hypochlorite (HOCl/OCl^-). Unlike free-radical oxidants, HOCl preferentially modifies the apolipoprotein moiety of LDL. HOCl-modified LDL induced a dose-dependent increase of thrombin- and ADP-induced platelet aggregation [106]. Irreversible platelet aggregation and secretion from the dense granules have also been reported [107, 108]. HOCl-LDL and native LDL bind to similar sites on platelets [108]. However, HOCl-LDL stimulated platelet plasma membrane Ca^{2+}-ATPase, which resulted in decreased $[Ca^{++}]i$ [109]. HOCl-modified epitopes are present in vivo, and HOCl-modified apoB-100 has been extracted from advanced human atherosclerotic lesions [110]. To date, it is not clear whether highly reactive chloramines and/or methionine sulfoxides or secondary radicals derived from chloramines of apoB-100 contributing to HOCl-induced lipid peroxidation of LDL are responsible for effects on platelets. In addition, myeloperoxidase uses nitrite, a major end product of nitric oxide metabolism, as a substrate to nitrate protein tyrosine residues and lipid peroxidation [111]. These observations support the fact that in addition to reactive oxygen species, reactive nitrogen species may also alter platelet function. Peroxinitrite generated from nitric oxide radicals and superoxide anion radicals is present in human atherosclerotic lesions (as indicated by the presence of nitrotyrosine), and nitration was observed in

early subintimal fatty streaks [112]. Peroxinitrite rapidly induces tyrosine nitration of platelet membrane proteins and may even prevent phosphorylation of signaling proteins involved in platelet activation, apparently by cyclic GMP (cGMP) independent pathways, leading to decreased platelet aggregability [113–115].

In conclusion, native LDL sensitizes platelets via a receptor-mediated signaling cascade and via lipid exchange. The result is an increase in sensitivity to platelet-activating agents and enhanced aggregation and secretion responses. Modification of native LDL makes LDL an independent platelet activator. Oxidation leads to formation of LPA, which activates platelets via the LPA receptor. Modification of lipoproteins resulting in HOCl-LDL and glycated LDL generates platelet-sensitizing particles. These mechanisms make LDL a potentially prothrombotic factor and explain the increased risk factor for atherosclerosis in patients with hypercholesterolemia.

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