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Low-Density Lipoprotein-Lowering Medication and Platelet Function

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Abstract
Elevated low-density lipoprotein (LDL) cholesterol (LDL-C) levels represent one of the most important risk factors for atherosclerosis and therefore cardiovascular morbidity and mortality. LDL-C operates at different levels and through various classic and non-classic mechanisms. In particular, increased or modified LDL enhances platelet function and increases sensitivity of platelets to several naturally occurring agonists. Agents that lower LDL-C in hypercholesterolemic patients have been shown to interfere with platelet function. Several studies, in fact, suggested that statins exert anti-thrombotic effects largely as a result of an anti-platelet activity. Among the other LDL-C-lowering agents those acting by interfering with cholesterol reabsorption from the gut (cholestyramine, colestipol) do not appear to interfere with platelet function, whereas peroxisome proliferator-activated receptor agonists (such as fibrates) can inhibit platelet function. The full potential of these drugs in vascular protection is only just being realized. Further studies are still needed to elucidate the full therapeutic benefits of these agents in plaque stabilization and thrombosis.

Introduction

The idea of an involvement of the inflammatory system in the pathogenesis of atherosclerosis was first raised by Wirchow in 1856, but it was only in 1973 that the emerging knowledge of vascular biology led to the formulation of the 'response-to-injury' hypothesis by Ross and Glomset [1], who emphasized the role of endothelial damage as the first step in atherosclerosis. Since then, a wide variety of clinical and experimental studies have accumulated, supporting the concept that atherosclerosis is the result of a chronic inflammation perpetuated by a dysfunctional endothelium [2].

Elevated low-density lipoprotein (LDL) cholesterol (LDL-C) levels represent one of the most important risk factors for atherosclerosis and therefore cardiovascular morbidity and mortality. LDL-C operates at different levels and through various classic and non-classic mechanisms. For example, it has been shown that LDL-C modulates the expression of various growth factors and growth factor receptors that are involved in the process of atherosclerosis and that both native and modified LDL itself are potent growth factors for vascular cells (recently reviewed in [3]). Moreover, LDL-C is capable of inhibiting the release of nitric oxide (NO) [4] and down-regulating the expression of NO synthase [5]. The reduced NO bioavailability leads to endothelial dysfunction characterized by a phenotypic switch from a non-adhesive, non-

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thrombogenic cellular interface to one that expresses and secretes adhesion molecules and chemoattractants able to recruit and activate other vascular cell types [6].

In this respect, a particular emphasis has been given to the interactions occurring between vascular cells and platelets. Platelets play an important, but often under-recognized role in cardiovascular disease. For example, the normal response of the platelet can be altered, either by increased pro-aggregatory stimuli or by diminished anti-aggregatory substances, to produce conditions of increased platelet activation, which is often associated with the major risk factors for atherosclerosis (e.g. smoking [7], diabetes [8, 9] and hypercholesterolemia [9–12]). Moreover, the utility of an increasing range of anti-platelet drugs further emphasizes the pivotal role platelets play in the pathogenesis of cardiovascular disease. However, other drugs currently used in the primary or secondary prevention of atherothrombotic diseases may also affect platelet function, with mechanisms that are independent of their main pharmacological targets. The objective of this review will be to provide an overview of the current literature on the anti-platelet effects of lipid-lowering drugs in patients with hypercholesterolemia.

Evidence of Platelet Activation in Hypercholesterolemia

Platelets represent a key element in the pathogenesis of atherosclerosis [13]. Platelets were initially thought to play a minor role, if any, in ongoing inflammation or vascular injury and repair beyond initial adhesion, secretion and eicosanoid synthesis. However, increasing evidence is accumulating, suggesting that they have inflammatory actions and are a rich source of cytokines (e.g., interleukin 1β [IL-1β], vascular endothelial growth factor, CD40 ligand [CD40L], etc.) [14–19] that in some cases are actively synthesized upon platelet activation [18]. The kinetic of this secretory response is not known yet, but Weyrich et al. [19] have shown that platelets adherent to extracellular matrix have a limited secretory response over several hours. If agonists such as thrombin are introduced, however, the secretory pool is markedly increased. This indicates that the environmental milieu in vivo probably governs the magnitude and kinetics of the constitutive secretory response. Regardless of these factors, platelet release of their secretome at some point may contribute to initiation and progression of atherosclerotic lesions [19].

Platelet-derived substances, in fact, have been shown to induce a variety of genes within endothelial cells involved in molecular mechanisms of early inflammation [2, 20], such as those encoding for adhesion molecules [17], the monocyte chemotactic protein-1 [21] and the pro-inflammatory cytokines IL-6 and IL-8 [17, 22], thus contributing to attachment of monocytes to the endothelial layers [23] and their migration across the vessel wall [24]. Furthermore, platelet-derived cytokines, especially CD40L, are capable of inducing the expression of the potent procoagulant tissue factor (TF) [25–30] on endothelial cells [31, 32] and monocytes/macrophages [28, 33] combined with unaltered TF pathway inhibitor protein and activity, indicating that TF/TF pathway inhibitor balance is shifted toward increased procoagulant activity [33].

Hypercholesterolemia is associated with hypercoagulability, as demonstrated by the findings of an increased rate of thrombin generation, as well as increased levels of fibrinogen and factor VIIc [34–36], which have been directly correlated with elevated cholesterol levels [34]. But the main determinant of the prothrombotic state associated with hypercholesterolemia appears to be enhanced platelet activation (fig. 1) [10, 37]. Lipoprotein disorders affect platelet functions, and hypersensitive platelets are observed in various stages of hyperlipidemia [38], and the occurrence of in vivo platelet activation has often been reported in hypercholesterolemic patients [36, 37, 39–47]. In particular, hypercholesterolemia has been associated with increased platelet α_2 adrenergic receptor density [40], changes in the composition of platelet membrane phospholipids and cholesterol [43] and increases in platelet cytosolic calcium [42]. LDL have been shown to enhance platelet function and increase sensitivity of platelets to several naturally occurring agonists via binding of apoB-100 to a receptor on the platelet membrane and via transfer of lipids to the platelet membrane [38]. Furthermore, it has been suggested that high LDL levels may increase platelet reactivity in association with enhanced thromboxane A_2 (TXA_2) biosynthesis, as evidenced by increased urinary excretion of 11-dehydro-TXB_2 (a major enzymatic metabolite of TxA_2) [11, 36, 37]. Besides this TX-dependent pathway, other mechanisms may be involved in the in vivo platelet activation in hypercholesterolemic patients, as suggested by the experimental observation that oxidative modified LDL (ox-LDL) may cause P-selectin expression on platelets and endothelial cells [40, 43] and by the clinical findings of elevated plasma soluble (s) P-selectin levels in hypercholesterolemia [44–46].

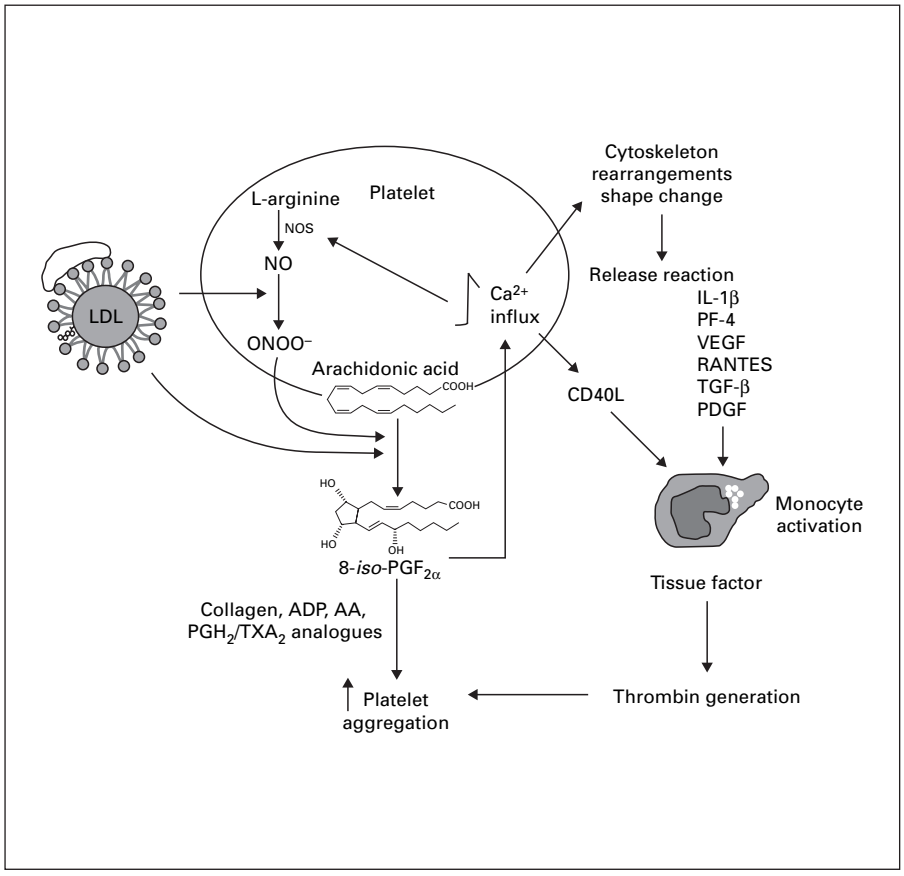

Fig. 1. Classic and non-classic mechanisms through which LDL-C induces enhanced platelet activation thus contributing to initiation and progression of atherosclerotic lesions. LDL-C is capable of inhibiting the release of NO and down-regulating the expression of NO synthase. The reduced NO bioavailability leads to endothelial dysfunction characterized by a phenotypic switch from a non-adhesive, non-thrombogenic cellular interface to one that expresses and secretes adhesion molecules and chemoattractants able to recruit and activate other vascular cell types. Increases in platelet cytosolic calcium induced by LDL-C stimulate cytoskeleton rearrangement and shape change with consequent release reaction, and expression of cytokines, especially CD40L, which are capable of inducing the expression of the potent procoagulant TF on endothelial cells and monocytes/macrophage, resulting in increased thrombin generation and platelet aggregation. Furthermore, LDL both directly and through enhanced formation of peroxinitrite ($ONOO^-$) stimulates non-enzymatic formation of 8-epi-$PGF_{2\alpha}$, which is a potent trigger for platelet activation and further promotes cytosolic calcium influx with additional CD40L expression and enhancement of the above-mentioned cascade.

Anti-Platelet Effects of Statins

Most of the recent advances in the pathophysiology of hypercholesterolemia are primarily due to the development of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or 'statins'. Landmark clinical trials have demonstrated that statins reduce the risk of cardiovascular events in primary or secondary prevention trials [48–52]. Risk reduction appears proportionate to the degree of LDL cholesterol reduction, and also appears to increase as the treatment duration increases [53, 54]. However, the clinical benefit of statins is manifest early in the course of treatment before plaque stabilization and/or regression could occur, and it has been suggested that the clinical benefits of these drugs are best explained by their direct effects on inflammatory and thrombotic mechanisms within arteries [55].

Among the pleiotropic effects of statins, stimulation of fibrinolysis [56], and modulation of the hemostatic system [57–62] have been described. Indeed, several studies suggested that statins can directly or indirectly influence coagulant functions of platelets, blood cells, vascular walls, as well as circulating coagulation factors or cofactors (fig. 2). A marked decrease in thrombin generation, as evidenced by reductions in plasma thrombin generation markers, such as prothrombin fragment 1 + 2 (F1 + 2), fibrinopeptide A and thrombin-anti-thrombin III complexes, has been reported in hypercholesterolemic subjects treated with pravastatin or simvastatin [59, 60]. Moreover, treatment with simvastatin at a dose that reduced LDL-cholesterol by 30–40% in patients with hypercholesterolemia resulted in normalization of altered platelet aggregation ex vivo and a 50% reduction in TXA_2 metabolite excretion when compared with placebo [11].

Despite this latter finding, it is unlikely that a reduction in TXA_2 per se explains the anti-thrombotic effects of lipid-lowering therapies as the clinical benefits of statins are evident even in patients receiving aspirin (which comprised 80% of patients in the LIPID trial) [52]. However, several studies suggest that ox-LDL can activate platelets directly [61]. The anti-platelet activity of simvastatin may also involve reduction in the platelet membrane cho-

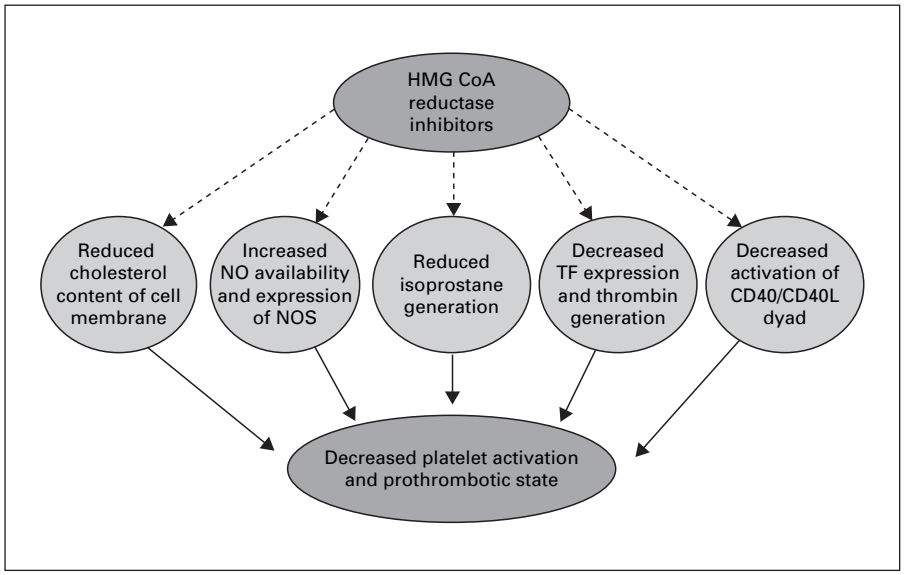

Fig. 2. Direct effects of HMGCoA reductase inhibitors on inflammatory and thrombotic mechanisms within arteries, leading to decreased platelet activation and prothrombotic state. In addition to their lipid-lowering effects, statins have proven effective in decreasing platelet activation and prothrombotic state through different mechanisms, including reduced cholesterol cell membrane content, blunting of the CD40L-CD40 dyad activation, decreased expression of TF and consequent thrombin generation, reduced isoprostane generation, and increased expression of NOS and NO availability.

lesterol content [62]. Furthermore, a series of isomers of prostaglandins (PGs) termed isoprostanes have been found in atherosclerotic tissue [63] and ox-LDL. These are generated non-enzymatically by free radical attack of arachidonic acid in cell membranes and so are insensitive to aspirin [64]. Several of these have biologic activity, including 8-epi-$PGF_{2\alpha}$, which is a potent platelet activator and vascular smooth muscle cell mitogen. Isoprostane formation is increased in patients with atherosclerosis, possibly reflecting an increase in oxidant tone [47]. The increase is normalized by treatment with a statin and this in turn may modulate platelet activity.

Of particular interest are the findings by Szczeklik et al. [65], who showed that simvastatin treatment was capable of inhibiting the generation of thrombin cleavage peptides in bleeding time blood (aspirated from the site of a skin incision). In the same study, the authors showed that aspirin had a similar effect, but there was no further reduction in thrombin cleavage peptides by the addition of simvastatin to aspirin, thus suggesting that the reduction in thrombin generation by simvastatin was secondary to an anti-platelet effect and specific inhibition of TXA_2. Likewise, Dangas et al. [66] showed that pravastatin reduced thrombus generation in an ex vivo model where the patient's blood was passed through a perfusion system, although plasma concentrations of fibrinopeptide and F1 + 2 were unaffected by treatment. The reduction in thrombosis was attenuated in patients on aspirin, again suggesting that aspirin and the statin operate through a similar pathway. Thus, both these studies suggested that statins exert anti-thrombotic effects largely as a result of an anti-platelet activity.

However, the nature and characteristics of statin-induced thrombin-lowering effects are still elusive, and there is great controversy about anti-thrombotic effects of statins [67, 68]. It remains to be determined which mechanisms produce a shift toward anti-coagulation in the hemostatic balance following statin treatment. Suppression of TF exposure appears to be a plausible explanation, as suggested by a prolongation of a lag phase in thrombin generation, which is determined by a concentration of the initiator of the extrinsic coagulation pathway, the complex TF-FVIIa [69]. However, until now, decreased TF expression, induced by statins, has been demonstrated solely on cultured human macrophages or monocytes [70]. TF-induced formation of thrombin on human monocytes, stimulated by lipopolysaccharides, was significantly decreased by simvastatin at final concentrations of 10 nmol/l to 10 μmol/l [70]. Postulated mechanisms by which simvastatin might change the coagulation reactions are: inhibition of the synthesis of isoprenoids, which are substrates for post-translational modification of numerous intracellular proteins, including GTP-binding proteins [71], or up-regulation of endothelial NO synthase [72], leading to an increase in NO production, which depresses TF expression in the endothelial cells [73]. Furthermore, it has been recently demonstrated that statins are able to decrease FVII coagulant activity in hyperlipidemic patients [74], which might also cause a reduced formation of the TF-FVIIa complexes.

Table 1. PPAR ligands

Endogenous ligands		Exogenous ligands	
PPAR-α	PPAR-γ	PPAR-α	PPAR-γ
Palmitic acid	Linoleic acid	Clofibrate	Rosiglitazone
Stearic acid	Arachidonic acid	Gemfibrozil	Pioglitazone
Palmitoleic acid	15-deoxy-delta12-14-PGJ2	Bezafibrate	Troglitazone
Oleic acid	9-HODE	Fenofibrate	Ciglitazone
Linoleic acid	13-HODE	Nafenopin	Isoxazolidinedione
Arachidonic acid	12-HETE		GW 7845
Eicosapentaenoic		Pioglitazone?	CDDO

CDDO = 2-Cyano-3,12-dioxoolean-1,9-dien-28-oic acid; 12-HETE = 12-hydroxyeicosatetraenoic acid; HODE = hydroxyoctadecadienoic acid; PPAR = peroxisome proliferator activated receptors.

A novel mechanism by which statins could exert their effects on the extrinsic pathway of coagulation might be represented by activation of the CD40L-CD40 dyad. Nowadays, several lines of evidence implicate CD40/CD40L signaling in the vascular pathology associated with hypercholesterolemia. In a substudy in the Atorvastatin versus Simvastatin on Atherosclerosis Progression trial, sCD40L levels were found to be about 27 times higher in 110 asymptomatic patients with familial hypercholesterolemia as compared to controls [75]. Statin therapy over 2 years markedly down-regulated sCD40L levels, regardless of the statin used ('aggressive' therapy with atorvastatin 80 mg/day, or 'conventional' therapy with simvastatin 40 mg/day) and, more important, with no correlation with the degree of cholesterol lowering. These results, coupled with the large sample size, the long follow-up period, the randomized, controlled nature of the trial, emphasizes the possibility that the influence of statins on CD40L may be explained by mechanisms other than the lipid lowering effects.

Patients with moderate hypercholesterolemia exhibit increased ex vivo expression of CD40L and P-selectin on platelets and elevated CD40 expression on monocytes [76]. Furthermore, increased plasma sCD40L levels have been positively associated with in vivo platelet activation, as reflected by plasma P-selectin and urinary 11-dehydro-TXB_2, and with procoagulant state, as reflected by FVIIa and F1 + 2, in 80 patients with hypercholesterolemia compared with healthy controls [12]. Short-term treatment with cerivastatin or pravastatin [12], as well as atorvastatin [77], was able to down-regulate both sCD40L and markers of a prothrombotic state. These observations provide evidence that sCD40L suppression by statins is associated with a prothrombotic state reduction in vivo, thus corroborating the hypothesis that statins exert their anti-thrombotic effects largely as a result of an anti-platelet activity.

Anti-Platelet Effects of Other LDL-Lowering Drugs

Agents that lower LDL-C in hypercholesterolemic patients by interfering with cholesterol reabsorption from the gut (cholestyramine, colestipol) do not appear to interfere with platelet hyperreactivity and do not change platelet-derived TX formation [78]. A different situation might exist with peroxisome proliferator activated receptors (PPARs) agonists (table 1) [79].

PPARs are ubiquitously expressed throughout the body. On activation by endogenously secreted PGs and fatty acids, they initiate transcription of an array of genes that are involved in energy homeostasis. So far, three major types have been identified, namely PPAR-α, PPAR-β/δ and PPAR-γ. PPAR-α and PPAR-γ are crucial for lipid and glucose metabolism, respectively [79]. PPAR-γ agonists are the thiazolidinediones, such as rosiglitazone and pioglitazone, insulin-sensitizing agents that are currently used for the treatment of type 2 diabetes mellitus. In addition, thiazolidinediones were shown to exert differing effects on lipid profile [80]. In particular, significant improvements in lipid profile were noted during therapy with pioglitazone, whereas none were detected with conversion to rosiglitazone [81]. Specifically, patients administered with pioglitazone experienced an average decrease in total cholesterol of 20 mg/dl [81]. These differences cannot be attributed to differences in their effects on serum free fatty acid concentrations, but piogli-

tazone seems to act like a partial PPAR-α agonist in vitro, whereas rosiglitazone seems to be a pure PPAR-γ agonist [80].

Both rosiglitazone and pioglitazone have been shown to reduce platelet activity by modifying phosphoinositide metabolism and increasing cyclic-adenosine monophosphate levels in platelets [82, 83], independently of any glucose-lowering action or insulin-sensitizing effect [84]. Furthermore, pioglitazone administration was capable of decreasing platelet aggregation and delaying intra-arterial thrombus formation in rats, at least partially, by an increase in the expression of cNOS and thrombomodulin [85].

Of major relevance for the present dissertation are the PPAR-α agonists, namely fibrates. The first reports on the effects of fibrates on platelet function date back to 1970, however the data available in the literature are scattered and sometimes conflicting. Gemfibrozil, for example, has shown some proactivating effects on platelet function in vitro, as evidenced by a doubled urinary excretion of 11-dehydro-TXB_2 and a slight increase of plasma beta-thromboglobulin levels during active treatment [86]. Nonetheless, these results were not confirmed in animal studies showing that gemfibrozil, at average therapeutic plasma levels (peak levels of 28 μg/ml), did not increase platelet mural thrombosis [87] and mildly reduced the increase in platelet-fibrinogen binding induced by hypercholesterolemia in a rat model [88]. More recently, Ammazzalorso et al. [89] demonstrated that gemfibrozil, as well as its chiral analogs, exert an anti-aggregating effect, as detected by the PFA-100 system, with different mechanism than that of aspirin.

Other fibrates commonly used in the treatment of combined hyperlipidemia are etofibrate and bezafibrate. Both were shown to influence platelet function. In particular, etofibrate can inhibit platelet function as evidenced by a reduced platelet aggregation in response to ADP and TXB_2 formation after 6 weeks of therapy [90], whereas bezafibrate is capable of reducing the sensitivity of platelets to the aggregatory effect of collagen, with no effect on TXB_2 production [90, 91]. Fibrinogen levels are also significantly lowered after bezafibrate treatment, the effect being more marked in patients with hyperfibrinogenemia [91, 92], but no differences in platelet-fibrinogen binding were observed in a double-blind, placebo-controlled cross-over trial with 400 mg bezafibrate once daily [93]. The level of platelet microparticles, activated platelets, and soluble adhesion molecules were all significantly decreased after 6 months of treatment with bezafibrate [94].

Conclusions

The lipid-modifying effects of statin and/or fibrate therapy have been well documented. However, the clinical benefit conveyed by these LDL-lowering drugs appears to extend beyond cholesterol lowering, and it has been suggested that these agents have cholesterol-independent or pleiotropic effects that may impact on thrombogenic responses. Although hypocholesterolemic drugs already provide clinicians with a powerful therapeutic tool, their full potential in vascular protection is only just being realized. Further studies on the interrelated action on endothelial dysfunction, platelet activation and thrombosis induced by statins, fibrates or thiazolidinediones will help to elucidate the full therapeutic benefits of these agents.

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