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Forum Review

Oxidant Stress and Platelet Activation in Hypercholesterolemia

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ABSTRACT

Hypercholesterolemia is the dominant risk factor associated with atherothrombotic disorders in the western world. Consequently, much attention has been devoted to defining its role in the pathogenesis of atherosclerosis. It is currently recognized that hypercholesterolemia induces phenotypic changes in the microcirculation that are consistent with oxidative and nitrosative stresses. Superoxide is generated via several cellular systems and, once formed, participates in a number of reactions, yielding various free radicals, such as hydrogen peroxide, peroxynitrite, or oxidized low-density lipoproteins. Once oxidant stress is invoked, characteristic pathophysiologic features ensue, such as platelet activation and lipid peroxidation, which are both involved in the initiation and progression of the atherosclerotic lesions. Thus, therapeutic strategies that act to maintain the normal balance in the oxidant status of the vascular bed may prove effective in reducing the deleterious consequences of hypercholesterolemia. *Antioxid. Redox Signal.* 6, 747–756.

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 (83), 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 (55).

Hypercholesterolemia is the dominant risk factor associated with atherosclerosis in the western world. Consequently, much attention has been devoted to defining its role as a putative mediator of endothelial dysfunction. However, only recently it has been discovered that hypercholesterolemia induces phenotypic changes in the microcirculation that are consistent with oxidative and nitrosative stresses (92).

All common risk factors for atherosclerosis are capable of increasing production of reactive oxygen species (ROS) by vascular cells. Although the underlying mechanisms are incompletely understood, it has been postulated that oxidative stress due to hypercholesterolemia may play a pivotal role in the pathogenesis of atherosclerosis. Indeed, ROS activate other signaling molecules, such as protein kinase C and nuclear transcription factor-κB, leading to transcription of genes encoding cytokines, growth factors, and adhesion molecules (39, 93), which are ultimately responsible for a switch of the endothelium from a nonadhesive, nonthrombogenic cellular interface to one that expresses and secretes several adhesion molecules and chemoattractants capable of recruiting and activating other vascular cell types (99).

In this respect, a particular emphasis has been given to the interactions occurring between vascular cells and platelets. Platelets represent a key element in the pathogenesis of atherosclerosis (26) and a prime target for ROS produced or released in the vascular lumen (3). At the same time, they are also capable of endogenous generation of oxidants (22, 29, 61, 89). Earlier studies on the effects of oxidants on platelet

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function yielded conflicting results, probably due to differences in the experimental conditions used in those studies, such as concentration and chemical nature of oxidants tested, or the effects of plasma components. However, it is now clear that ROS can affect several key steps of platelet function, and therefore their net effect on the final process of aggregation may depend on various factors.

The objective of this review will be to summarize the current literature that addresses the mechanisms by which oxidant stress affects platelet function and ultimately contributes to vascular disease in the context of hypercholesterolemia.

SOURCES OF ROS IN THE VASCULAR BED

Humans are commonly exposed to exogenous (occupational or environmental) agents that generate ROS, such as air pollutant particles or ozone. Moreover, lifestyle can strongly influence the oxidant status. This is the case with cigarette smoke, which is a highly concentrated source of both ROS and reactive nitrogen species, and with diet, in which the types and amounts of antioxidants may contribute to the oxidative balance. Indeed, the contribution of exogenous ROS to human diseases is extensive and definitely deserves a separate dissertation. Thus, we will presently focus on the endogenous production of ROS by vascular cells, which can be activated during hypercholesterolemia.

Several enzyme systems contribute to production of ROS in vascular tissues, including xanthine oxidase, the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, arachidonic acid (AA) pathway enzymes lipoxygenase (LOX) and cyclooxygenase (COX), mitochondrial sources, and nitric oxide synthase (NOS) (5, 37, 48, 73, 103) (Fig. 1). Among them, the NOS, in particular the endothelial isoform of NOS (eNOS), is now recognized as an important source of superoxide (O_2^-) in several clinical settings, including hypercholesterolemia. eNOS is a cytochrome p450 reductase-like enzyme that requires tetrahydrobiopterin as a cofactor for transfer of electrons from a heme group within the oxygenase domain to L-arginine to form L-citrulline and nitric oxide (NO). If either tetrahydrobiopterin or L-arginine is absent, the electrons from heme reduce oxygen to form O_2^- (97). Furthermore, eNOS can generate O_2^- rather than NO in response to atherogenic stimuli such as low-density lipoprotein (LDL) particles (79). These findings have led to the concept of "NOS uncoupling," where the activity of the enzyme for NO production is decreased, in association with an increase in NOS-dependent O_2^- production. Uncoupling of eNOS in the endothelium may lead to oxidative stress and endothelial dysfunction via at least three mechanisms. First, the enzymatic production of NO is diminished, allowing the radicals that it normally might react with to attack other cellular targets. Second, the enzyme begins to produce O_2^-, contributing to oxidative stress. Finally, it is likely that eNOS can become partially uncoupled, such that both O_2^- and NO are produced simultaneously. Under this circumstance, eNOS may become a peroxynitrite ($ONOO^-$) generator, leading to a dramatic increase in oxidative stress, because $ONOO^-$ formed by the NO-superoxide reaction has additional detrimental effects on vascular function, due to the oxidation of cellular proteins and lipids, as well as LDL particles (102). Oxidation of LDL is a progressive process leading initially to the formation of mildly oxidized LDL (mox-LDL), which may be defined by a low content of lipid peroxidation derivatives and little modification in apolipoprotein B (25, 105). It is well known that oxidized LDL (ox-LDL) inhibits the release of NO and endothelium-derived hyperpolarizing factor *in vitro* (23) and down-regulates the expression of NOS (98). Furthermore, Weidtmann *et al.* (101) have demonstrated that mox-LDL induces platelet aggregation through activation of phospholipase A_2 and enhanced thromboxane A_2 (TXA_2) secretion from the platelets, resulting in release of several potent growth factors (4, 28, 101). Finally, mox-LDL may act synergistically with TXA_2 in stimulating cell proliferation probably as a result of oxidative components (44).

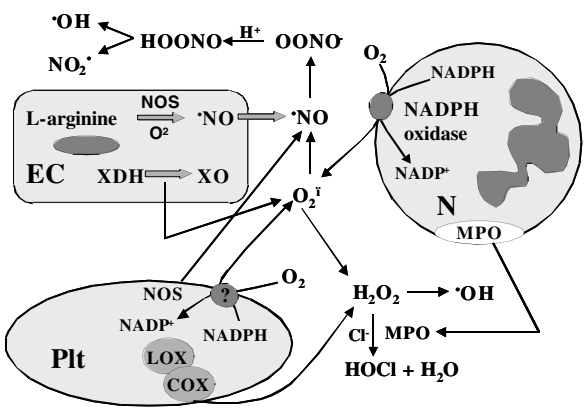

FIG. 1. Sources of ROS in vascular cells. Nitric oxide synthase (NOS) and xanthine oxidase (XO) in endothelial cells (EC), NADPH oxidases in neutrophils (N), or lipoxygenase (LOX) and cyclooxygenase (COX) in platelets (Plt) may all be responsible for superoxide (O_2^-) generation in health and disease.

As mentioned above, platelets also produce ROS, such as O_2^- and hydrogen peroxide (H_2O_2), during activation (22, 29, 61, 89). Both constitutive and inducible NOS have been identified in human platelets (59), and many studies report NO release from aggregating platelets (30, 63). Thus, the availability of both O_2^- and NO has raised the possibility that $ONOO^-$ may be formed endogenously in platelets (85, 95). In particular, stimulation of platelets with thrombin is capable of causing the formation of $ONOO^-$, whereas stimulation with collagen and AA does not seem to have any effect (57). Furthermore, in platelets, ROS are intermediate metabolites produced from COX and LOX reactions, and participate in cell signaling processes and their regulations. It is well established that COX activity is controlled by the peroxide tone of the cell, *i.e.,* its content in lipid hydroperoxides (47). On the other hand, although the structure of LOXs has been characterized extensively, the biological importance and physiological relevance of LOX-derived products remains poorly defined (45). This point certainly deserves attention considering that LOX may play a role in the development of atherosclero-

sis *in vivo* (90). Recent studies suggested that 12-hydroperoxyeicosatetraenoic acid [12(S)-HpETE], the main hydroperoxide formed from AA via the 12-lipoxygenase in platelets, lowers the threshold for platelet activation by AA, potentiating platelet aggregation and increasing TXA_2 formation, which is indicative of COX activation (10). Furthermore, nanomolar concentrations of 12-HpETE increase AA availability in platelets coincubated with nonaggregating concentrations of collagen via the activation of the p38 mitogen-activated protein kinase and phosphorylation of the cytosolic phospholipase (11, 15), which is in agreement with the current concept that activation of certain stress-activated protein kinases represents key signals in intracellular oxidant-induced signaling pathways (1, 8).

ROLE OF H_2O_2 AND O_2^- IN PLATELET FUNCTION

The relationship between ROS and platelet function has been extensively investigated (3). However, the question whether these species act as activators or inhibitors of platelet functions remains unresolved, because largely controversial results have been obtained depending on the oxidant doses or the experimental conditions used. Several studies report that H_2O_2 does not affect or inhibit platelet aggregation (12, 53, 94), whereas some authors found that H_2O_2 or O_2^- may enhance platelet aggregation (22, 38, 87, 88). To date, it is accepted that low levels of H_2O_2 may promote TXA_2 synthesis and aggregation. In fact, AA-dependent aggregation is almost completely suppressed in the presence of catalase, a scavenger of H_2O_2 (2). Furthermore, exogenously administered H_2O_2 markedly shortens the lag phase that precedes aggregation by AA (2), and low concentrations of H_2O_2 potentiate the aggregatory response to AA or collagen (22, 77). On the other hand, exposure to larger albeit not toxic, concentrations of exogenous H_2O_2 may inhibit aggregation to several agonists via stimulation of guanylate cyclase and increased cyclic guanosine monophosphate (cGMP) formation (2, 68). cGMP is known to activate cGMP kinase 1, which reduces Ca^{2+}, inhibits platelets, and initiates the phosphorylation of vasodilator-stimulated phosphoprotein (VASP) (82), the inositol trisphosphate receptor (40), and the TX receptor (100). Recently, it has been demonstrated that H_2O_2 even at submicromolar concentrations is able to induce phosphorylation of VASP without a rise in ambient cGMP concentrations (84). This suggests that other events are essential to connect VASP phosphorylation with inhibition of platelet activation, events that may be activated by low concentrations of NO (see below).

The complexity of the effects of ROS on platelets observed *in vitro* is also reflected in experiments conducted *in vivo*. The role of endogenous oxidants on intravascular thrombus formation has been examined in animal models of arterial stenosis (41, 106), and the results obtained were consistent with the two major observations obtained in *in vitro* platelet studies, showing that low levels of oxidants may promote aggregation (2, 22, 38, 77, 87, 88), whereas exposure to high concentrations of exogenous H_2O_2 may result in platelet inhibition (2, 12, 52, 94). However, the *in vivo* situation is clearly more complex, because the net effect of ROS on intravascular thrombosis is also dependent on the integrity of the endothelium, as well as on oxidant-mediated alterations of other major players in thrombosis, such as coagulation factors.

NO, $ONOO^-$, AND PLATELET FUNCTION

The balance between NO and O_2^- at the endothelial cell surface has been implicated in the pathogenesis of atherothrombotic disorders, and enhanced $ONOO^-$ formation has been demonstrated on reperfusion (51, 56, 74). Of particular relevance is the interaction of platelets with $ONOO^-$. Several studies have investigated the effects of $ONOO^-$ on platelets, and evidence from biochemical studies indicates that $ONOO^-$ can exert both nitrosative and oxidative stress (96). The nitrosative stress is of interest because this can generate NO donors, based on modification of polyhydroxylated compounds and thiols (62, 65, 66, 104). These reactions may explain the studies with intact cells or intact organ systems that reveal a biochemical response that is essentially identical to that of NO and suggests that $ONOO^-$ could exert a cytoprotective function (52, 70).

Earlier studies have indicated that platelets are very sensitive to low amounts of $ONOO^-$ (42, 64), and that $ONOO^-$ may inhibit (64, 65, 107) or stimulate platelet function (65). Moreover, it has been suggested that platelet activation by thrombin might cause $ONOO^-$ formation in platelets (58). However, only recently, the processes through which $ONOO^-$-dependent protein tyrosine nitration may modulate platelet structure and function have begun to be elucidated. Presently, it is postulated that $ONOO^-$ resulting from platelet activation, or diffusing into human platelets via the HCO_3^-/Cl^- transporter (9), can modulate AA metabolism via at least three mechanisms (Fig. 2). First, nitration of critical COX tyrosine residues is likely to inhibit enzymatic activity and/or alter the conformation of COX and its ability to recognize and bind to the substrate, thus leading to decreased formation of proaggregatory prostanoids (*e.g.,* TXA_2) (9). Furthermore, it has been recently suggested that low concentrations of $ONOO^-$ reversibly inhibit platelet aggregation by preventing the phosphorylation of key signaling proteins (57, 60, 69, 85). Secondly, as a hydroperoxide substrate for the COX peroxidase activity, $ONOO^-$ has the potential to increase prostaglandin formation. Indeed, $ONOO^-$ appears to be a better substrate for the peroxidase of both COX-1 and COX-2 (46). Finally, another aspect of $ONOO^-$ interaction with platelets is the observation that unique eicosanoids might originate from free radical processes. Indeed, increasing doses of $ONOO^-$ induced formation of 8-*iso*-prostaglandin $F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$) and *trans*-arachidonic acids (9), which are likely to contribute to the complexity of biological effects induced by $ONOO^-$ because both are biologically active in platelets (7, 67). Thus, formation of $ONOO^-$ from NO and O_2^- may function as a platelet hormone-like COX regulatory mechanism in inflammatory processes in which large amounts of these molecules are produced.

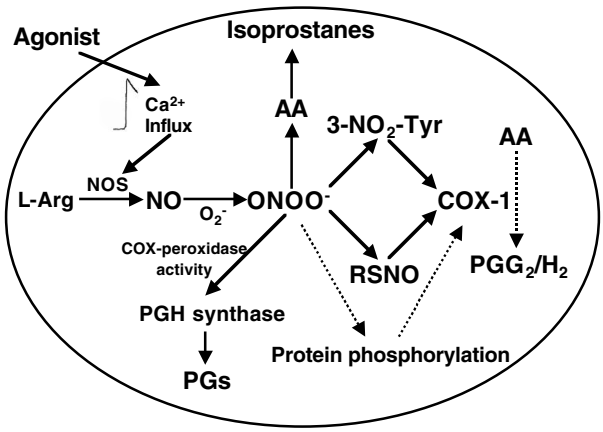

FIG. 2. Effects of peroxynitrite on platelet function. Nitric oxide (NO) derived from platelets following activation and Ca^{2+} influx reacts with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$), which, in turn, can react with tyrosyl residues in proteins to form 3-nitrotyrosyl residues (3-NO_2-Tyr) and with thiols to form *S*-nitrosothiols (RSNO). Nitration of critical cyclooxygenase-1 (COX-1) tyrosine residues is likely to inhibit enzymatic conversion of arachidonic acid (AA) to prostaglandins G_2 and H_2 (PGG_2/H_2), leading to decreased formation of proaggregatory prostanoids. On the other hand, $ONOO^-$ may act as a substrate for the COX peroxidase activity, leading to increased prostaglandin formation. Solid arrows indicate activation, and dashed arrows inhibition.

LIPID PEROXIDATION AND PLATELET ACTIVATION

In vivo studies on animal models have shown that atherogenesis is accompanied by an increased lipid peroxidation (for recent reviews, see 35, 36). As in hypercholesterolemic rodents, patients with hypercholesterolemia have evidence of increased ROS generation. Traditional approaches to the assessment of oxidative stress in humans relied on *in vivo* indices of questionable veracity, such as thiobarbituric acid reactive substances (14) and plasma malondialdehyde (76). However, these compounds can be formed nonspecifically. In particular, malondialdehyde, a by-product of COX activation, is generated during platelet activation and, thus, its measurement can be seriously confounded by *ex vivo* platelet activation during sample withdrawing and processing (13).

More recently, the determination of urinary isoprostanes (iPs), such as 8-*iso*-$PGF_{2\alpha}$ (also known as $iPF_{2\alpha}$-III), by mass spectrometry has been indicated as a reliable index of increased ROS generation and lipid peroxidation in human disease (54). By using 8-*iso*-$PGF_{2\alpha}$, a condition of oxidative stress has been demonstrated in adults with mild to severe hypercholesterolemia (80, 81) (Table 1). In particular, urinary 8-*iso*-$PGF_{2\alpha}$ was two- to threefold higher in type IIa hypercholesterolemic patients compared with control subjects, and its rate of excretion was directly correlated with LDL cholesterol levels and inversely related to the vitamin E content of LDL (20). Furthermore, Reilly *et al.* (81) demonstrated that both urinary 8-*iso*-$PGF_{2\alpha}$ and $iPF_{2\alpha}$-VI levels were elevated in patients with homozygous and heterozygous familial hypercholesterolemia. Excretion of the two iPs was highly correlated within both groups of hyperlipidemic patients. Interestingly, levels of 8-*iso*-$PGF_{2\alpha}$ esterified in circulating LDL correlated with urinary levels of this iP in patients with familial homozygous hypercholesterolemia (81). Furthermore, it has been shown that both statins (21, 50) and vitamin E (43, 86) (Fig. 3), by reducing the rate of lipid peroxidation, may interfere with oxidative stress, as demonstrated by a significant reduction in 8-*iso*-$PGF_{2\alpha}$ levels. Analysis of urinary iPs levels in hypercholesterolemic children yielded conflicting results. In fact, in a study by Cracowski and colleagues (16), it was shown that iPF_2 urinary levels in children with type IIa hypercholesterolemia did not differ from those of age-and sex-matched control children and were not correlated to blood lipid parameters. These results prompted the authors to suggest that hypercholesterolemia is not associated with increased lipid peroxidation in childhood (16) (Table 1). However, it has been reported recently that antioxidant therapy with vitamins C and E restores endothelial function in children with familial hypercholesterolemia or the phenotype of familial combined hyperlipidemia probably by increasing local NO bioavailability (24). Further studies are needed to define better the interplay between hypercholesterolemia and oxidative stress in childhood.

A major determinant of the prothrombotic state associated with hypercholesterolemia appears to be related to enhanced platelet activation. Platelets, in fact, play a fundamental role in atherogenesis and in the pathophysiology of atherothrom-

TABLE 1. URINARY EXCRETION RATES OF 8-*ISO*-$PGF_{2\alpha}$ IN HYPERCHOLESTEROLEMIC PATIENTS COMPARED WITH HEALTHY SUBJECTS

Clinical setting	*Patients*	*Controls*	*Reference*
Homozygous familial hypercholesterolemia (n = 38 vs. 38)	85 ± 5.5*	58 ± 4.2*	81
Moderate hypercholesterolemia (n = 24 vs. 24)	81 ± 10*	59 ± 4*	81
Hypercholesterolemia (n = 25 vs. 12)	441 ± 113†	141 ± 82†	50
Hypercholesterolemia (n = 40 vs. 40)	473 ± 305‡	205 ± 95‡	20
Children with type IIa hypercholesterolemia (n = 15 vs. 15)	84.7 ± 37*	96 ± 35*	16

Values are expressed as means ± SD.
*pmol/mmol creatinine.
†pg/ml creatinine.
‡pg/mg creatinine.

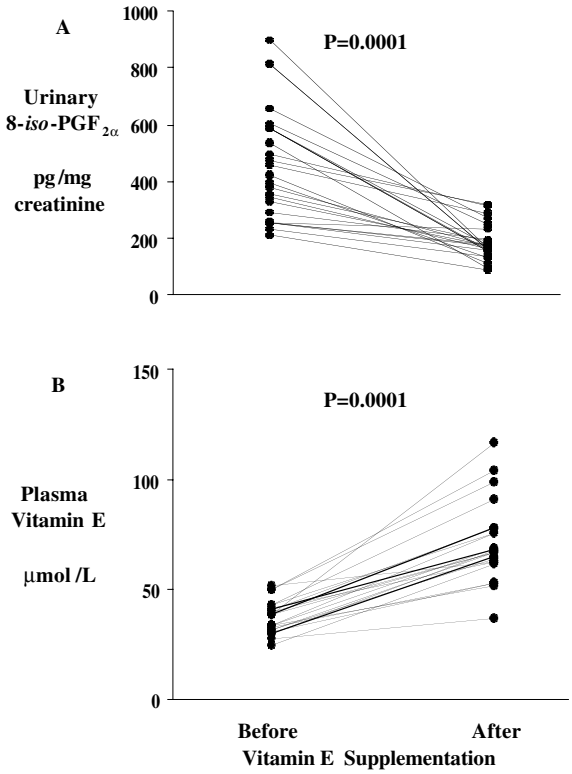

FIG. 3. Effects of vitamin E supplementation on urinary excretion of 8-*iso*-$PGF_{2\alpha}$ (upper panel) in hypercholesterolemic patients (n = 22). Eicosanoid levels were measured in hypercholesterolemic patients before (baseline) and at the end of a 2-week daily dosing with 600 mg of vitamin E. Plasma vitamin E concentrations at the same time points are reported for comparison (**lower panel**). Modified from Davì *et al*. (20).

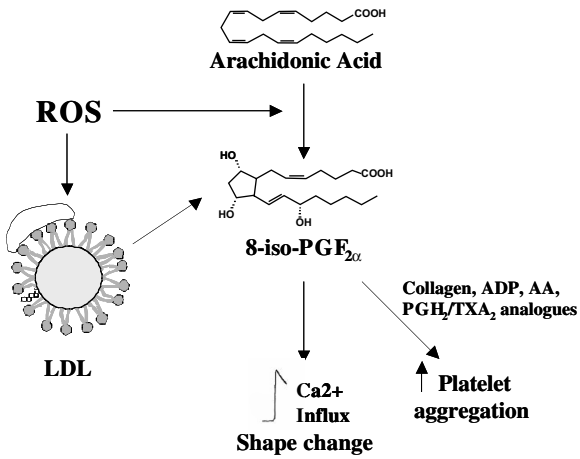

FIG. 4. Generation of 8-*iso*-$PGF_{2\alpha}$ either from AA or circulating LDL may modify aspects of platelet function, acting by a dose-dependent increase in Ca^{2+} release from intracellular stores and platelet shape change, as well as by its ability to amplify the aggregation response to subthreshold concentrations of platelet agonists. AA, arachidonic acid; PGH_2/TXA_2 endoperoxides/thromboxane analogues.

botic disorders (55). The occurrence of *in vivo* platelet activation has been reported in type IIa hypercholesterolemic patients (19, 27, 71), suggesting that high LDL levels, through changes in the composition of platelet membrane phospholipids and cholesterol, may increase platelet reactivity with enhanced TXA_2 biosynthesis, as reflected by increased urinary excretion of 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2) (18), one of the major stable metabolites of TXA_2.

As already mentioned, generation of 8-*iso*-$PGF_{2\alpha}$ may modify aspects of platelet function, acting by a dose-dependent increase in platelet shape change, Ca^{2+} release from intracellular stores, and metabolism of inositol phosphates (67, 78) (Fig. 4). Moreover, 8-*iso*-$PGF_{2\alpha}$ causes dose-dependent, irreversible platelet aggregation in the presence of concentrations of exogenous agonists that, when acting alone, fail to aggregate platelets (78). This ability of 8-*iso*-$PGF_{2\alpha}$ to amplify the aggregation response to subthreshold concentrations of platelet agonists may be relevant to hypercholesterolemia, where platelet activation and enhanced free-radical formation coincide (78).

In patients with hypercholesterolemia, enhanced formation of 8-*iso*-$PGF_{2\alpha}$ and other bioactive iPs appears to contribute to persistent platelet activation (20) (Fig. 5). Paired

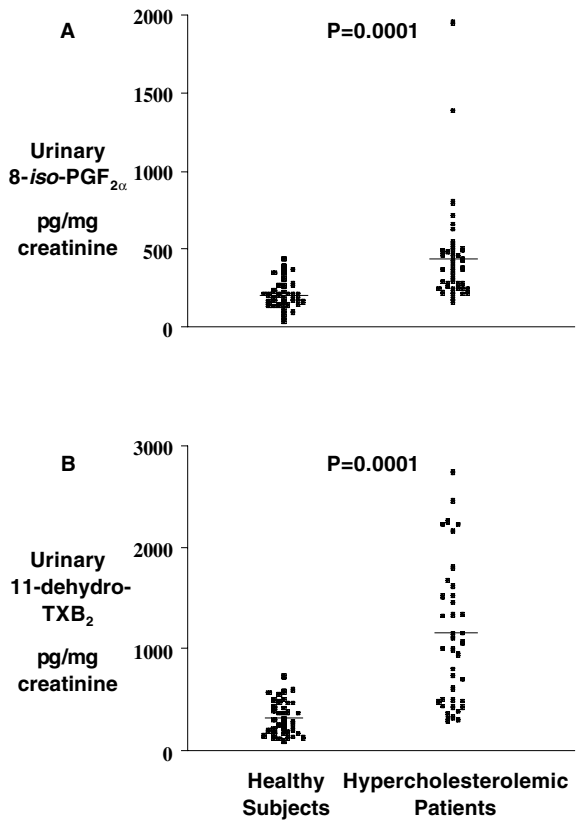

FIG. 5. Scatterplot distribution of urinary excretion of 8-*iso*-$PGF_{2\alpha}$ (upper panel) and 11-dehydro-TXB_2 (lower panel) in patients with hypercholesterolemia (n = 40) and in age- and sex-matched healthy subjects (n = 40). Modified from Davì *et al.* (20).

measurements of 8-*iso*-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 in hypercholesterolemic patients and age- and gender-matched control subjects revealed a highly significant linear correlation between the two (20).

Of interest, urinary immunoreactive 8-*iso*-$PGF_{2\alpha}$ was unchanged following 2-week dosing with low-dose aspirin or indobufen, a reversible COX inhibitor, despite complete suppression of TX metabolite excretion. Consistent with the hypothesis of enhanced 8-*iso*-$PGF_{2\alpha}$ formation contributing to platelet activation in this setting, dose-dependent suppression of the former by vitamin E supplementation was associated with comparable reductions in 11-dehydro-TXB_2 excretion (20). Thus, enhanced nonenzymatic peroxidation of AA may provide a biochemical link between oxidant stress and platelet activation in the setting of hypercholesterolemia. In this respect, of particular interest is the hypothesis recently raised by Csiszar *et al.* who suggested that an increase in COX-independent iP formation in platelets may contribute to aspirin resistance in patients with cardiovascular disorders (17, 75).

EFFECTS OF ROS ON THE COAGULATION CASCADE

In addition to their direct effects on platelet function, ROS might affect thrombus formation within the vasculature through an enhanced activation of the extrinsic coagulation cascade, ultimately leading to thrombin formation, via their combined effects on stimulation of tissue factor (TF) activity and inhibition of fibrinolytic pathways. Indeed, a brief period of exposure to ROS resulted in a significant increase in TF mRNA levels, accompanied by the appearance of large TF procoagulant activity (33), a phenomenon that was not confined to endothelial cells *in vitro*, but was also confirmed in an animal model of coronary artery occlusion and reperfusion in which endogenous production of large amounts of oxygen radicals was accompanied by a marked increase in TF activity in the coronary circulation and a significant reduction of myocardial perfusion (33). Moreover, this effect was abolished by oxygen radical scavengers (33) or NO (31). Besides this effect, ROS may also promote intravascular thrombus formation by interfering with mechanisms that normally inhibit activation of the coagulation pathway. For example, lipid peroxides formed as a consequence of oxygen radical attack on circulating lipoproteins can increase the amount of thrombin produced and can slow down the rate of thrombin decay (6). Both effects are consequent to inhibition of plasma antithrombin (34). Similar susceptibility to ROS-mediated inactivation has been reported for other key antithrombotic factors, such as α2-antiplasmin (91), plasminogen activator (49), and thrombomodulin (32). More recently, it has been shown that the reaction of $ONOO^-$ with fibrinogen results in both structural modifications and altered biological properties of this glycoprotein (72). Furthermore, $ONOO^-$-treated fibrinogen in comparison with the native molecule had a reduced ability to mediate platelet adhesion and aggregation, and the percentage of ADP-induced platelet aggregation decreases as a function of $ONOO^-$-mediated modification of the fibrinogen molecule (72).

CONCLUDING REMARKS

Oxidation of LDL cholesterol by O_2^- generated by vascular cells is critical to the pathogenesis of endothelial dysfunction in hypercholesterolemia. The latter, in turn, potentiates LDL oxidation by increasing substrate and promoting LDL conformations that are more susceptible to oxidation. Furthermore, ox-LDL are capable of mediating a number of redox-sensitive processes that are deleterious to endothelial function. In particular, ox-LDL reduces NO bioavailability through inhibition of eNOS and NOS uncoupling. In addition, ox-LDL promotes an inflammatory phenotype through activation of nuclear factor-κB, triggering the release of inflammatory cytokines and the expression of adhesion molecules through redox-sensitive pathways. Inflammatory mediators may, in turn, activate enzymatic sources of ROS, thus sustaining the already established oxidant stress.

The hypothesis that oxidative stress mediates atherosclerotic endothelial dysfunction would implicate a potential for antioxidant therapies to ameliorate and perhaps reverse vascular pathology. Numerous clinical studies have attempted to validate the salutary effects of antioxidant drugs or to characterize better the antioxidant effects of existing therapeutic agents. Indeed, the ameliorative effect of vitamin E on *in vivo* platelet activation and endothelial dysfunction, together with a reduction in serum markers of lipid peroxidation, has been observed in patients with hypercholesterolemia. On the other hand, it has been proposed that lipid-lowering drugs can disrupt the oxidative stress/inflammation cycle.

Further elucidation of the role of oxidants in vascular disease will facilitate the development of antioxidant therapies that target pathologic oxidative mechanisms by sparing homeostatic functions dependent on oxidative signaling. Future studies are warranted to develop novel therapeutic strategies that, while acting to maintain the normal balance in the oxidant status of the vascular bed, will prove effective in reducing the deleterious consequences of hypercholesterolemia.

ABBREVIATIONS

AA, arachidonic acid; cGMP, cyclic guanosine monophosphate; COX, cyclooxygenase; 11-dehydro-TXB_2, 11-dehydrothromboxane B_2; eNOS, endothelial nitric oxide synthase; H_2O_2, hydrogen peroxide; HpETE, 12-hydroperoxy-eicosatetraenoic acid; iP, isoprostane; 8-*iso*-$PGF_{2\alpha}$, 8-*iso*-prostaglandin $F_{2\alpha}$; LDL, low-density lipoprotein; LOX, lipoxygenase; mox-LDL, mildly oxidized low-density lipoprotein; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NOS, nitric oxide synthase; O_2^- superoxide; $ONOO^-$, peroxynitrite; ox-LDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; TF, tissue factor; TX, thromboxane; VASP, vasodilator-stimulated phosphoprotein.

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