Increased Levels of Soluble P-Selectin in Hypercholesterolemic Patients

Giovanni Davi, MD; Mario Romano, MD; Andrea Mezzetti, MD; Antonio Procopio, MD; Stefano Iacobelli, MD; Teresa Antidormi, MD; Tonino Bucciarelli, MD; Paola Alessandrini, MD; Franco Cuccurullo, MD; Gabriele Bittolo Bon, MD

Background—Hypercholesterolemia is considered a major risk factor for the development of atherosclerosis. Enhanced lipid peroxidation and persistent platelet activation can be observed in vivo in hypercholesterolemic patients and may have pathophysiological implications in the occurrence of cardiovascular events. P-selectin may play an important role in the pathogenesis of multicellular events, including atherosclerosis. We studied the impact of hypercholesterolemia and oxidative stress on plasma levels of P-selectin.

Methods and Results—Plasma levels of P-selectin were measured by means of an enzyme immunoassay in 20 hypercholesterolemic patients with no clinical evidence of cardiovascular disease and in 20 sex- and age-matched normocholesterolemic subjects. Hypercholesterolemic patients had higher levels of P-selectin compared with that of control subjects (98 ± 61 versus 56 ± 14 ng/mL; P = .001). They also displayed increased von Willebrand Factor (vWF) levels (176 ± 22 versus 119 ± 12%; P = .0001). A direct correlation was observed between P-selectin and LDL cholesterol levels (r = .453). Administration of vitamin E (600 mg/d for 2 weeks) to hypercholesterolemic patients significantly reduced plasma P-selectin (40%), and an inverse correlation was observed between vitamin E and P-selectin plasma levels (r = -.446).

Conclusions—Hypercholesterolemia is associated with elevated plasmatic P-selectin. Altered oxidative processes leading to endothelial dysfunction and persistent platelet activation may contribute to increased soluble P-selectin levels. P-selectin may be proposed as a marker of endothelial dysfunction in hypercholesterolemic patients. (Circulation. 1998;97:953-957.)

Key Words: platelets ■ hypercholesterolemia ■ antioxidants ■ cell adhesion molecules

Hypercholesterolemia is considered a major risk factor for the development of atherosclerosis.1 Cholesterol-rich LDL may play a critical role in the onset and further progression of the atherosclerotic lesion. LDL become pathogenic when subjected to oxidation. ox-LDL are in fact no longer recognized by the LDL receptor; instead they are taken up by a scavenger receptor, which is not subjected to regulation by the intracellular cholesterol level.2 As a consequence, subendothelial macrophages that possess the scavenger receptor become engulfed with LDL and are transformed into foam cells, which represent the first stage of the atherosclerotic lesion.3 Also, ox-LDL may impair vascular functions, resulting in increased risk of occlusive thrombotic events.4 Recently, we have obtained evidence of enhanced in vivo lipid peroxidation in hypercholesterolemic patients.5 These findings emphasize the role that lipid peroxidation may have in the pathogenesis of atherosclerosis.

P-selectin is a glycoprotein contained in the platelet α-granules and in the Weibel-Palade bodies of endothelial cells, from where it is mobilized to the cell surface after activation.6,7 The P-selectin ligand P-selectin glycoprotein ligand 1 is abundant in circulating monocytes and polymorphonuclear leukocytes,8 and there is accumulating evidence that P-selectin mediates leukocyte adhesion to platelets and endothelial cells during inflammation, thrombosis, and atherosclerosis.9 Alternative splicing of P-selectin mRNA generates a soluble form of the protein that can be measured in human plasma.10 Increased levels of plasma P-selectin have been observed in several vascular diseases such as unstable angina, myocardial infarction, thrombotic thrombocytopenic purpura, and in the coronary sinus after coronary spasm.11-14 It has been proposed that plasma P-selectin may reflect the functional status of platelets and endothelial cells.14

Because hypercholesterolemic patients display signs of persistent platelet activation in vivo15 and high cholesterol levels are frequently associated with enhanced lipid peroxidation and endothelial dysfunction, we investigated whether plasma levels of P-selectin could be altered in a group of hypercholesterolemic patients with no clinical evidence of cardiovascular disease.

In this report, we show that hypercholesterolemic patients have higher levels of plasma P-selectin compared with that of sex- and age-matched normocholesterolemic subjects. In ad-
dition, we show that an antioxidant treatment with vitamin E significantly reduces plasma P-selectin levels.

Methods

Subjects
Twenty hypercholesterolemic patients (14 women and 6 men; age, 53±6 years) and 20 healthy sex- and age-matched normocholesterolemic subjects were asked to participate in the study. Patients were on an American Heart Association step I diet without drug therapy for at least 2 months and none of the subjects was taking drugs, vitamins, or dietary supplements. The hypercholesterolemic patients were not taking lipid-lowering drugs either because they were unwilling to do so or because they were still on a diet preceding drug treatment.

None of the patients had clinical evidence of cardiovascular disease (by clinical history, physical examination, and ECG). Exclusion criteria for all subjects included renal insufficiency or proteinuria, altered hepatic function, and alcohol abuse. Patients with diabetes mellitus (fasting blood glucose level >115 mg/dL or treatment with a hypoglycemic agent) or hypertension (systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or treatment with an antihypertensive agent) and smokers were excluded.

The study was approved by the local Ethics Committees, and the patients signed a written informed consent. Some of the subjects enrolled in this study had been previously involved in another study.5

The plasma lipid profiles of patients and control subjects are shown in the Table.

Baseline Characteristics of Hypercholesterolemic Patients and Age- and Sex-Matched Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n=20)</th>
<th>Control Subjects (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>53±6</td>
<td>53±6</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6/14</td>
<td>6/14</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Smoking</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clinical atherosclerosis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>291±34</td>
<td>183±24*</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>207±31</td>
<td>114±22*</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>141±60</td>
<td>78±24</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>55±9</td>
<td>52±11</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<.0001 vs control.

Statistics

The data were analyzed by nonparametric methods to avoid assumptions about the distribution of the measured variables. Comparisons between groups were made with the Mann-Whitney U test. The differences between baseline and posttreatment values were analyzed with the Wilcoxon signed-rank test. The association of measurements with other biochemical parameters was assessed by the Spearman rank correlation test. All values are reported as mean±1 SD. Statistical significance was considered to be indicated by a value of P<.05. All calculations were made with the Stat View II computer program (Abacus Concepts).

Results

Baseline characteristics of hypercholesterolemic patients and control subjects are shown in the Table. Patients had a significantly higher total cholesterol and LDL cholesterol levels compared with those of control subjects.

As shown in Fig 1, plasma P-selectin was significantly increased in hypercholesterolemic patients compared with that
in normocholesterolemic subjects (98±61 versus 56±14 ng/mL; P=.001). Hypercholesterolemic patients also had higher levels of vWF compared with those of control subjects (176±22 versus 119±12%; P=.0001) (Fig 1).

Plasma P-selectin directly correlated with either cholesterol (ρ=.453; P=.0046), LDL cholesterol (ρ=.513; P=.0017) (Fig 2), or vWF (ρ=.654; P=.0001) levels.

Because hypercholesterolemic patients have increased lipid peroxidation in vivo, we next examined whether supplementation with the antioxidant vitamin E could lower plasma levels of P-selectin in our group of patients. To this end, the 20 hypercholesterolemic patients were treated with vitamin E at the dosage of 600 mg/d for 2 weeks. As shown in Fig 3 after vitamin E administration, a significant reduction in P-selectin (98±61 versus 59±34 ng/mL [-40%], P=.001) and vWF (175±22 versus 152±14 [-13%], P=.001) was observed.

Vitamin E supplementation was associated with a statistically significant increase in vitamin E plasma levels (from 37.9±7.4 to 73.3±17.8 nmol/L; P=.0001) and in the lag time for LDL oxidation (from 40.5±14.3 to 74.5±23.4 minutes; P=.0001). In the 20 hypercholesterolemic patients, plasma P-selectin was inversely related to plasma vitamin E levels (ρ=−.446; P=.0067) (Fig 4) but not to the lag time for LDL-oxidation (results not shown).

Vitamin E inhibits platelet aggregation and adhesion; therefore we examined the contribution of platelet activation to the effect of vitamin E on soluble P-selectin. To this end, the urinary excretion of 11-dehydro-TXB₂, which is an established marker of in vivo platelet activation, was measured in our group of patients before and after vitamin E administration. Interestingly, vitamin E significantly lowered the urinary excretion of 11-dehydro-TXB₂ (from 1271±632 to 654±197 pg/mg creatinine; P=.0015) (Fig 5), which suggests that the impact of vitamin E on soluble P-selectin levels could be, at least in part, related to a reduction in the degree of in vivo platelet activation.

No significant change in plasma lipid levels, including LDL cholesterol, were detected during vitamin E supplementation.

Discussion

P-selectin is an adhesion molecule that is contained in the α-granules of platelets and in the Weibel-Palade bodies of endothelial cells.14 P-selectin mediates interactions among platelets, leukocytes, and endothelial cells and may play a central role in the pathophysiology of multicellular vascular events such as thrombosis, inflammation, and atherosclerosis. Alternative splicing of P-selectin mRNA gives rise to a soluble form that lacks the transmembrane domain and is detectable with immunologic methods in human plasma.10 This isoform is also contained in human platelets.15 An increase in the levels of plasma P-selectin has been observed in several pathological conditions of the vascular system,11-14 and it has been proposed that soluble P-selectin may reflect the total upregulation of this glycoprotein in platelets and endothelial cells.14

In this report, we show that plasma P-selectin is increased in hypercholesterolemic patients compared with that in sex- and
Along these lines, activated platelets and endothelial cells generate, dysfunction and platelet activation causing P-selectin expression.28 and in the occluded arteries of rat heart allografts pressure is increased in endothelial cells overlying atherosclerotic plaques.26 On the other hand, the platelet inhibitory properties of vitamin E are well documented.223 Because P-selectin is expressed by activated platelets4 and hypercholesterolemic patients display increased in vivo platelet activation,15 it might be hypothesized that vitamin E reduced soluble P-selectin levels in hypercholesterolemic patients by correcting their higher degree of platelet activation. Indeed, in our group of patients (Fig 5), vitamin E administration significantly lowered the urinary excretion of 11-dehydro-TXB2, which is an established marker of in vivo platelet activation.24 This is consistent with previous results from our group5 and indicates that the reduced levels of soluble P-selectin observed after vitamin E treatment in hypercholesterolemic patients might be, at least in part, related to the inhibitory activity of this vitamin on platelet activation.

The pathophysiological significance of an increase in P-selectin plasma levels in hypercholesterolemic patients still remains unclear. Other soluble cell adhesion molecules such as intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1 (sVCAM-1), and E-selectin have been found to represent the equivalent of the plasma isoform, have shown that P-selectin induces tissue factor expression in human circulating monocytes.39 Similarly, measurements of soluble P-selectin, which originates from both platelets and endothelial cells, may be proposed as a marker of endothelial atherosclerotic damage.32 These findings suggest that a correlation might exist between P-selectin expression and oxidative stress in hypercholesterolemic patients. If this hypothesis was correct, the administration of antioxidants would have had an impact on the elevated P-selectin plasma levels found in hypercholesterolemic patients. Indeed vitamin E supplementation lowered significantly both P-selectin and vWF plasma levels (Fig 3), which indicates that enhanced oxidation contributes to determine the development of endothelial dysfunction in hypercholesterolemia. Interestingly, an inverse correlation between P-selectin and vitamin E plasma levels was observed (Fig 4), but there was no correlation between P-selectin and the lag time for LDL oxidation. Taken together these results suggest that oxidative events may have a quite complex impact, not solely restricted to LDL oxidation rate, on the mechanisms of P-selectin release. In this regard it would be interesting to determine whether there is a correlation between plasma P-selectin and urinary 8-epi-prostaglandin F2α levels. On the other hand, the platelet inhibitory properties of vitamin E are well documented.223 Because P-selectin is expressed by activated platelets4 and hypercholesterolemic patients display increased in vivo platelet activation,15 it might be hypothesized that vitamin E reduced soluble P-selectin levels in hypercholesterolemic patients by correcting their higher degree of platelet activation. Indeed, in our group of patients (Fig 5), vitamin E administration significantly lowered the urinary excretion of 11-dehydro-TXB2, which is an established marker of in vivo platelet activation.24 This is consistent with previous results from our group5 and indicates that the reduced levels of soluble P-selectin observed after vitamin E treatment in hypercholesterolemic patients might be, at least in part, related to the inhibitory activity of this vitamin on platelet activation.

The pathophysiological significance of an increase in P-selectin plasma levels in hypercholesterolemic patients still remains unclear. Other soluble cell adhesion molecules such as intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1 (sVCAM-1), and E-selectin have been found to represent the equivalent of the plasma isoform, have shown that P-selectin induces tissue factor expression in human circulating monocytes.39 Similarly, measurements of soluble P-selectin, which originates from both platelets and endothelial cells, may be proposed as a marker of endothelial atherosclerotic damage.32 These findings suggest that a correlation might exist between P-selectin expression and oxidative stress in hypercholesterolemic patients. If this hypothesis was correct, the administration of antioxidants would have had an impact on the elevated P-selectin plasma levels found in hypercholesterolemic patients. Indeed vitamin E supplementation lowered significantly both P-selectin and vWF plasma levels (Fig 3), which indicates that enhanced oxidation contributes to determine the development of endothelial dysfunction in hypercholesterolemia. Interestingly, an inverse correlation between P-selectin and vitamin E plasma levels was observed (Fig 4), but there was no correlation between P-selectin and the lag time for LDL oxidation. Taken together these results suggest that oxidative events may have a quite complex impact, not solely restricted to LDL oxidation rate, on the mechanisms of P-selectin release. In this regard it would be interesting to determine whether there is a correlation between plasma P-selectin and urinary 8-epi-prostaglandin F2α levels. On the other hand, the platelet inhibitory properties of vitamin E are well documented.223 Because P-selectin is expressed by activated platelets4 and hypercholesterolemic patients display increased in vivo platelet activation,15 it might be hypothesized that vitamin E reduced soluble P-selectin levels in hypercholesterolemic patients by correcting their higher degree of platelet activation. Indeed, in our group of patients (Fig 5), vitamin E administration significantly lowered the urinary excretion of 11-dehydro-TXB2, which is an established marker of in vivo platelet activation.24 This is consistent with previous results from our group5 and indicates that the reduced levels of soluble P-selectin observed after vitamin E treatment in hypercholesterolemic patients might be, at least in part, related to the inhibitory activity of this vitamin on platelet activation.

The pathophysiological significance of an increase in P-selectin plasma levels in hypercholesterolemic patients still remains unclear. Other soluble cell adhesion molecules such as intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1 (sVCAM-1), and E-selectin have been found to represent the equivalent of the plasma isoform, have shown that P-selectin induces tissue factor expression in human circulating monocytes.39 Similarly, measurements of soluble P-selectin, which originates from both platelets and endothelial cells, may be proposed as a marker of endothelial atherosclerotic damage.32 These findings suggest that a correlation might exist between P-selectin expression and oxidative stress in hypercholesterolemic patients. If this hypothesis was correct, the administration of antioxidants would have had an impact on the elevated P-selectin plasma levels found in hypercholesterolemic patients. Indeed vitamin E supplementation lowered significantly both P-selectin and vWF plasma levels (Fig 3), which indicates that enhanced oxidation contributes to determine the development of endothelial dysfunction in hypercholesterolemia. Interestingly, an inverse correlation between P-selectin and vitamin E plasma levels was observed (Fig 4), but there was no correlation between P-selectin and the lag time for LDL oxidation. Taken together these results suggest that oxidative events may have a quite complex impact, not solely restricted to LDL oxidation rate, on the mechanisms of P-selectin release. In this regard it would be interesting to determine whether there is a correlation between plasma P-selectin and urinary 8-epi-prostaglandin F2α levels. On the other hand, the platelet inhibitory properties of vitamin E are well documented.223 Because P-selectin is expressed by activated platelets4 and hypercholesterolemic patients display increased in vivo platelet activation,15 it might be hypothesized that vitamin E reduced soluble P-selectin levels in hypercholesterolemic patients by correcting their higher degree of platelet activation. Indeed, in our group of patients (Fig 5), vitamin E administration significantly lowered the urinary excretion of 11-dehydro-TXB2, which is an established marker of in vivo platelet activation.24 This is consistent with previous results from our group5 and indicates that the reduced levels of soluble P-selectin observed after vitamin E treatment in hypercholesterolemic patients might be, at least in part, related to the inhibitory activity of this vitamin on platelet activation.
in part reduced by vitamin E treatment. On the basis of these results, we propose that circulating levels of P-selectin may represent in this clinical setting an in vivo marker of endothelial dysfunction and/or platelet activation and that the administration of an antioxidant might be beneficial to reduce the risk of cardiovascular events in hypercholesterolemic patients.

Acknowledgments

This study was supported by grants from Consiglio Nazionale delle Ricerche (CNR), Progetto Finalizzato Prevenzione e Controllo dei Fattori di Malattia (SP8:94.00560.PF41 and 95.00807.PF41). We wish to thank Domenico De Cesare and Salvatore Roccaforte for technical assistance.

References


Increased Levels of Soluble P-Selectin in Hypercholesterolemic Patients
Giovanni Davi?, Mario Romano, Andrea Mezzetti, Antonio Procopio, Stefano Iacobelli, Teresa Antidormi, Tonino Bucciarelli, Paola Alessandrini, Franco Cuccurullo and Gabriele Bittolo Bon

Circulation. 1998;97:953-957
doi: 10.1161/01.CIR.97.10.953

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/97/10/953

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/