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Susceptibility of Apolipoprotein B-Containing Lipoproteins to Oxidation and Antioxidant Status in Acute Coronary Syndromes

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Summary

Background: Oxidized lipoproteins may play an important role in the pathogenesis of atherosclerosis, and it has been shown that antioxidants have a protective effect against the progression of atherosclerosis.

Hypothesis: The aim of this study was to investigate the oxidative susceptibility of apolipoprotein B-containing lipoproteins and antioxidant status in patients with acute coronary syndromes and chronic stable angina pectoris.

Methods: The study population included 70 patients with acute coronary syndromes (14 with recent acute myocardial infarction and 56 with unstable angina pectoris), 105 patients with stable angina pectoris, and 75 control subjects. In addition to conventional lipid and lipoprotein analysis, the susceptibility of apolipoprotein B-containing lipoproteins to in vitro oxidation (lag phase) and plasma vitamin E and total carotene levels was measured.

Results: The lag phase was significantly shorter in patients with acute coronary syndromes (45 ± 12 min) than in patients with stable angina pectoris (51 ± 10 min) and in control subjects (58 ± 9 min) ($p < 0.0001$). Both plasma vitamin E and total carotene levels were lowest in patients with acute coronary syndromes (1.11 ± 0.32 mg/dl and 119 ± 32 μg/dl, respectively), followed by patients with stable angina pectoris (1.25 ± 0.37 mg/dl and 132 ± 37 μg/dl) and then controls (1.52 ± 0.31 mg/dl and 167 ± 41 μg/dl).

Conclusions: These data suggest that there is an intense oxidative process and a lower antioxidant status in acute coronary syndromes. This may lead to plaque instability due to the activation of the inflammatory response in coronary atherosclerotic lesions.

Key words: lipoprotein oxidation, antioxidants, acute coronary syndromes, stable coronary artery disease

Introduction

The evidence that oxidative modification of lipoproteins may play an important causative role in atherosclerosis has been increasing rapidly over the past several years.

Oxidized low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) exert several biological effects that may contribute to the initiation progression of the atherosclerotic lesion.[1–3] Oxidized LDL and VLDL share some common properties: both are chemoattractant and cytotoxic, and both can be taken up by the scavenger receptors.[4–7] These findings suggest the possibility that oxidized LDL and VLDL may be atherogenic, not only because of their ability to induce foam cell formation, but also by triggering a chronic inflammatory process and cell hyperplasia in the vessel wall.[8]

Several studies have associated concentrations of low alpha tocopherol, which is the principal lipid soluble antioxidant in plasma, with the development of atherosclerosis.[9–11] Alpha tocopherol has been shown to protect lipoproteins from oxidation.[3] In addition, Esterbauer *et al.* have shown that carotenoids provide auxiliary antioxidant defenses with respect to LDL after alpha tocopherol.[9]

The aim of this study was to compare the oxidative susceptibility of apolipoprotein B-containing lipoproteins and antioxidant status in patients with acute coronary syndromes and chronic stable angina pectoris.

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Materials and Methods

We studied 250 consecutive patients undergoing diagnostic coronary angiography. The study population included 176 patients with angiographically documented coronary artery disease (CAD). Seventy patients were considered to have acute coronary syndromes [14 with recent myocardial infarction (MI) and 56 with unstable angina] and 105 patients chronic stable angina pectoris. Seventy-five patients with angiographically normal coronary arteries were considered control subjects. Recent MI was diagnosed retrospectively if at least two of the following criteria were met: typical chest pain, diagnostic electrocardiographic (ECG) changes, cardiac enzymes at least twice the upper reference limit. Unstable angina was diagnosed in the absence of ECG and cardiac enzyme changes diagnostic of an MI if one of the following criteria was met: crescendo angina, angina pectoris of new onset brought on by minimal exertion, and angina pectoris at rest. Chronic stable angina was defined as typical exertional chest pain relieved either by rest, sublingual nitrates, or both, and with no change in symptoms in the preceding 3 months.

Conventional risk factors for CAD (smoking, hypertension, family history of premature cardiovascular disease, diabetes, hyperlipidemia), height, and weight were recorded in all patients. Venous blood samples were collected from patients in the fasting state before angiography. Total cholesterol and triglyceride were measured by conventional enzymatic methods. High-density lipoprotein cholesterol (HDL-C) was quantified by the same enzymatic method after precipitation of apolipoprotein B-containing lipoproteins with dextran sulphate-magnesium chloride.[12] Low-density lipoprotein cholesterol (LDL-C) was determined using the Friedewald formula.[13]

Vitamin E was extracted into xylene and analyzed with ferric chloride/D-alpha-alpha dipyridyl reagent.[14] Serum total carotene was measured by using the spectrophotometric method described by Neeld and Person.[15]

The susceptibility of apolipoprotein B-containing lipoproteins to in vitro oxidation was assessed by the thiobarbituric acid reactive substance (TBARS) test. In this test, the chromogen is formed by the reaction of one molecule of malondialdehyde (MDA) with two molecules of thiobarbituric acid (TBA). The method involves heating the sample with TBA under acidic conditions and reading the absorbance of the MDA-TBA adduct formed at 532 nm.[16] The lag phase was defined as the intercept of the tangent of the slope of the absorbance curve in the propagation phase with the baseline, and was expressed in minutes.[17]

Diagnostic coronary angiography was performed using the Judkins technique in all patients. Films were evaluated by experienced angiographers who were blinded to the patients' conditions. Coronary artery disease was defined as >50% narrowing of lumen diameter of a major coronary artery. A quantitative method (the Gensini scoring system) was used to evaluate the extent of lesions.[18]

Statistical analysis: Data are expressed as mean value ± standard deviation. Control subjects and patients were compared, and analysis of variance (ANOVA) was used for comparison of the mean values of lag phase, alpha tocopherol, total carotene concentrations, and other lipid parameters. The Spearman correlation test was used for comparison of the severity of CAD and the lag phase. The correlation between the lag phase and vitamin E and total carotene was analyzed by Spearmen correlation test as well. A value of $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics and conventional coronary risk factors of the patients are summarized in Table I.

As expected, patients with CAD had significantly higher cholesterol, triglyceride, and LDL-C, and lower HDL-C levels than controls (Table II).

The susceptibility of apolipoprotein B-containing lipoproteins to in vitro oxidation (lag phase) was 58 ± 9 min in control subjects. These values were significantly lower than in patients with chronic stable angina pectoris (51 ± 10 min, $p < 0.0001$) and in those with acute coronary syndromes (45 ± 12 min, $p < 0.0001$). The lag phase was also significantly longer in patients with chronic stable angina pectoris than in those with acute coronary syndromes ($p < 0.001$) (Table III).

TABLE I Clinical characteristics of study groups

Variable	Group 1 (n = 75)	Group 2 (n = 105)	Group 3 (n = 70)
Age (years)	55 ± 10	57 ± 9	57 ± 10
Male/female	30/45	84/21	60/10
BMI (kg/m^2)	28 ± 4.7	26 ± 3.9	27 ± 4.1
Hypertension (%)	26 (35)	42 (40)	28 (39)
Diabetes mellitus (%)	10 (13)	19 (18)	11 (15)
Smoking (%)	34 (45)	58 (55)	34 (48)
Family history (%)	36 (48)	45 (43)	31 (44)

Group 1: Patients without coronary artery disease.
Group 2: Patients with chronic stable angina.
Group 3: Patients with acute coronary syndromes.
Abbreviation: BMI = body mass index.

TABLE II Serum lipid and lipoprotein levels in study groups

	Group 1	Group 2	Group 3
Total cholesterol (mg/dl)	194 ± 41	228 ± 43[a]	220 ± 40[b]
LDL-C (mg/dl)	127 ± 35	145 ± 31[a]	139 ± 35[b]
HDL-C (mg/dl)	40 ± 7	32 ± 6[a]	33 ± 6[b]
Triglycerides (mg/dl)	146 ± 45	195 ± 63[a]	200 ± 78[b]

[a] $p < 0.01$ (Group 1 vs. Group 2).
[b] $p < 0.01$ (Group 1 vs. Group 3).
Group definition as in Table I.
Abbreviations: LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol.

TABLE III The oxidative susceptibility of apolipoprotein B-containing lipoproteins and antioxidant status

	Lag phase (min)	Vitamin E (mg/dl)	Total carotene (μg/dl)
Group 1 (n = 75)	58 ± 9	1.50 ± 0.31	167 ± 41
Group 2 (n = 105)	51 ± 10[a]	1.25 ± 0.37[a]	132 ± 36[a]
Group 3 (n = 70)	45 ± 12[b,c]	1.10 ± 0.32[b,c]	119 ± 32[c,d]

[a] $p < 0.01$ (Group 1 vs. Group 2).
[b] $p < 0.01$ (Group 1 vs. Group 3).
[c] $p < 0.01$ (Group 2 vs. Group 3).
[d] $p < 0.05$ (Group 2 vs. Group 3).
Group definition as in Table I.

Plasma vitamin E levels were 1.5 ± 0.31 mg/dl in control subjects, 1.25 ± 0.37 mg/dl in patients with stable CAD, and 1.1 ± 0.32 mg/dl in patients with acute coronary syndromes. Plasma levels of vitamin E were significantly lower in patients with acute coronary syndromes than in those with stable CAD and in control subjects (Table III).

In control subjects and in patients with stable angina and acute coronary syndromes, plasma total carotene levels were 167 ± 41, 132 ± 36, and 119 ± 32 μg/dl, respectively. A positive correlation was found between lag phase and vitamin E levels ($r = 0.38$, $p < 0.0001$) and between lag phase and total carotene levels ($r = 0.15$, $p < 0.01$). A significant inverse correlation ($r = -0.4$, $p < 0.001$) was found between lag phase and severity of coronary atherosclerosis (Gensini scoring system).

Discussion

The present study demonstrates that the lag phase was significantly shorter in patients with acute coronary syndromes than in those with stable CAD, suggesting that increasing the oxidative susceptibility of apolipoprotein B-containing lipoproteins may lead to plaque instability.

Oxidized LDL and VLDL are cytotoxic and could promote endothelial dysfunction.[2,5] Oxidized LDL can stimulate expression of some cytokines, such as interleukin-1, in the arterial wall.[19] Interleukin-1 has been shown to induce smooth muscle cell proliferation, promote a procoagulant state, and stimulate leukocyte-endothelial cell adhesion.[20] Oxidized LDL can adversely affect coagulation by stimulating tissue factor and plasminogen activator inhibitor-1 synthesis.[2,21,22] In addition, it inhibits endothelium-derived relaxation factor-mediated vasodilation,[2,21,22] may contribute to the initiation and progression of the atherosclerotic process, and may also lead to plaque instability with these biological effects.

In our study we found that apolipoprotein B-containing lipoproteins were more susceptible to oxidation in patients with acute coronary syndromes than in patients with stable CAD and in control subjects. Kostner *et al.*[23] found that conjugated diens were significantly higher in patients with unstable than with stable angina pectoris. They suggested that lipid peroxidation parameters are increased in patients with unstable angina pectoris and discriminate stable from unstable angina pectoris.

In another study, plasma levels of oxidized LDL were very similar in patients with stable CAD and with acute coronary syndromes.[24] They suggest that their increases were independent of plaque instability. In contrast, they found that plasma levels of MDA-modified LDL were significantly higher in patients with acute coronary syndromes than in those with stable CAD.

There are numerous methods for the measurement of lipoprotein oxidation. We used an indirect method for the susceptibility of apolipoprotein B-containing lipoproteins to in vitro oxidation (lag phase). In the other studies different methods were used for the measurement of LDL oxidation; this may lead to different results.

The oxidative susceptibility of LDL (lag phase) has been shown to correlate with the severity of coronary atherosclerosis, as evaluated by coronary angiography. Regnström *et al.*[25] found that in male survivors of MI the susceptibility of LDL to oxidation (as measured by lag phase) correlated with the severity of coronary atherosclerosis. We also found that susceptibility of apolipoprotein B-containing lipoproteins to oxidation is associated with the severity of coronary atherosclerosis both in acute and chronic CAD.

Antioxidants such as alpha tocopherol, probucol, and diphenyl phenylenediamine have been shown to decrease the degree of LDL oxidation and atheromatous lesions in animal models of atherosclerosis.[21,26] Alpha tocopherol is the predominant lipophilic antioxidant in tissue and LDL;[7] it inhibits LDL oxidation in vitro. Numerous human studies have shown that alpha tocopherol supplementation reduced LDL oxidizability in healthy subjects.[26,27] Also, alpha-tocopherol supplementation was shown to reduce recurrent MI by 77% in a recent study.[28] One can reasonably speculate that alpha tocopherol can slow the progression of atherosclerosis by reducing lipoprotein oxidation. In our study, E vitamin level was lowest in patients with acute coronary syndromes, followed by patients with stable angina pectoris and control subjects.

Conclusion

These data suggest that there is an intense oxidative process in acute coronary syndromes. This may lead to plaque instability by activation of the inflammatory response in coronary atherosclerotic lesions.

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