Clinical Significance of Antibody Against Oxidized Low Density Lipoprotein in Patients With Atherosclerotic Coronary Artery Disease

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OBJECTIVES
This study was designed to establish the clinical significance of antibodies against oxidized low density lipoprotein (anti-Ox-LDL) titer in atherosclerotic coronary artery disease (CAD).

BACKGROUND
Oxidative modification of LDL, which plays a key role in the development of atherosclerosis, induces immunogenic epitopes in the LDL molecule, and the presence of anti-Ox-LDL has been demonstrated in human sera.

METHODS
Anti-Ox-LDL titer was measured by enzyme-linked immunosorbent assay in 108 patients who had angiographically verified CAD, and 31 patients who had chest pain but no significant CAD, as controls.

RESULTS
The anti-Ox-LDL titer was higher (p<0.01) in patients with multivessel CAD (19.4±10.1 AcU/ml, n=68) than in the controls (9.8±4.1). However, no significant difference was shown between the single-vessel CAD group (15.1±6.4, n=40) and the controls, or between the multivessel CAD group and the single-vessel CAD group. The titer was higher in patients with unstable angina (21.5±11.8 AcU/ml, n=20, p<0.01), or in patients with acute myocardial infarction (23.1±12.0, n=20, p<0.01) than in patients with stable-effort angina or old myocardial infarction (12.2±8.6, n=68). Multiple logistic regression analysis indicated that the anti-Ox-LDL titer most powerfully discriminated CAD patients from controls (odds ratio [OR]: 1.20, 95% confidence interval [CI]: 1.07–1.33, p=0.0006) and acute coronary syndrome from chronic CAD (OR: 1.09, 95% CI: 1.04–1.14, p=0.0008).

CONCLUSIONS
Serum anti-Ox-LDL titer not only can predict a presence of atherosclerotic CAD but also may be a marker of plaque instability. Low density lipoprotein oxidation may play an important role in the development of plaque instability. (J Am Coll Cardiol 2001;37:775–9)

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Oxidized low density lipoprotein (LDL) is believed to play a key role in the development of atherosclerosis (1). It has been proposed that oxidative modification of LDL is a prerequisite for rapid accumulation of LDL in macrophages and for foamy cell formation. The LDL extracted from atherosclerotic lesions certainly shows biochemical and immunoreactive properties similar to those of in vitro oxidized LDL (2). Oxidative modification of LDL induces immunogenic epitopes in the LDL molecule (3), and the presence of antibodies against oxidized LDL (anti-Ox-LDL) has been demonstrated in human sera (4,5). Results of several studies demonstrated the increased titer of anti-Ox-LDL in patients with atherosclerotic coronary artery disease (CAD) (6–8) as well as cerebral (9) or peripheral (10) artery disease. Conversely, other studies reported that no positive relationship was observed between anti-Ox-LDL titers and the extent of atherosclerosis (11–13). The purpose of this study was to establish the clinical significance of measuring anti-Ox-LDL titer in atherosclerotic CAD.

METHODS

Patient Selection and Angiographic Assessment
In this study we enrolled 108 patients who underwent initial diagnostic coronary angiography and had significant atherosclerotic CAD. Diagnostic criteria of angiographically significant CAD included organic-discrete stenotic lesions as indicating more than 75% diameter stenosis. The criterion for single-vessel CAD or multivessel CAD was based upon the number of arteries with a more than 75% diameter stenosis. Eighty-eight patients underwent scheduled coronary angiography. These included 68 patients with stable-effort angina or old myocardial infarction (OMI) and 20 patients with unstable angina, which was defined as an increase in the frequency and/or severity of chest pain or new onset of symptoms suggesting myocardial ischemia. In patients with unstable angina, only those who were scheduled for coronary angiography within seven days from the last ischemic evidence were enrolled. The remaining 20 patients included those with acute myocardial infarction.
(AMI) who underwent coronary angiography in the emergent situation. Excluded were patients who underwent prior coronary angioplasty or coronary bypass surgery and those with other cardiac or vascular diseases including cardiomyopathy, congenital or valvular heart disease, atherosclerotic peripheral artery diseases, or cerebrovascular diseases. Thirty-one patients who complained of chest pain but had no angiographically detectable CAD (defined as having neither discrete stenosis nor vessel wall irregularity) and who showed negative acetylcholine provocation test and whose age and gender were matched with the CAD patients were selected as controls. The study protocol was approved by the Dokkyo University Institutional Review Board, and written informed consent was obtained from each patient.

Lipid Profile

Venous blood samples were taken in the fasting state in 88 patients with CAD and 31 control subjects early in the morning on the day of coronary angiography. In 20 patients with AMI, blood samples were taken on admission before the start of emergent coronary angiography. Routine lipid profile and glucose metabolism were examined. The residual serum was frozen at −80°C until the analysis of anti-Ox-LDL. Serum total cholesterol and triglyceride levels were determined by automated enzymatic assays (14,15). Low density lipoprotein cholesterol was assayed by enzymatic measurement, and high density lipoprotein (HDL) cholesterol was determined by a precipitation method. Apolipoprotein (apo) A-I, apo B and apo E were quantified with a turbidimetric immunoassay. The assay of lipoprotein (a) [Lp(a)] was conducted by a latex agglutination immunoassay. The presence of hypertension or diabetes mellitus and smoking habits were treated as categorical variables. The levels of total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol and the anti-Ox-LDL titer were treated as continuous variables. A multiple logistic regression model was used for discriminating CAD patients from control subjects or discriminating patients with acute coronary syndrome from patients with chronic CAD. Of the independent variables, the remaining risk factors were identical among the three patient groups of control, single-vessel CAD and multivessel CAD. Comparisons of conventional coronary risk factors (hypertension, diabetes mellitus, smoking habits and the lipid profile) among the patient groups of control, single-vessel CAD and multivessel CAD are shown in Table 1. The HDL-cholesterol level was lower in the multivessel CAD group than in the controls. The remaining risk factors were identical among the three patient groups. The anti-Ox-LDL titer was 19.4 ± 10.1 AcU/ml in the multivessel CAD group, which was higher than the value of 9.8 ± 4.1 AcU/ml in the controls (p < 0.01). No significant difference in the values of anti-Ox-LDL titer was seen between the single-vessel CAD group (15.1 ± 6.4) and the controls, and between the multivessel CAD group and the single-vessel CAD group (Fig. 1). Multiple logistic regression analysis indicated that the anti-Ox-LDL titer as well as the HDL cholesterol level was a more powerful discriminating factor than the HDL cholesterol level (OR: 0.89, 95% CI: 0.81–0.97, p = 0.003) (Table 2).

Abbreviations and Acronyms

AMI = acute myocardial infarction
ANOVA = one-way analysis of variance
anti-Ox-LDL = antibodies against oxidized low density lipoprotein
apo = apolipoprotein
CAD = coronary artery disease
CI = confidence interval
ELISA = enzyme-linked immunosorbent assay
HDL = high density lipoprotein
LDL = low density lipoprotein
Lp(a) = lipoprotein (a)
MDA = malonic dialdehyde
OMI = old myocardial infarction
OR = odds ratio
p-NPP = p-nitrophenyl phosphate

RESULTS

Of 108 patients, 40 had single-vessel CAD, and 68 had multivessel (double- or triple-vessel) CAD. Comparisons of conventional coronary risk factors (hypertension, diabetes mellitus, smoking habits and the lipid profile) among the patient groups of control, single-vessel CAD and multivessel CAD are shown in Table 1. The HDL-cholesterol level was lower in the multivessel CAD group than in the controls. The remaining risk factors were identical among the three patient groups. The anti-Ox-LDL titer was 19.4 ± 10.1 AcU/ml in the multivessel CAD group, which was higher than the value of 9.8 ± 4.1 AcU/ml in the controls (p < 0.01). No significant difference in the values of anti-Ox-LDL titer was seen between the single-vessel CAD group (15.1 ± 6.4) and the controls, and between the multivessel CAD group and the single-vessel CAD group (Fig. 1). Multiple logistic regression analysis indicated that the anti-Ox-LDL titer as well as the HDL cholesterol level was a more powerful discriminating factor than the HDL cholesterol level (OR: 0.89, 95% CI: 0.81–0.97, p = 0.003) (Table 2).
Table 1. Comparison of Coronary Risk Factors in Three Patient Groups of Single-Vessel CAD, Multivessel CAD and Controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 31)</th>
<th>Single-Vessel CAD (n = 40)</th>
<th>Multivessel CAD (n = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>61 ± 10</td>
<td>62 ± 11</td>
<td>61 ± 10</td>
</tr>
<tr>
<td>Male gender</td>
<td>18 (58%)</td>
<td>31 (78%)</td>
<td>53 (78%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (13%)</td>
<td>7 (18%)</td>
<td>13 (19%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>12 (39%)</td>
<td>12 (30%)</td>
<td>26 (38%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>18 (58%)</td>
<td>28 (70%)</td>
<td>44 (65%)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>194 ± 18</td>
<td>202 ± 19</td>
<td>203 ± 24</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>104 ± 43</td>
<td>102 ± 42</td>
<td>101 ± 46</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>45 ± 12</td>
<td>43 ± 10</td>
<td>38 ± 8*</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>116 ± 29</td>
<td>123 ± 32</td>
<td>129 ± 28</td>
</tr>
<tr>
<td>Apo A-I (mg/dl)</td>
<td>113 ± 20</td>
<td>113 ± 19</td>
<td>106 ± 18</td>
</tr>
<tr>
<td>Apo B (mg/dl)</td>
<td>92 ± 21</td>
<td>92 ± 21</td>
<td>98 ± 15</td>
</tr>
<tr>
<td>Apo E (mg/dl)</td>
<td>3.3 ± 0.8</td>
<td>3.3 ± 0.8</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>21 ± 26</td>
<td>23 ± 31</td>
<td>23 ± 25</td>
</tr>
</tbody>
</table>

*p < 0.01.

CAD = coronary artery disease; HDL = high density lipoprotein; LDL = low density lipoprotein; apo = apolipoprotein; Lp(a) = lipoprotein (a).

Within the 108 CAD patients, the anti-Ox-LDL titer was higher in patients with unstable angina (21.5 ± 11.8 AcU/ml, p < 0.01) or AMI (23.1 ± 12.0 AcU/ml, p < 0.01) than in patients with stable-effort angina or OMI (12.2 ± 8.6) (Fig. 2). In the multiple logistic regression model, the anti-Ox-LDL titer as well as smoking habits but not other conventional coronary risk factors could discriminate 40 patients with acute coronary syndrome (i.e., unstable angina or AMI) from 68 patients with chronic CAD (i.e., stable-effort angina or OMI). The anti-Ox-LDL (OR: 1.09, 95% CI: 1.04–1.14, p = 0.0008) was a more powerful discriminating factor than smoking habits (OR: 3.74, 95% CI: 1.22–11.44, p = 0.02) (Table 3).

DISCUSSION

Immunological Response to Oxidized LDL

There has been considerable interest recently in the contribution of oxidative process of LDL (Ox-LDL) to the development of atherosclerosis (1). Whereas native LDL does not cause cholesterol ester accumulation in macrophages, modified LDL by oxidation does (16). Ox-LDL has also been implicated in other mechanisms potentially involved in the development of atherosclerosis—that is, cytotoxic (17) or chemotactic action for monocytes (18) and inhibition of macrophage motility (19). Furthermore, the involvement of oxidation in atherosclerosis is supported by the results of clinical studies. Both elevated lipid peroxide levels (20) and increased susceptibility of LDL to in vitro oxidation (21) are associated with atherosclerosis, and increased dietary consumption of antioxidants appears to be associated with a decreased risk for CAD (22,23).

To evaluate this phenomenon further, reliable assays for LDL oxidation state are necessary. However, the direct measurement of Ox-LDL in serum or plasma is complicated by the potential for in vivo modification of the sample and also by the possibility that the primary location of the analyses of the interest may not be the circulation. In addition, oxidation leads to an array of potential forms, which appear at different stages of the oxidation process and may have varied significance (24). In contrast, the measurement of anti-Ox-LDL titer can provide us stable data. Specific immunological epitopes expressed on Ox-LDL were found in atherosclerotic lesions both in animals with experimental atherosclerosis and in humans (2,3). Expression of such epitopes in vitro can be generated by various procedures, including incubation with endothelial cells and macrophages, oxidation in the presence of copper ions and the treatment with MDA (3). Ox-LDL can interact with scavenger receptors of monocyte-derived macrophages. It is suggested that these interactions can induce the formation of anti-Ox-LDL (25). Therefore, anti-Ox-LDL can be considered a marker of LDL oxidation in the level of tissues or cells.

Antibody Against Oxidized LDL as a Predictor of CAD

We demonstrated in this study that anti-Ox-LDL titer was higher in patients with multivessel CAD than in patients with single-vessel CAD or in control patients, although it showed no significant difference in single-vessel CAD patients and controls. This result indicated that the anti-Ox-LDL titer level was elevated in patients with severe CAD but not in patients with mild CAD. The multiple logistic regression analysis showed that the anti-Ox-LDL titer and HDL cholesterol level but not any other conventional coronary risk factors could discriminate CAD patients from control subjects. In addition, the anti-Ox-LDL
titer was a more powerful predicting factor than HDL cholesterol. These results suggest that the anti-Ox-LDL may be a potent predictor of CAD. Elevated levels of anti-Ox-LDL titer in patients with CAD have also been reported elsewhere. Maggi et al. (7) demonstrated that high titer of anti-Ox-LDL was observed not only in patients with CAD but also in patients without clinically relevant signs of CAD but considered at risk. Their results indicate that the anti-Ox-LDL titer may be useful in predicting early-stage CAD, whereas our results suggest that the anti-Ox-LDL titer may be a marker of advanced CAD. Antibodies against oxidized LDL titer also increased in other atherosclerotic disease besides CAD. Bergmark et al. (10) demonstrated by a case-controlled study that anti-Ox-LDL titer discriminated patients with atherosclerotic peripheral vascular disease. A prospective ultrasound observation of carotid atherosclerosis by Salonen et al. (9) indicated that anti-Ox-LDL titer was correlated with the rate of lesion progression but not with the baseline intima-media thickness.

**Antibody against oxidized LDL as a marker of plaque instability.** In our study, patients with unstable angina or patients with AMI showed higher titer of anti-Ox-LDL than patients with stable-effort angina or OMI. Furthermore, our multiple regression analysis results showed the anti-Ox-LDL titer could most strongly discriminate acute coronary syndrome (i.e., unstable angina or AMI) from chronic CAD (i.e., stable-effort angina or OMI). These results suggest that the anti-Ox-LDL may be a marker of plaque instability. Several recent studies suggested that Ox-LDL was related not only to onset or progression of coronary atherosclerosis but also to plaque instability (26,27). In the present study, we employed a commercial kit for anti-Ox-LDL measurement. In this assay, we measured an antibody against an epitope of MDA-induced-Ox-LDL. Holvoet et al. (28) demonstrated that the plasma level of MDA-modified LDL was threefold higher in patients with acute coronary syndrome than in patients with stable angina as well as normal volunteers. Ryan et al. (29) reported that high levels of MDA-LDL antibodies were found in patients with AMI and that the highest level was seen within 48 h from its onset. In addition, Puurunen et al. (6) and Wu et al. (30) presented data indicating that antibodies against MDA-induced Ox-LDL could predict the occurrence of AMI. These results suggest that the anti-Ox-LDL titer may be a marker of unstable plaque presence and that MDA-induced LDL oxidation may play an important role in the development of plaque instability.

**CONCLUSIONS**

Finally, from this study we suggest that serum anti-Ox-LDL titer not only can predict a presence of atherosclerotic CAD but may also be a marker of plaque instability. The LDL oxidation on local vascular wall seems to be associated with plaque instability. Lipid anti-oxidation as well as lipid lowering should be considered as a therapeutic strategy to inhibit plaque instability in atherosclerotic CAD.
Table 3. Multiple Logistic Regression Analysis for Discriminating Acute Coronary Syndrome From Chronic CAD

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>Wald χ²</th>
<th>p Value</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>−0.470</td>
<td>0.009</td>
<td>0.922</td>
<td>0.95 (0.37–2.45)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>−0.656</td>
<td>0.934</td>
<td>0.334</td>
<td>0.52 (0.13–1.96)</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.319</td>
<td>5.346</td>
<td>0.021</td>
<td>3.74 (1.22–11.44)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.001</td>
<td>0.003</td>
<td>0.991</td>
<td>1.00 (0.97–1.03)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.003</td>
<td>0.250</td>
<td>0.617</td>
<td>0.99 (0.98–1.01)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−0.010</td>
<td>1.123</td>
<td>0.275</td>
<td>0.99 (0.94–1.05)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>−0.020</td>
<td>1.911</td>
<td>0.167</td>
<td>0.98 (0.95–1.02)</td>
</tr>
<tr>
<td>Anti-Ox-LDL</td>
<td>0.085</td>
<td>11.298</td>
<td>0.0008</td>
<td>1.09 (1.04–1.14)</td>
</tr>
</tbody>
</table>

Anti-Ox-LDL = antibodies against oxidized low density lipoprotein; CAD = coronary artery disease; CI = confidence interval; HDL = high density lipoprotein; LDL = low density lipoprotein.

Acknowledgments

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