Elevated Plasma Levels of Oxidized Low-Density Lipoprotein Relate to the Presence of Angiographically Detected Complex and Thrombotic Coronary Artery Lesion Morphology in Patients With Unstable Angina

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Background  Increased levels of oxidized low-density lipoprotein (ox-LDL) are related to plaque instability, so the aim of the present study was to investigate whether there is a relationship between angiographic coronary plaque morphology in patients with unstable angina pectoris (UAP) and the level of ox-LDL.

Methods and Results  Plasma ox-LDL levels were measured in 149 patients with UAP and in 88 control subjects, using a highly sensitive enzyme-linked immunosorbent assay method. Angiographic morphology of the culprit lesion was classified as either simple or complex based on the Ambrose classification. Plasma ox-LDL levels in patients with Braunwald class III were significantly higher than in patients with class I (p < 0.0001) or in control subjects (p < 0.0001). In each of the 3 Braunwald classes, plasma ox-LDL levels in patients with a complex lesion were significantly higher than in patients with a simple lesion. Multivariate logistic regression analysis revealed that ox-LDL level and Braunwald class III were independent factors associated with angiographically detected complex lesions.

Conclusion  In each Braunwald class of UAP, elevated plasma levels of ox-LDL closely relate to the presence of angiographically detected complex and thrombotic lesion morphology. (Circ J 2007; 71: 681–687)

Key Words: Angina pectoris; Atherosclerosis; Coronary artery disease; Lipoproteins
human coronary atherosclerosis, the question arises whether an elevation in the plasma level of ox-LDL in UAP patients could relate to the presence of unstable plaque with thrombosis, angiographically represented by complex and thrombotic lesion morphology. The present study was designed to address this question.

Methods

Patients

The study was approved by the hospital ethics committee, and informed consent was given by all patients before the study.

The study population contained 149 consecutive UAP patients who had undergone coronary angiography within 3 days of admission and had angiographically documented narrowing of at least 70% of the luminal diameter of a major coronary artery.14 We excluded patients with variant angina, concomitant inflammatory diseases or malignant tumors, and those undergoing dialysis. Variant angina was defined as recurrent attacks of chest symptoms occurring spontaneously at rest, with ST-segment elevation on the ECG, which were rapidly relieved by nitroglycerin. All patients with variant angina had proven coronary spasm as demonstrated by chest symptoms and ST-segment elevation after intracoronary injection of acetylcholine. The UAP patients were classified according to Braunwald’s criteria.15 Class I indicates new onset severe or accelerated exertional angina within 2 months; class II indicates angina at rest during the previous month before admission but not within the last 48 h before admission; and class III indicates angina at rest during the last 48 h before admission. All patients had primary UAP, corresponding to subclass “B” of the Braunwald classification: 54 were in Braunwald class I, 19 were in class II, and 76 were in class III. On admission, all patients were on aspirin (81 mg) therapy, and antianginal treatment (β-blockers, nitrates or calcium-channel blockers, alone or in combination) was prescribed for 82% of the patients. Moreover, patients with high risk were also being treated with intravenous heparin, but none had received thrombolytic agents. Before admission, lipid-lowering agents were administered to 30 patients, but none used antioxidant drugs. Age- and gender-matched healthy volunteer blood donors served as controls (51 men, 37 women; age 59±13 years, mean±SD). Of them, none had hypercholesterolemia or diabetes mellitus, 13 had hypertension and 20 were smokers. All 13 hypertensive controls were stage I hypertensive according to the WHO Study Group, 21 and hypercholesterolemia as defined by Japan Atherosclerotic Society Guideline22).

Coronary Angiography Analysis

Cardiac catheterization was performed using the brachial or femoral approach using standard catheters and techniques. The culprit vessel was identified on the basis of clinical, ECG, and angiographic data. In patients with single-vessel disease, the culprit lesion was considered to be the most severely stenosed lesion in the affected vessel. In patients with multivessel disease, the culprit lesion was defined as the most severe stenosis in the artery that supplied the ischemic area as identified by ECG changes or noninvasive testing results. Angiography was performed so that each lesion could be viewed from at least 2 angles. In all UAP patients, off-line quantitative coronary angiography was conducted to reveal the highest degree of stenosis. Calculation was performed using the Cardiovascular Measurement System (CMS-MEDIS Medical Imaging System) by an operator who was unaware of the patients’ clinical situation or the presence of thrombus. Intracoronary thrombus was identified by the presence of intraluminal filling defects surrounded by contrast on at least 3 sides with or without contrast staining. In our analysis, total occlusions and intracoronary thrombus were considered complex lesions, and were not classified as separate angiographic variables.18

Pre-intervention angiographic morphology was evaluated by 2 independent observers, who were unaware of the clinical situation. In cases of disagreement, consensus was reached by further joint reading. Angiographic morphology was classified as either simple or complex based on the Ambrose classification.13 Simple lesions were concentric or eccentric and characterized by a smooth border and a broad neck. Complex lesions were eccentric with a narrow neck, irregular borders or overhanging edges, including ulceration or the presence of thrombus. Intracoronary thrombus was identified by the presence of intraluminal filling defects surrounded by contrast on at least 3 sides with or without contrast staining. In our analysis, total occlusions and intracoronary thrombus were considered complex lesions, and were not classified as separate angiographic variables.18

The interobserver agreement for angiographic morphology was 90% and the intraobserver agreement was 93%.

Blood Sampling and Assays

Venous blood samples of all patients were obtained on admission to hospital. For measuring TC, HDL- and LDL-cholesterol levels, blood samples were obtained after an overnight fast. The concentration of CRP was measured by latex agglutination photometric immunoassay with an automated immunochemistry analyzer (LX-6000; Eiken Chemical Co, Tokyo, Japan) with normal values <0.3 mg/dl. The serum TnT level was determined by ELISA using an ES-300 immunoassay analyzer (Boehringer-Mannheim, Mannheim, Germany) with normal values <0.1 lg/L.
Statistical Analysis

Statistical analyses were performed with StatView 5.0 (Abacus Concepts, Calabasus, CA, USA) and results expressed as mean±SD. When the data were normally distributed, the 2 groups were compared with an unpaired Student’s t-test. Otherwise, the Mann-Whitney U-test was used. Statistical comparisons between more than 3 groups were performed by one-way analysis of variance (ANOVA) and post-hoc multiple comparison, using Scheffe’s test. Levels of ox-LDL and CRP did not distribute normally, so transformed values of ox-LDL and CRP in logarithm were used as variables for statistical analyses. Categorical variables were compared by chi-square test.

Results

Ox-LDL Level and Clinical Severity of UAP

Table 1 shows the relationship between the patients’ clinical and angiographic characteristics and Braunwald classification. TC levels in patients with Braunwald class III were significantly lower than in patients with class I (ANOVA: p<0.05). With regard to inflammatory markers, neutrophil counts in patients with class III were significantly higher than in patients with Braunwald class I (ANOVA: p<0.05).

Plasma levels of ox-LDL showed a weak positive correlation with CRP levels (R=0.204, p<0.05). Fig 1 shows plots of the plasma levels of ox-LDL in UAP patients with Braunwald class I, II or III, with reference to either the angiographically detected complex or simple lesion group. Plasma ox-LDL levels in patients with class III were significantly higher than in patients with class I (p<0.0001) or in control subjects (p<0.0001). The levels of plasma ox-LDL in patients with class II were also significantly higher than those in control subjects (p<0.005) (class III: 1.25±0.91, class II: 1.10±0.83, class I: 0.72±0.46, control: 0.58±0.20 ng/5 μg LDL protein, Table 1).

Ox-LDL Levels and Coronary Angiographic Morphology

In this cohort, 84 of 149 culprit lesions in the UAP patients showed angiographic evidence of a complex lesion. Among the 3 Braunwald groups, the frequency of the type of angiographic morphology was significantly different. In patients with class II or III, a complex lesion was more frequent.
quent than in those with class I (class I: 37%, class II: 63%, class III: 68%, p<0.0001). Total occlusions were observed in 3 patients (16%) with class II and in 10 patients (13%) with class III vs in 1 patient (2%) with class I. Intracoronary thrombus was detected in 16% of class II and 12% of class III vs in 4% of class I.

Table 2 shows the patients’ characteristics in the angiographically detected simple vs complex lesion groups. Plasma levels of ox-LDL in patients with a complex lesion were significantly higher (Mann-Whitney U: p<0.0001) than in those with a simple lesion (complex lesion: 1.37±0.88, simple lesion: 0.61±0.38 ng/5 g LDL protein). Moreover, as shown in Fig 2, plasma ox-LDL levels in patients with a complex lesion were significantly higher than in those with a simple lesion in each of the 3 Braunwald classes (class I: simple 0.59±0.40, complex 0.94±0.49; class II: simple 0.55±0.26, complex 1.40±0.90; class III: simple 0.58±0.36, complex 1.49±0.93 ng/5 g LDL protein).

Fig 3 shows the ROC curves of ox-LDL and C-reactive protein (CRP) for detection of angiographic complex lesions. True-positive fraction (sensitivity as y axis) is plotted vs false-positive fraction (1-specificity as x axis) by changing cutoff values for test.

Table 2 Clinical and Angiographic Characteristics Between Angiographically Detected Simple vs Complex Lesion Groups

<table>
<thead>
<tr>
<th></th>
<th>Simple lesion (n=65)</th>
<th>Complex lesion (n=84)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>65±11</td>
<td>65±10</td>
<td>NS</td>
</tr>
<tr>
<td>Male (%)</td>
<td>82</td>
<td>74</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>69</td>
<td>61</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia (%)</td>
<td>43</td>
<td>50</td>
<td>NS</td>
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<tr>
<td>Diabetes mellitus (%)</td>
<td>34</td>
<td>32</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>57</td>
<td>56</td>
<td>NS</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>200±31</td>
<td>198±41</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>45±10</td>
<td>46±12</td>
<td>NS</td>
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<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>126±26</td>
<td>127±34</td>
<td>NS</td>
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<tr>
<td>QCA analysis</td>
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<tr>
<td>MLD (mm)</td>
<td>0.58±0.30</td>
<td>0.58±0.45</td>
<td>NS</td>
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<tr>
<td>DS (%)</td>
<td>84±9</td>
<td>87±11</td>
<td>NS</td>
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<tr>
<td>Leukocyte count (/mm³)</td>
<td>7,020±2,030</td>
<td>7,039±2,518</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophil count (/mm³)</td>
<td>4,137±1,624</td>
<td>4,768±2,390</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.39±0.78</td>
<td>0.59±1.06</td>
<td>NS</td>
</tr>
<tr>
<td>ox-LDL (ng/5 g LDL protein)</td>
<td>0.61±0.38</td>
<td>1.37±0.88</td>
<td>&lt;0.0001</td>
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Data are mean±SD or percentages.
See Table 1 for abbreviations.
Statistical comparisons were performed by unpaired Student’s t-test or Mann-Whitney U-test. Categorical variables were compared by chi-square test.

Fig 2. Relationship between plasma levels of oxidized low-density lipoprotein (ox-LDL) and coronary angiographic morphology (simple or complex) in patients with Braunwald class I, II or III (Mann-Whitney U-test). Open bars, simple lesion. Solid bars, complex lesion.

Fig 3. Receiver-operating characteristic curves of oxidized low-density lipoprotein (ox-LDL) and C-reactive protein (CRP) for detection of angiographic complex lesions. True-positive fraction (sensitivity as y axis) is plotted vs false-positive fraction (1-specificity as x axis) by changing cutoff values for test.
Relationship Between Plasma Ox-LDL Level and Myocardial Necrosis

Of the 149 patients, 118 (79%) had negative TnT levels (<0.1 μg/L); 50 patients were in Braunwald class I, 15 were in class II and 53 were in class III. There was no significant difference in the plasma levels of ox-LDL between UAP patients with positive TnT or negative TnT (positive TnT: 1.03±0.69, negative TnT: 1.04±0.82 ng/5μg LDL protein).

Factors Related to Angiographically Detected Complex Lesions

Multivariate analysis revealed that ox-LDL level and Braunwald class III were independent factors associated with angiographically detected complex lesions (Table 3).

Discussion

To the best of our knowledge, this is the first study to demonstrate that in each Braunwald class of UAP, increased levels of plasma ox-LDL closely relate to the presence of angiographically detected complex and thrombotic lesion morphology.

In recent years, increasing evidence has shown an important role of ox-LDL in the genesis of intraplaque inflammatory processes. We have previously demonstrated that elevated levels of plasma ox-LDL show a significant positive correlation with the severity of ACS. Similarly, Tsimikas et al recently reported that temporal increases in the plasma level of ox-LDL strongly reflect the presence of ACS. These data have led us to hypothesize that increased levels of plasma ox-LDL are related to plaque instability with thrombosis in humans and our present study results from 149 UAP patients support this view and provide new information about the relationships among plasma ox-LDL level, Braunwald class and angiographic coronary plaque morphology in UAP.

In angiographic studies of stenoses responsible for UAP, the term “complex lesion” has been angiographically defined in different ways. In the present study, we incorporated complex morphology, intracoronary thrombus and total occlusions into the general angiographic definition of a complex lesion, as previously described by Dangas et al.

A number of previous angiographic studies have reported that complex and thrombotic lesions are prevalent in UAP. Pathological studies of postmortem material revealed that these angiographic appearances were caused by plaque disruption with thrombosis. Moreover, a recent immunohistochemical study using atherectomy specimens demonstrated that in UAP patients specimens from angiographic complex lesions had a greater percentage of macrophages than those from angiographic simple lesions. Those findings strongly suggest that angiographically detected complex lesions reflect the presence of unstable plaque associated with intraplaque inflammation, disruption, and thrombus formation.

Furthermore, a correlation between the Braunwald classification and coronary morphology has been described. Dangas et al found that the incidence of angiographically detected complex and thrombotic lesions was higher in Braunwald class III than in class I. Rupprecht et al also reported that the incidence of angiographic evidence of complex lesions and/or thrombosis rose progressively with higher UAP class. These observations clearly indicate an association between high Braunwald score and the incidence of angiographic findings of complex and thrombotic lesions. Our present observation that angiographically detected complex lesions were more frequent in patients with class II or III than in those with class I, is in agreement with these previous studies. Our results, moreover, demonstrated that plasma ox-LDL levels in patients with class III were significantly higher than in those with class I, suggesting a similar association between high Braunwald class and increased levels of plasma ox-LDL in UAP patients. Moreover, at the same time, the present study clearly showed that even in Braunwald class I, plasma ox-LDL levels in patients with a complex lesion were significantly higher than in those with a simple lesion. Furthermore, our multivariate regression analysis revealed that an angiographically detected complex lesion was an independent predictor of increased plasma ox-LDL levels in UAP patients. These observations are of considerable interest because it appears that the presence of angiographic complex and thrombotic lesion morphology, rather than a high Braunwald classification, is more strongly associated with an increase in the plasma ox-LDL level in UAP.

A recent study by Segev et al has shown that plasma levels of OxLDL-E06 did not correlate with angiographic complexity; however, in their study the patient cohort had only SAP. More recently, Anselmi et al measured circulating levels of ox-LDL using Mercodia ox-LDL ELISA kit in patients with UAP (n=26) and SAP (n=29), and demonstrated that plasma ox-LDL levels correlated with the presence of angiographically complex plaques. In that study, however, only patients in Braunwald class III B were included. Therefore, a relationship between angiographically detected complex lesion morphology and plasma ox-LDL levels in each Braunwald class of UAP was not seen.

Previous studies have demonstrated that reactive oxygen species are formed at an accelerated rate in postischemic myocardium, which raises the possibility that myocardial necrosis may induce an increase in plasma ox-LDL levels. In the present study, there was no significant difference in the plasma level of ox-LDL between UAP patients with positive TnT or negative TnT, which suggests that the elevation of plasma ox-LDL levels, detected with our method, does not relate to the presence of myocardial necrosis in UAP patients.

What causes the increased plasma level of ox-LDL in UAP patients? As mentioned before, culprit lesions obtained from UAP patients contain increased numbers of macrophages and T lymphocytes, and previous in vitro studies have shown that macrophages and lymphocytes promote...

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<th>Table 3 Multivariate Logistic Regression Analysis</th>
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<td>Braunwald class II</td>
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<td>ox-LDL</td>
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OR, odds ratio; CI, confidence interval. See Table 1 for other abbreviations.
LDL oxidation. Moreover, we demonstrated previously that the number of ox-LDL-positive macrophages is significantly increased in the culprit lesions of UAP patients. It is conceivable, therefore, that one of the sources of ox-LDL in the blood of UAP patients is direct release from disrupted or ruptured coronary atherosclerotic plaque. Our previous study, moreover, demonstrated that an infiltration of myeloperoxidase (MPO)-positive neutrophils occurs in the culprit lesions of UAP patients and recent studies have shown that MPO, a strong pro-oxidant enzyme released from activated neutrophils, plays a key role in promoting LDL oxidation in vivo. Together with our present findings, the results suggest that the progression of plaque instability and thrombosis in association with infiltration of MPO-positive neutrophils at culprit lesions of UAP patients may induce excessive generation of pro-oxidants leading to a pro-oxidant/antioxidant imbalance in the blood and contribute to further enhancement of LDL oxidation.

Our recent experimental study has shown that minimally modified LDL (MM-LDL) is a type of ox-LDL enriched with ox-PC, and that the DLH3 antibody binds not only to fully ox-LDL but also to MM-LDL. We speculated, therefore, that our measurement method using the DLH3 antibody, which recognizes specifically ox-PC, may enhance the behavior of ox-PC particles as part of ox-LDL and MM-LDL in humans.

Study Limitations

Angiography offers only visual information, and its sensitivity for the detection of intracoronary thrombus is limited. Furthermore, the time of angiography was not standardized but was left up to the decision of the primary cardiologist. Therefore, we could not assess exactly the incidence of intracoronary thrombus. More importantly, regarding features related to plaque vulnerability, angiography has limitations compared with angiography and intravascular ultrasound. However, recent studies have reported that angiographically complex lesions are strongly associated with disrupted plaques and/or thrombus as assessed by ultrasound and by angiography in patients with UAP. These observations support the concept that angiographically detected complex lesions may be used as markers of plaque instability. Our ox-LDL-measuring method with separation of the LDL fraction from every plasma sample is time consuming, but is a good way of avoiding interference from other plasma constituents, such as ox-VLDL, anti-ox-LDL autoantibodies, and anti-phospholipid antibodies. We think that our method, which could monitor the behavior of ox-PC particles as part of ox-LDL and MM-LDL, is important for establishing the clinical relevance of ox-LDL and MM-LDL measurement in ACS. Finally, our present finding that there was no association between plasma ox-LDL level and diabetes mellitus was not consistent with our previous study and the discrepancy may have occurred because of the small numbers of patients in the previous study.

Conclusion

Thrombus formation on a ruptured, disrupted, or eroded coronary atherosclerotic plaque is widely considered as the most important pathophysiologic mechanism of the acute or severe presentations of UAP. Our present study demonstrates that in each Braunwald class of UAP, elevated plasma levels of ox-LDL closely relate to the presence of angiographically detected complex and thrombotic lesion morphology.

References

1. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 1994; 89: 36–44.
22. Working Committee on JAS Guideline for Diagnosis and Treatment of Hyperlipidemias. Report of the Japan Atherosclerosis Society
Ox-LDL and Complex Lesions


