Elevated concentrations of oxidized lipoprotein(a) are associated with the presence and severity of acute coronary syndromes

Jun-jun Wang a,⁎,1, Chun-ni Zhang a,1, Yang Meng a, Ai-zhong Han a, Jian-bin Gong b, Ke Li a

a Department of Biochemistry, Jinling Hospital, School of Medicine, Nanjing University, 210002, Nanjing, PR China
b Department of Cardiology, Jinling Hospital, PR China

A R T I C L E   I N F O

Article history:
Received 23 May 2009
Received in revised form 20 July 2009
Accepted 22 July 2009
Available online 29 July 2009

Keywords:
Atherosclerosis
Coronary artery disease
Acute coronary syndromes
Lipoproteins
Lipoprotein(a)
Oxidation
Risk factor

A B S T R A C T

Objective: To investigate possible mechanisms and association of increased oxidized Lp(a) [ox-Lp(a)] levels with presence and extent of acute coronary syndromes (ACS).

Methods: Ox-Lp(a) levels were studied in 96 patients with ACS, 89 patients with stable coronary artery disease (CAD), and 100 control subjects.

Results: Compared to control, ox-Lp(a) levels increased in stable CAD patients (P<0.001), and especially in ACS (P<0.001) (ACS, 16.29±13.80 μg/ml; stable CAD, 10.04±10.32 μg/ml; control, 7.10±9.16 μg/ml). The ratio of ox-Lp(a) to Lp(a) was higher in the ACS than those in the stable CAD (P<0.05) and control (P<0.001). Ox-Lp(a) levels were found associated with a graded increase in extent of angiographically documented CAD in the ACS (R=0.275, P=0.007), while not in the stable CAD (R=0.070) accounted for 11.1% of the variation in the extent of angiographically documented CAD in ACS patients; Lp(a) (β=0.415, P=0.000) and extent of CAD (β=0.193, P=0.071) accounted for 21.5% of that in ox-Lp(a) levels.

Conclusion: Elevated ox-Lp(a) levels are associated with presence and severity of ACS, and may be useful for identification of patients with ACS.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Lipoprotein(a) [Lp(a)] is an atherogenic particle that structurally resembles a low density lipoprotein (LDL) particle but contains a molecule of apolipoprotein(a) [apo(a)] attached to apoB-100 by a disulfide bond [1]. Elevated plasma concentrations of Lp(a) have been shown to be one of the independent risk factor for atherosclerosis [2–4]. Oxidized Lp(a) [ox-Lp(a)] has been reported to play more potent role than native Lp(a) in atherosclerosis. Ox-Lp(a) is a ligand for scavenger receptors [5,6] and might reasonably be expected to contribute to foam cell formation. Ox-Lp(a) may also induce adhesion molecular expression on monocytes, promoting their recruitment and adhesion to the endothelium [7], and modified forms of Lp(a), some resembling oxidized Lp(a), have been identified in human atheromatous lesions [8]. In addition, in vitro oxidative modification increases the inhibitory effect of Lp(a) on plasminogen binding to cell surfaces, which could attenuate fibrinolytic activity by reducing plasminogen activation [9].

Several lines of evidence support the concept that oxidized LDL (ox-LDL) plays a pivotal role in the development of atherosclerosis [10–19]. Ox-LDL concentrations have been reported to be a useful marker for identifying patients with coronary artery disease (CAD) and to be positively related with the severity of acute coronary syndromes (ACS) [10–15]. Oxidized phospholipid has also been found preferentially associated with Lp(a) [16] and correlates with both the presence and extent of angiographically documented CAD [17,18], and their concentrations increase after acute coronary syndromes (ACS) [14] and immediately after percutaneous coronary intervention [19]. Ox-Lp(a), autoantibodies, and Lp(a) immune complexes have all been detected in vivo [20–25]. Interestingly, our previous studies have shown increased concentrations of ox-Lp(a) and its immune complexes in patients with CAD [22,24]. Therefore, we hypothesized that circulating ox-Lp(a) may be associated with the presence and severity of CAD, specifically in patients with ACS, and be useful for the identification of patients with ACS. Hence we designed this study to evaluate the relationship between circulating ox-Lp(a), ACS, and stable CAD, and to explore possible mechanisms of increased ox-Lp(a) in stable and unstable syndromes.

Footnotes:
⁎ These authors have “equal contributions” to this work.
1 These authors have “equal contributions” to this work.
2. Materials and methods

2.1. Study subjects

The present study included 96 patients with ACS, 89 patients with stable CAD, and 100 control subjects. The control subjects selected from routine health examination were found normal in physical and electrocardiography and laboratory tests, and without diseases such as hypertension, hyperlipidemia, diabetes mellitus, or any other evident sign of atherosclerosis. All the subjects were living in Nanjing of China, Han population, which is the major ethnic group in China.

The CAD patients in this study were selected from admitted patients under the department of cardiology of Jinling Hospital between January 2005 and December 2008, who were undergoing clinically indicated coronary angiography. Angiograms of all the CAD patients showed at least 50% stenosis of 1, 2, or 3 coronary arteries. Ninety-six patients with ACS included acute myocardial infarction (MI) patients and unstable angina (UA) with Braunwald classification IIb or III. Eighty-nine patients with angiographically documented CAD and no cardiac events/procedures for >1 y were considered to have stable CAD. The exclusion criteria of the CAD patients included mild disease of angiography (a stenosis of 10–50% of the luminal diameter in all the 3 coronary arteries), prior coronary revascularization, and the presence of renal disease.

In patients with ACS, blood samples were taken on admission. Blood samples were collected at least 12 h after fasting from control subjects and patients with stable CAD. The blood sample was collected into EDTA (1 mg/ml) containing tube and plasma was separated immediately and stored at –70 °C until analysis. All laboratory assays were conducted within 1 year of blood sampling. This study protocol was approved by the Ethics Committee of Jinling Hospital, and all the subjects provided written informed consent.

2.2. Angiographic analysis

Catheterization was performed by either the Sones or the Judkins technique. Multiple views including angulated views were obtained, and the angiograms were evaluated. The extent of angiographically documented CAD was quantified in the left anterior descending coronary artery, the left circumflex artery, or the right coronary artery as follows: normal coronary arteries (smooth, with no stenosis or a stenosis of <10% of the luminal diameter), mild disease (a stenosis of 10–50% of the luminal diameter in ≥1 coronary arteries), or 1, 2, or 3- vessel disease, defined as a stenosis of >50% of the luminal diameter. To eliminate bias of judgment, angiographic observation and laboratory assays were conducted by different investigators in a double-blind way until all results were recorded and ready for statistical analysis.

2.3. Assays

Ox-Lp(a) was measured by a sandwich enzyme-linked immunosorbent assay (ELISA), using polyclonal antibody against ox-LDL as the capture antibody and quantitating with monoclonal anti-apo(a) enzyme conjugate as previously described [22]. Antibodies to ox-LDL were obtained by immunization of New Zealand white female rabbits with ox-LDL. The resulting rabbit antiserum was first fractionated by affinity chromatography with immobilized protein G, and the IgG fractions were then absorbed in a column of immobilized native LDL. The washout from the column of immobilized native LDL, containing antibodies to ox-LDL and irrelevant IgG, was used to capture ox-LDL, which had no reactivity with native LDL. A pooled fresh-frozen plasma sample (mixed plasma from 50 healthy subjects) was used as reference plasma of ox-Lp(a). The value of ox-Lp(a) was determined by the ELISA repeatedly, based on the concentration of copper ion oxidized Lp(a) as the standard. Lp(a) concentration was also detected by a "sandwich" ELISA as previously described [26]. With the use of monoclonal anti-apo(a) as capture antibody, bound Lp(a) particles were quantitated with polyclonal anti-apo(a) enzyme conjugates. Polyclonal sheep anti-apo(a) was self-made. Antibodies against LDL, plasminogen and other apolipoproteins in the Lp(a) antisera were removed by absorbing on an affinity chromatographic column of Sepharose 4B (Pharmacia) coupled with plasminogen and pooled sera obtained from Lp(a)-negative subjects. Reference Lp(a) was from Immuno AG Vienna. Total cholesterol, triglyceride, high density lipoprotein (HDL) cholesterol (Daichi Pure Chemicals, Japan) were measured on a Hitachi 7600 analyzer. LDL cholesterol was estimated with the use of the Friedewald formula [27].

2.4. Statistical analysis

Statistical analyses were performed with SPSS 11.5. The values were expressed as mean± standard deviation. Lp(a) and ox-Lp(a) concentrations of non-normal distributions were logarithmically transformed. The differences of variants among groups were analyzed by ANOVA, and the differences between groups were subsequently determined by Fisher LSD test when appropriate. Correlations between variables were calculated by the non-parametric Spearman rank coefficient test. Multiple linear regression analysis was used to estimate the associations among ox-Lp(a), degree of CAD, lipid measurements and patients’ characteristics. Values of P<0.05 were considered statistically significant.

3. Results

3.1. Base clinical characteristics and lipid concentrations in the study groups

The baseline clinical characteristics of the patients and control subjects, indications for coronary angiography, and lipid concentrations are shown in Table 1. The distributions of Lp(a), and ox-Lp(a) concentrations in all the studied patients and control subjects (n=285) were skewed toward lower values. In the entire population, a strong correlation was noted between Lp(a) and ox-Lp(a) concentrations (R=0.329, P<0.000).

3.2. Association with presence of ACS and stable CAD

Compared to control subjects, ox-Lp(a) and Lp(a) concentrations were found increased in both ACS and stable CAD patients. Furthermore, ox-Lp(a) concentrations were significantly higher in patients with ACS as compared to the patients with stable CAD. To exclude the influence of Lp(a), we also examined the association of the ratio of ox-Lp(a) to Lp(a) [ox-Lp(a)/Lp(a)] with the presence of ACS and stable CAD. The ratios of ox-Lp(a)/Lp(a) were found higher in the patients with ACS than in the patients with stable CAD as well as controls, while they remained similar between the stable CAD and control (Table 2).

3.3. Associations among ox-Lp(a), extent of angiographically documented disease, lipid parameters and clinical characteristics

The associations of ox-Lp(a) and Lp(a) concentrations with a graded increase in the extent of CAD were shown in Table 3. In detail, ox-Lp(a) concentrations were found significantly higher in the ACS patients with 3-vessel disease than those both in the patients with 1 and 2-vessel disease, while Lp(a) concentrations remained unchanged in the three groups. In the patients with stable CAD, no significant difference was found in ox-Lp(a) and Lp(a) concentrations among the three groups with different extents of vessel disease.

To further study the associations among ox-Lp(a), extent of angiographically documented disease, lipid parameters and clinical characteristics, simple linear correlation (Table 4) and multiple linear regression analysis were performed in the studied ACS patients (Table 5). Lp(a) concentrations were found positively correlated with age, smoking, and hypertension in the entire population. The distribution of Lp(a) tended to be shifted to higher levels in the ACS patients and the stable CAD patients than in the control subjects. With the use of monoclonal anti-apo(a) as capture antibody, ox-Lp(a) was measured by a sandwich ELISA as previously described [26]. They were significantly higher in patients with ACS than in the patients with stable CAD. The ratios of ox-Lp(a)/Lp(a) were found higher in the patients with ACS than in the patients with stable CAD as well as controls, while they remained similar between the stable CAD and control.

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>ACS (n=96)</th>
<th>Stable CAD (n=89)</th>
<th>Control (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>60.1±9.8</td>
<td>62.6±9.8</td>
<td>59.3±10.8</td>
</tr>
<tr>
<td>Male/female</td>
<td>65/31</td>
<td>52/37</td>
<td>58/41</td>
</tr>
<tr>
<td>Coronary risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>67 (70)</td>
<td>68 (76)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>31 (33)</td>
<td>33 (37)</td>
<td>35 (35)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>15 (16)</td>
<td>28 (31)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Family history of coronary artery disease</td>
<td>6 (6)</td>
<td>5 (6)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>51 (53)</td>
<td>53 (60)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other hypcholesterolemic agents</td>
<td>4 (4)</td>
<td>3 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>66 (69)</td>
<td>54 (61)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Past medical history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>10 (10)</td>
<td>31 (35)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>39 (41)</td>
<td>38 (43)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Ld cholesterol (mmol/l) 5.40±1.31 5.12±1.07 4.71±0.83
LDL cholesterol (mmol/l) 1.26±0.23 1.04±0.93 2.64±0.66
HDL cholesterol (mmol/l) 1.27±0.26 1.26±0.29 1.39±0.30
Triglyceride (mmol/l) 1.89±0.97 1.79±1.08 1.48±0.73

Data are presented as the mean value ± SD or number (%) of subjects. Comparred with control: *P<0.05, †P<0.01, ‡P<0.001.

Differences are not shown between the data expressed as number of subjects.
total cholesterol, LDL-C and ox-Lp(a) concentrations; ox-Lp(a) concentrations were found positively correlated with Lp(a) and the extent of CAD; and the extent of CAD was found positively correlated with only ox-Lp(a). No association of Lp(a), ox-Lp(a) and extent of angiographically documented disease was found with clinical characteristics, including age, sex, coronary risk factors (hypertension, tobacco use, diabetes mellitus, family history of CAD), medications (statins, other hypcholesterolemic agents, Beta-blockers), and past medical history (MI, congestive heart failure) (data not shown). We next performed the multiple linear regression analysis for the extent of CAD (or ox-Lp(a)) vs each factor. A backward elimination procedure of stepwise analysis was used; ox-Lp(a) [or the extent of CAD], Lp(a), lipid parameters and clinical characteristics were treated as independent variables. Consequently, ox-Lp(a), age and TG were found to account for 11.1% of the variation in the extent of CAD in the ACS patients. Furthermore, Lp(a) and extent of CAD were found to account for 21.5% of the variation in the concentration of ox-Lp(a). No association of the extent of CAD was found with ox-Lp(a) (R = 0.090, P = 0.402) or Lp(a) (R = 0.057, P = 0.597) in the patients with stable CAD.

4. Discussion

This study for the first time shows plasma ox-Lp(a) concentrations associated with the presence and extent of angiographically documented CAD in patients with ACS. Compared to control subjects, the plasma concentrations of ox-Lp(a) significantly increased in both ACS and stable CAD patients. Furthermore, ox-Lp(a) concentrations in ACS patients were significantly higher than stable CAD patients, suggesting that the increased ox-Lp(a) concentrations might be one of the major contributing factors for the occurrence of ACS.

Recently, ox-Lp(a) has been reported to play more potent role than native Lp(a) in atherosclerosis [5–9]. Theoretically, apoB and apo(a) proteins of Lp(a) molecule can both be oxidatively modified in vivo. The degree of oxidized apo(a) or apoB protein of Lp(a) has been detected to estimate circulating ox-Lp(a) concentrations [20,21]. We previously have also developed 2 ELISAs for measuring plasma ox-Lp(a) concentration, using human autoantibodies against ox-Lp(a) or specific rabbit antiserum against human ox-LDL as the capture antibody and quantitating with monoclonal anti-apo(a) enzyme conjugate, respectively. As the autoantibodies isolated from human mixed plasma can recognize both apo(a) and apoB epitopes of ox-Lp(a), the developed ELISA for ox-Lp(a) by using autoantibody may accurately reflect the state of Lp(a) oxidation in vivo. Furthermore, a significantly positive relationship was found between ox-Lp(a) concentrations detected by our two assays [22]. Ox-LDL can’t be detected by the ELISAs, because of using monoclonal antibody against apo(a) as quantitating antibodies. In addition, plasma ox-Lp(a) concentrations are low and as the samples are diluted, plasma ox-LDL has no influence on the assays.

Our previous studies have reported ox-Lp(a) concentrations increased in CAD patients [22], and in rheumatoid arthritis patients with excessive cardiovascular events [28]. In addition, hypertensive patients with complications showed a significantly higher concentration of serum apo(a) epitope of ox-Lp(a) than did normotensive subjects, whereas there was no significant difference in native Lp(a) [20]. ApoB protein of plasma Lp(a) was also found oxidized and was a characteristic of the patients with end-stage renal disease undergoing continuous ambulatory peritoneal dialysis [21]. In this study, ox-Lp(a) concentrations were found increased in both the ACS and stable CAD patients. Interestingly, ox-Lp(a) concentrations were found significantly higher in the ACS patients than those in the stable CAD patients. Furthermore, the ratios of ox-Lp(a)/Lp(a) were also found higher in the patients with ACS than in the patients with stable CAD as well as control, while they remained similar between the stable CAD and control, which suggest that the increased ox-Lp(a) concentrations might mainly be attributable to the occurrence of ACS. Therefore, plasma ox-Lp(a) concentrations may represent a better biochemical risk marker for ACS than plasma Lp(a). The diagnostic implications of the assay remain to be established in further studies.

Ox-Lp(a) concentrations and ox-Lp(a)/Lp(a) ratio were also noted associated with a graded increase in the extent of CAD in the patients with ACS, while not in stable CAD. The multiple linear regression analysis showed that ox-Lp(a), age and TG were significantly associated with the extent of CAD, adjusted for lipid parameters and clinical characteristics, but the associations were weak and remained to be studied. The above results are also supported by the studies about oxidized phospholipids. Recently, a series of studies have demonstrated convincingly that a key oxidized phospholipid is preferentially associated with Lp(a) [16] and correlates with both the presence and extent of angiographically documented CAD [17,18], and their concentrations increase after ACS [14] and immediately after percutaneous coronary intervention [19].

The source of plasma ox-Lp(a) is unknown. Holvoet et al. [29] isolated ox-LDL from the plasma of patients with post-transplant CAD and analyzed its characteristics, which suggested that it did not

Table 2

Lp(a), Ox-Lp(a) concentrations and ratio of ox-Lp(a) to Lp(a) in ACS, stable CAD and control groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>ACS</th>
<th>Stable CAD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=96)</td>
<td>(n=88)</td>
<td>(n=100)</td>
</tr>
<tr>
<td>Lp(a) mg/l</td>
<td>206.91±107.49 †</td>
<td>181.30±83.03 †</td>
<td>151.69±95.96</td>
</tr>
<tr>
<td>Ox-Lp(a) µg/ml</td>
<td>16.29±13.80 †‡</td>
<td>10.04±10.32 †‡</td>
<td>7.10±5.16</td>
</tr>
<tr>
<td>Ox-Lp(a)/Lp(a)</td>
<td>0.084±0.068 †‡</td>
<td>0.060±0.052 †‡</td>
<td>0.049±0.041</td>
</tr>
</tbody>
</table>

Compared with control: †P<0.05, ‡P<0.01, †P<0.001; compared with stable CAD: †P<0.05, ‡P=0.007

Table 3

Association of ox-Lp(a) and Lp(a) concentrations with extent of CAD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>3-vessel disease</th>
<th>2-vessel disease</th>
<th>1-vessel disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) mg/l</td>
<td>206.91±107.49 †</td>
<td>181.30±83.03 †</td>
<td>151.69±95.96</td>
</tr>
<tr>
<td>Ox-Lp(a) µg/ml</td>
<td>16.29±13.80 †‡</td>
<td>10.04±10.32 †‡</td>
<td>7.10±5.16</td>
</tr>
<tr>
<td>Ox-Lp(a)/Lp(a)</td>
<td>0.084±0.068 †‡</td>
<td>0.060±0.052 †‡</td>
<td>0.049±0.041</td>
</tr>
</tbody>
</table>

Compared with 1-vessel disease: †P<0.05; compared with 2-vessel disease: †P<0.05, ‡P<0.01

Table 4

Simple linear correlations between Lp(a), ox-Lp(a), the Extent of CAD, lipid parameters in ACS patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total cholesterol</th>
<th>Triglyceride</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
<th>Ox-Lp(a)</th>
<th>Extent of CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a)</td>
<td>0.323</td>
<td>0.100</td>
<td>0.039</td>
<td>0.315</td>
<td>0.241</td>
<td>0.160</td>
</tr>
<tr>
<td>P=0.001</td>
<td>P=NS</td>
<td>P=NS</td>
<td>P=0.002</td>
<td>P=0.018</td>
<td>P=NS</td>
<td></td>
</tr>
<tr>
<td>Ox-Lp(a)</td>
<td>−0.017</td>
<td>−0.063</td>
<td>0.029</td>
<td>−0.013</td>
<td>0.241</td>
<td>0.275</td>
</tr>
<tr>
<td>P=NS</td>
<td>P=NS</td>
<td>P=NS</td>
<td>P=NS</td>
<td>P=0.018</td>
<td>P=0.007</td>
<td></td>
</tr>
<tr>
<td>Degree</td>
<td>0.188</td>
<td>0.138</td>
<td>−0.019</td>
<td>0.153</td>
<td>0.160</td>
<td>0.275</td>
</tr>
<tr>
<td>P=NS</td>
<td>P=NS</td>
<td>P=NS</td>
<td>P=NS</td>
<td>P=NS</td>
<td>P=NS</td>
<td></td>
</tr>
</tbody>
</table>

NS, not significant.
Table 5

<table>
<thead>
<tr>
<th>Outcome variable/model</th>
<th>Beta</th>
<th>SE</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent of CAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ox-Lp(a)</td>
<td>0.271</td>
<td>0.006</td>
<td>0.019</td>
<td>0.111</td>
</tr>
<tr>
<td>Age</td>
<td>0.244</td>
<td>0.010</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.213</td>
<td>0.103</td>
<td>0.070</td>
<td></td>
</tr>
<tr>
<td>Ox-Lp(a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.415</td>
<td>0.014</td>
<td>0.000</td>
<td>0.215</td>
</tr>
<tr>
<td>Extent of CAD</td>
<td>0.193</td>
<td>1.928</td>
<td>0.071</td>
<td></td>
</tr>
</tbody>
</table>

References