Percutaneous coronary intervention results in acute increases in native and oxidized lipoprotein(a) in patients with acute coronary syndrome and stable coronary artery disease

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A B S T R A C T

Objective: To investigate possible changes of native and oxidized lipoprotein(a) [ox-Lp(a)] levels after percutaneous coronary intervention (PCI).

Design and methods: Lp(a), ox-Lp(a), and Lp(a) immune complexes (IC) and autoantibody levels were studied in 111 patients with acute coronary syndrome (ACS) and 68 patients with stable coronary artery disease (CAD) before and after PCI.

Results: Compared with pre-PCI, Lp(a), ox-Lp(a), and Lp(a)-IC levels acutely increased, while the autoantibody decreased in both the ACS and stable CAD patients. They all returned toward baseline by 1 to 2 days. The absolute change of ox-Lp(a) was found positively related with both the diameter of stenosis (R=−0.273, P=0.004) and the number of vessel disease (R=−0.312, P=0.001) in the ACS patients, while not in the stable CAD patients.

Conclusion: PCI results in acute plasma increases of ox-Lp(a) and Lp(a). Ox-Lp(a) may be present in ruptured or permeable plaques and be released into the circulation by PCI.

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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 [2,3]. Oxidized Lp(a) [ox-Lp(a)] has been reported to play more potent roles than native Lp(a) in atherosclerosis [4–7]. Ox-Lp(a), autoantibodies, and Lp(a) immune complexes (IC) have all been detected in vivo [8–16], and modified forms of Lp(a), some resembling ox-Lp(a), have been identified in human atheromatous lesions [17].

Several lines of evidence support the concept that oxidized LDL (ox-LDL) plays a pivotal role in the development of atherosclerosis [18–26]. 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) [18–22]. Oxidized phospholipid has also been found preferentially associated with Lp(a) [23] and correlated with both the presence and extent of angiographically documented CAD [24,25], and their concentrations increase after ACS [21] and immediately after percutaneous coronary intervention (PCI) [26]. Our previous studies have shown that ox-Lp(a) are present in newborns and children [10], while elevated ox-Lp(a) levels are found associated with presence of stable CAD and ACS, and with severity of ACS [11,12]. In the present study, we measured plasma levels of Lp(a) and several ox-Lp(a) markers immediately before and serially up to 6 months after PCI to further evaluate the clinical value of ox-Lp(a) in atherosclerotic cardiovascular disease.

Materials and methods

Study subjects

The present study included 111 patients with ACS, 68 patients with stable CAD, and 29 control subjects. The ACS and stable CAD patients were all performed stent placement as PCI. The control group only underwent diagnostic coronary angiography without PCI. The CAD patients in this study were selected from admitted patients under the Department of Cardiology of Jinling Hospital between September 2008 and November 2009, 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. ACS included acute myocardial infarction patients and unstable angina (UA) with Braunwald classification II or III. The patients with angiographically documented CAD and no cardiac events/procedures for more than 1 year were considered to have stable CAD. The exclusion criteria of the CAD patients included mild disease of angiography (a stenosis of 10% to 50% of the luminal diameter in all the three coronary arteries), prior coronary revascularization, and the presence of renal disease.

Venous blood in EDTA was obtained before PCI and immediately after PCI in all the patients, and the blood follow-up was obtained 24 hours, 2 days, 3 days, and 6 months after PCI from 15 of the ACS patients; the blood sample from control was obtained immediately before and after angiography. The 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 Jining Hospital, and all the subjects provided written informed consent.

**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 either no stenosis or a stenosis of <10% of the luminal diameter), mild disease (a stenosis of 10%–50% of the luminal diameter in one or more coronary arteries), or one- vessel, two-vessel, or three-vessel disease, defined as a stenosis of more than 50% of the luminal diameter in one, two, or three coronary arteries. 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.

**Lp(a) assay**

Lp(a) level was also detected by a “sandwich” enzyme-linked immunosorbent assay (ELISA) as previously described [27]. With the use of monoclonal anti-lp(a) as capture antibody, bounded Lp(a) particles were detected 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) particles were detected 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 serum of Lp(a) was from Immuno AG Vienna.

**Determination of ox-Lp(a), Lp(a)-IC levels, and ox-Lp(a) autoantibody (Ab) titters**

OX-Lp(a) was measured by a “sandwich” ELISA, using polyclonal antibody against ox-LDL as the capture antibody and detecting with monoclonal anti-apo(a) enzyme conjugate as previously described [28]. 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)-IC was measured by a sandwich ELISA, using anti-human IgG as the capture antibody and detecting with monoclonal anti-apo(a) enzyme conjugate as previously described [15]. A pooled fresh-frozen plasma sample (from 50 healthy subjects) was used as reference plasma of Lp(a)-IC, and the value was expressed as 1 relative absorbance unit (AU).

Ox-Lp(a) autoantibody was also measured by an ELISA. Briefly, one half of a microtiter ELISA plate (Corning, USA) was coated with copper ion oxidized Lp(a) and the other half with native Lp(a), each at a concentration of 10 μg/ml in sodium carbonate/bicarbonate buffer. The plates were incubated for 2 hours at 37 °C and then overnight at 4 °C. After washing three times with PBS containing 0.05% Tween 20 (PBS–Tween 20), plates were blocked with 1% fatty acid–free bovine serum albumin (BSA, Sigma) in PBS for 1 hour at room temperature. Plasma samples diluted 1:100 in PBS–Tween 20 containing 1% BSA (1% BSA–PBS–Tween 20) were incubated for 2 hours at room temperature. The wells were washed, and color was developed by adding substrate (3′,5′-tetramethylbenzidine). After 15 min at room temperature, the enzyme reaction was stopped by sulfuric acid, and the absorbance of each well was read at 450 nm. ox-Lp(a) was prepared as previously described [15]. Results were expressed as the absorbance unit (AU), and antibody titer against ox-Lp(a) was calculated by subtracting binding to native Lp(a) from binding to ox-Lp(a). Intra- and interassay coefficients of variation for all assays were 4.6%–7.2% and 9.2%–10.6%, respectively.

**Statistical analysis**

Statistical analyses were performed with SPSS 13.0. The values were expressed as mean ± standard deviation. The differences of variants among groups were analyzed by ANOVA, and the differences between groups were subsequently determined by Fisher LSD test when appropriate. The differences of paired variants were analyzed by paired-samples t test. Correlations between variables were calculated by the nonparametric Spearman rank coefficient test. Values of P < 0.05 were considered statistically significant.

**Results**

**Angiographic characteristics and lipid levels in the study patients**

There were no significant differences in lipid levels among the ACS and stable CAD patients who had PCI and the angiography-only control group, while the extent of angiographically documented CAD in both the ACS and stable CAD patients was greater than that in control subjects (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>ACS</th>
<th>Stable CAD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>111</td>
<td>68</td>
<td>29</td>
</tr>
<tr>
<td>Age (y)</td>
<td>63.21±12.38</td>
<td>65.63±10.58</td>
<td>60.86±0.64</td>
</tr>
<tr>
<td>Male/female</td>
<td>80/31</td>
<td>47/21</td>
<td>18/11</td>
</tr>
<tr>
<td>Angiographic analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter of stenosis (%)</td>
<td>0.87±0.13 †</td>
<td>0.81±0.15 ‡</td>
<td>0.14±0.24</td>
</tr>
<tr>
<td>Number of vessel disease</td>
<td>2.05±0.92 †</td>
<td>1.93±0.85 ‡</td>
<td>0.14±0.35</td>
</tr>
<tr>
<td>Lipid levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.27±0.91</td>
<td>4.09±0.90</td>
<td>4.04±0.86</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.20±0.88</td>
<td>2.00±0.83</td>
<td>2.03±0.70</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.18±0.25</td>
<td>1.20±0.25</td>
<td>1.24±0.32</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.89±1.28</td>
<td>1.94±1.23</td>
<td>1.72±0.83</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD or number (%) of subjects. Compared with control: †P < 0.001; compared with stable CAD: ‡P < 0.01.
Lp(a), ox-Lp(a), Lp(a)-IC, and ox-Lp(a)-Ab levels before and immediately after PCI

Compared with pre-PCI levels, Lp(a), ox-Lp(a), and Lp(a)-IC levels significantly increased, while ox-Lp(a)-Ab levels decreased in both ACS and stable CAD patients (Table 2). A strong correlation was noted between Lp(a) and ox-Lp(a) in all the patients plasma samples (n = 179) obtained before (R = 0.70, P < 0.0001) and immediately after PCI time points (R = 0.69, P < 0.0001; Fig. 1), respectively. In contrast, in the angiography-only control group, no significant changes were noted in Lp(a), ox-Lp(a), Lp(a)-IC, and ox-Lp(a)-Ab levels between the pre- and postangiography samples (Table 3).

Relative changes in Lp(a), ox-Lp(a), Lp(a)-IC, and ox-Lp(a)-Ab levels

Lp(a), ox-Lp(a), Lp(a)-IC, and ox-Lp(a)-Ab levels were followed up 24 hours, 2 days, 3 days, and 6 months after PCI in 15 of the ACS patients (Fig. 2). The absolute values of Lp(a) and ox-Lp(a) markers at baseline had a wide range and were not normally distributed. Therefore, we expressed each patient’s changes in its levels in response to PCI as a percent change compared with the pre-PCI samples, and mean percent changes were calculated. Lp(a), ox-Lp(a), Lp(a)-IC, and ox-Lp(a)-Ab levels acutely increased or decreased after PCI, and returned toward baseline by 1 to 2 days. For ox-Lp(a), there was a 94% mean percent increase after PCI compared with before PCI (P < 0.0001), and the post-PCI change was significantly different from that at 2- and 3-day and 6-month time points, respectively. In parallel, Lp(a) levels increased 54% in the post-PCI time point (P < 0.0001), and the post-PCI change was significantly different from that at 2- and 3-day and 6-month time points, respectively. Compared with before PCI, Lp(a)-IC increased 73% (P < 0.0001), and ox-Lp(a)-Ab decreased 18% (P < 0.0001) in the post-PCI time point.

Relation of angiographic variables to ox-Lp(a) and Lp(a) levels

The absolute change of ox-Lp(a) before and immediately after PCI, as well as pre- and post-PCI ox-Lp(a) levels were all found positively related with both the diameter of stenosis and the number of vessel disease in the ACS patients, respectively, while no significant association of angiographic variables was noted with the absolute change of ox-Lp(a) in the stable CAD patients. Similarly, no significant association of angiographic variables was found with the absolute change of Lp(a) in the ACS patients, although pre- or post-PCI Lp(a) levels were related with angiographic variables, respectively. In addition, no associations of angiographic variables were found with pre- and post-PCI Lp(a) levels and with the absolute change in stable CAD patients (Table 4).

Discussion

This study for the first time shows that PCI results in acute plasma increases of Lp(a) and ox-Lp(a) in both ACS and stable CAD patients. Interestingly, the changes of ox-Lp(a) levels are found to correlate with the extent of angiographically documented disease in ACS. These observations support the hypothesis that ox-Lp(a) are present in ruptured or permeable plaques and are released into the circulation by PCI.

Ox-Lp(a) has been reported to play more potent role than native Lp(a) in atherosclerosis [4–7]. 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) levels [8,9]. We previously have developed two ELISAs for measuring plasma ox-Lp(a) level by using two different capture antibodies: human autoantibodies against ox-Lp(a) or specific rabbit antiserum against ox-LDL [28]. As the autoantibodies isolated from human mixed plasma can recognize both apo(a) and apoB epitopes of ox-Lp(a), the developed ELISA by using autoantibody may accurately reflect the state of Lp(a) oxidation in vivo, and ox-Lp(a) levels detected by the assay using autoantibody may represent a better risk marker than those using antibodies against ox-LDL for ACS and stable CAD. Furthermore, a significantly

![Fig. 1. Relation of Lp(a) and ox-Lp(a) levels in all patients at before and after PCI.](image_url)
positive relationship was noted between ox-Lp(a) levels detected by the two assays [12].

Oxidized lipoprotein results from exposure of lipoprotein to oxidizing species, such as superoxide anion and hydrogen peroxide derived from all cells present in the artery wall, particularly macrophages, as well as enzymes such as lipoperoxides and products of myeloperoxidase, resulting in oxidation of the lipid and protein components [29]. The source of plasma ox-Lp(a) is unknown. Holvoet et al. [20] isolated ox-LDL from the plasma of patients with post-transplant CAD and analyzed its characteristics, which suggested that it did not originate from extensive metal ion-induced oxidation of LDL but that it might be generated by cell-associated oxidative enzymatic activity in the arterial wall. It was also demonstrated in animal models that the oxidation of LDL indeed occurs in the arterial wall and not in the blood [31]. Our previous studies showed that the increased ox-Lp(a) levels were associated with the presence of stable CAD and ACS and with the extent of angiographically documented CAD in ACS patients [11], which suggest that ox-Lp(a) particles in the blood come from the arterial wall, such as directly released from ruptured or permeable plaques.

What causes the rise of ox-Lp(a) and Lp(a) after PCI? The main possibility is that plasma ox-Lp(a) and Lp(a) are derived from ruptured or permeable plaque contents. Ox-Lp(a) and Lp(a) have both been identified in human atheromatous lesions [8,17]. It has also been clearly documented that PCI results in plaque compression, redistribution, or disruption, and intimal and medial dissection; that emptied plaque cavities are noted in patients with spontaneous plaque rupture; and that placing stents in patients with unstable angina leads to a marked reduction in plaque burden, suggesting compression and embolization of plaque material [32]. Another possibility for the rise in plasma ox-Lp(a) but probably not Lp(a) is a consequence of transient oxidative stress secondary to ischemia/reperfusion occurring during PCI causing increased lipid peroxides. It was observed that balloon occlusion of the left anterior descending coronary artery resulted in transient elevation of free lipid peroxides in the coronary sinus [33]. Thus, Lp(a) may expose to oxidizing species generated secondary to such ischemia/reperfusion in the vessel wall or even in plasma, and increased plasma ox-Lp(a) may be subsequently detected. This study showed that Lp(a) levels acutely increased 54% after PCI, which suggests that this amount of Lp(a) be unlikely derived from release of plaque disruption alone. The possibility for the acute increase of Lp(a) is rapid synthesis of apo (a) by the liver, and where it may bind to LDL, forming Lp(a) particles, or release of apo(a) from a preformed hepatic pool. It is also possible that Lp(a) distribution changes between intravascular and extravascular regions during PCI. In fact, Lp(a) is an acute phase reactant [21,26,34], so it is possible that liver increases synthesis of Lp(a) like that of C-reactive protein, which is upregulated by released cytokines during PCI. The apo(a) gene was reported to have an IL-6 response element leading to enhanced transcription [35]. Our previous study has also shown that increased native, oxidized Lp(a) and Lp(a)-IC concentrations were all positively related with C-reactive protein in active rheumatoid arthritis patients [13]. In addition, ox-Lp(a) level was also found related with Lp(a), which seems that increased concentrations of Lp(a) results in more Lp(a) having chance to be oxidized in vivo [11,12].

Recently, a series of studies have demonstrated convincingly that a key oxidized phospholipid is preferentially associated with Lp(a) [23] and correlates with both the presence and extent of angiographically documented CAD [24,25], and their concentrations increase after ACS [21]. Tsimikas et al. [26] also found oxidized phospholipids concentrations immediately increased after PCI. Their patient cohort was composed only of patients with stable angina, while not of patients with ACS. In addition, no significant associations of oxidized phospholipids or Lp(a) were found with angiographic characteristics. In present study, the changes of native and oxidized Lp(a) during PCI in stable CAD were found similar to those in ACS. Interestingly, the extent of CAD was found related with pre-, post-ox-Lp(a) levels, and change of ox-Lp(a) levels before and after PCI in the ACS patients, respectively, which further supports that ox-Lp(a) are present in ruptured or permeable plaques and are released into the circulation by PCI. Our previous studies have found that elevated ox-Lp(a) levels are associated with severity of angiographically documented disease in ACS, while not in stable CAD [11]. Similarly, the change of ox-Lp(a) during PCI is found to correlate with the extent of angiographically documented disease only in ACS patients, while not in stable CAD. It is not clear what causes the above difference. One possibility is that ACS episode causes sustained rise of ox-Lp(a), Tsimikas et al. [21] also reported that indirect and direct plasma markers of oxidized phospholipids show significant temporal elevations following ACS, but not in patients with stable CAD or in subjects without CAD. The oxidized phospholipids levels were found increased by 54% in the myocardial infarction patients at hospital discharge and still by 36% at 30 days.

The present study also shows that acute changes of Lp(a)-IC and ox-Lp(a)-Ab after PCI. New increased ox-Lp(a) enters the circulation, it may bind with ox-Lp(a)-Ab, resulting in immediate decline in free autoantibody levels and increase in IC formation. Tsimikas et al. [26] also reported ox-LDL autoantibody decreased, whereas apoB-IC levels increased after PCI, but both returned to baseline by 6 hours. Subsequently, IgM autoantibodies were found increased and peaked at 1 month and then returned to baseline, whereas IgG autoantibodies increased steadily over 6 months. They thought that the long-term increase in IgG and IgM autoantibodies presumably reflected an anamnestic response or synthesis of totally new species of ox-LDL antibodies and was consistent with acute presentation of ox-LDL to a previously sensitized immune system. The present study has not studied the long-term responses of Lp(a)-IC and ox-Lp(a)-Ab and only show that they returned to baseline at 6 months.

Limitations of this study include the fact that direct capture and analysis of embolized debri for native and oxidized Lp(a) were not performed. In addition, the long-term responses of Lp(a)-IC and ox-Lp(a)-Ab were not studied.

Table 3

<table>
<thead>
<tr>
<th>Time point</th>
<th>Lp(a) (mg/L)</th>
<th>Ox-Lp(a) (g/L)</th>
<th>Lp(a)-IC (AU)</th>
<th>Ox-Lp(a)-Ab (AU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>191.07±80.07</td>
<td>4.98±4.22</td>
<td>2.55±2.33</td>
<td>0.77±0.24</td>
</tr>
<tr>
<td>After</td>
<td>196.55±82.08</td>
<td>5.21±4.32</td>
<td>2.25±1.66</td>
<td>0.75±0.25</td>
</tr>
<tr>
<td>Change</td>
<td>7.48±30.25</td>
<td>0.23±1.23</td>
<td>-0.30±1.92</td>
<td>-0.02±0.11</td>
</tr>
</tbody>
</table>

Fig. 2. Changes of Lp(a), ox-Lp(a), Lp(a)-IC, and ox-Lp(a)-Ab levels after PCI. The change is expressed as mean percent change from pre-PCI levels. *P<0.001 by ANOVA. Lp(a): P<0.001 compared with before and 6-month time points; P<0.05 compared with 2- and 3-day time points. Ox-Lp(a): P<0.001 compared with before, 3-day, and 6-month time points; P<0.05 compared with 2- and 3-day time points. Lp(a)-IC: P<0.05 compared with before, 3-day, and 6-month time point. Ox-Lp(a)-Ab: P<0.001 compared with before and 6-month time points; P<0.01 compared with 2- and 3-day time points.
In conclusion, PCI results in acute elevations of native and oxidized Lp(a), as well as the changes of Lp(a)-IC and ox-Lp(a)-Ab. Furthermore, the changes of ox-Lp(a) levels in ACS patients are related with extent of angiographically documented disease. These observations provide impetus to further investigate the clinical value of ox-Lp(a) in atherosclerotic cardiovascular disease and to explore the exact pathogenic role of ox-Lp(a).

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