Acute Reduction of Lipoprotein(a) by Tissue-Type Plasminogen Activator

Robert A. Hegele, MD; Michael R. Freeman, MD; Anatoly Langer, MD; Philip W. Connely, PhD; and Paul W. Armstrong, MD

Background. Lipoprotein(a) [Lp(a)] is a low density lipoprotein–like particle whose apolipoprotein B (apo B) moiety is disulfide-linked to apo(a), a plasminogen-like inhibitor of fibrinolysis in vitro. We hypothesized that plasma concentrations of Lp(a) are acutely affected by intravenous tissue-type plasminogen activator (t-PA).

Methods and Results. Patients with unstable angina were randomized to receive either intravenous t-PA (n = 15) or placebo (n = 11). Two-way ANOVA using repeated measures revealed a significant effect of t-PA on concentrations of Lp(a) (p = 0.026). There was a 48% fall in Lp(a) from baseline concentrations in the t-PA group at 12 hours (p = 0.031) but not at 72 hours. Lp(a) in the placebo group was unchanged.

Conclusions. We conclude that t-PA produces a sharp and substantial but reversible reduction in plasma Lp(a). These data suggest that Lp(a) concentration is not as static in vivo as had been believed and might be acutely modifiable through some mechanism that induces its removal from the freely circulating state. (Circulation 1992;85:2034–2038)

Key Words • apolipoproteins • atherosclerosis • thrombolysis • coagulation

Lipoprotein(a) [Lp(a)] is a low density lipoprotein (LDL)–like particle with a characteristic polymeric glycoprotein called apo(a) that is disulfide-linked to the apolipoprotein B (apo B) moiety of LDL.1 High plasma Lp(a) concentrations are strongly associated with increased risk of atherosclerotic diseases.1 Pharmacological reduction of Lp(a) has been achieved with neomycin,2 niacin,2,3 and N-acetylcysteine4 but not with bile acid–binding resins or HMG CoA reductase inhibitors.2,5

Lp(a) inhibits fibrinolysis at endothelial cell surfaces in vitro5,6 and accumulates within the cytoplasm of cultured macrophages,8,9 probably after it binds to surface plasminogen receptors.10 Lp(a) in the presence of plasmin in vitro binds avidly to fibrin and fibrinogen.11 The in vivo effect of plasmin on Lp(a) is unknown.

Recombinant tissue-type plasminogen activator (t-PA) increases myocardial salvage and reduces mortality from myocardial infarction by opening acutely occluded coronary vessels.12,13 t-PA facilitates the conversion of plasminogen to plasmin by directly binding to fibrin.14 Compared with streptokinase, t-PA selectively enhances fibrinolytic activity at the surface of a fibrin clot, diminishes the total activation of plasminogen in plasma, and spares the proteolysis of fibrinogen.14

To test whether in vivo plasminogen activation to plasmin by t-PA affects Lp(a), we measured serial levels of Lp(a) and other lipoproteins for 72 hours in patients who were part of a randomized trial that examined the efficacy of t-PA in unstable angina.

Methods

Subjects and Treatment

The patients studied were part of a randomized double-blind placebo-controlled trial of t-PA in unstable angina approved by the University of Toronto Ethics Committee.13 Subjects were randomized to either placebo or double-chain t-PA (Wellcome) administered at a dose of 0.49 MU/kg for 1 hour followed by 0.07 MU/kg for 9 hours. All patients received intravenous heparin commencing with a bolus of 4,000 units followed by an infusion at 1,000 units/hour. Intravenous heparin was continued for at least 96 hours, and acetylsalicylic acid (325 mg) was administered daily beginning 72 hours after admission for the remainder of the patients’ hospitalization. Medical therapy was standardized. Individuals randomized to the placebo group received no t-PA, but their treatment was otherwise the same as the t-PA group. Blood samples at four time points (at 0, 4, 12, and 72 hours after initiation of treatment) were available from the final 26 of the overall total of 70 patients who entered the trial (15 from the t-PA group and 11 from the placebo group).

Biochemical Determinations

Plasma was drawn immediately before (“0 hours”) and at 4, 12, and 72 hours after the commencement of either t-PA or placebo and was immediately frozen at −70°C. Plasma concentrations of total cholesterol, triglycerides, and apo A-I and apo B were determined in the Lipid Research Laboratory at St. Michael’s Hospital using reported methods.16 The means of duplicate de-
Table 1. Baseline Characteristics of Study Subjects (Mean±SEM) and Their Treatment Outcomes

<table>
<thead>
<tr>
<th>Treatment assignment</th>
<th>t-PA</th>
<th>Placebo</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number (women)</td>
<td>15 (4)</td>
<td>11 (2)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.3±9.8</td>
<td>51.8±8.2</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.2±2.8</td>
<td>22.1±3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.59±1.06</td>
<td>5.67±1.37</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.95±0.99</td>
<td>2.36±1.55</td>
<td>NS</td>
</tr>
<tr>
<td>Apo B (g/l)</td>
<td>1.37±0.07</td>
<td>1.41±0.13</td>
<td></td>
</tr>
<tr>
<td>Apo A-I (g/l)</td>
<td>1.33±0.21</td>
<td>1.23±0.21</td>
<td>NS</td>
</tr>
<tr>
<td>Lp(a) (mg/dl)</td>
<td>10.2±3.8</td>
<td>11.5±5.9</td>
<td></td>
</tr>
</tbody>
</table>

- t-PA, tissue-type plasminogen activator; Apo, apolipoprotein; Lp(a), lipoprotein(a).

Terminations of plasma levels of Lp(a) were obtained using a commercial ELISA (Terumo Inc., Elkton, Md.). The anti-Lp(a) antibody (1D1) used in our studies has been demonstrated to be highly specific to epitopes on Lp(a) and does not cross-react with plasminogen.17

Statistical Analysis

The results are presented as mean±SEM. Baseline comparisons were performed using Student's t-test. Because the results of Lp(a) concentrations had a non-Gaussian distribution, logarithmic transformation was used before statistical analysis. The change with time in Lp(a) and the other biochemical variables was compared between the two groups using two-way ANOVA for repeated measures and the Duncan test.18

When significant differences were identified, means from the two groups at various time points were compared with a t test for unpaired data. The Lp(a) value of each subject at each time point was classified as either "decreased" or "unchanged or increased" from baseline. χ² analysis was performed on 4-, 12-, and 72-hour values comparing the number of subjects in each group who had a decrease in Lp(a).

Results

Study Sample

Baseline clinical characteristics and treatment outcomes of the 26 subjects studied are shown according to treatment assignment in Table 1. There were no significant differences between t-PA and placebo groups in baseline biochemical values (Table 1), nor were there differences in prevalence of diabetes or hypertension, smoking history, numbers of past myocardial infarctions, medications, and numbers of coronary vessels with >50% stenoses. When the baseline characteristics of the 26 subjects in this study were compared with those of the other 44 patients in the overall study, there were no significant differences except for a younger mean age in our study sample of 26 (52.7±10.7 versus 58.0±9.0 years, mean±SEM; p=0.04).

Levels of Plasma Lipids and Lipoproteins

The mean±SEM concentrations of Lp(a) are plotted against the time course of the experiment as shown in Figure 1. Lp(a) at baseline was 11.5±5.9 mg/dl (range, 0.5–66.5 mg/dl) in the placebo group and 10.2±3.8 mg/dl (range, 0.5–57.3 mg/dl) in the t-PA group (NS). Lp(a) in the placebo group did not differ significantly from baseline at any time. In the t-PA group, however, Lp(a) fell by 48% from baseline at 4 and 12 hours and returned to within 15% of baseline at 72 hours (p=0.026, Duncan test). Pairwise comparisons showed that Lp(a) concentration in the t-PA group at 12 hours (6.2±4.1 mg/dl) was significantly different both from the concentration at baseline (p=0.031) and from the Lp(a) concentration in the placebo group at 12 hours (11.9±6.0 mg/dl, p=0.027). At 12 hours, 13 of 15 t-PA recipients had a decrease from their baseline Lp(a) concentration, and 4 of 11 placebo recipients had a decrease (χ²=5.49, p<0.05).

The mean±SEM concentrations of apo B are plotted against the time course of the experiment as shown in Figure 2. Apo B at baseline was 1.41±0.13 g/l in the placebo group and 1.37±0.07 g/l in the t-PA group (NS). Apo B in the placebo group did not differ significantly from baseline at any time. In the t-PA group, however, apo B fell significantly (by 20%) at 12 hours and returned to baseline at 72 hours (p=0.001, Duncan test). Pairwise comparisons showed that apo B concentration in the t-PA group at 12 hours (1.07±0.09 g/l) was significantly different both from the concentration at baseline (p=0.01) and from the apo B concentration in the placebo group at 12 hours (1.22±0.09 g/l).
These levels and in substantial acute, statistical of small NS). 26 our to t-PA thrombus intracoronary 15ations > 1.5 of triglyceride cholesterol (Table 1) showed that levels can be rapidly modulated. In addition to its lipid-binding component, Lp(a) has lysine-binding structures that interact with fibrinogen and fibrin in the presence of plasmin.11 We thus hypothesize that the acute reduction of circulating Lp(a) during t-PA administration might be related to a plasmin-mediated increase in binding of Lp(a) to intravascular fibrinogen, fibrin, or other receptive site. It could be argued that the acute decline in Lp(a) might be artificial, related to the confounding effects of t-PA in the Lp(a) assay. However, given the short plasma half-life of t-PA (90 seconds) and the persistence of the reduced Lp(a) levels hours after cessation of t-PA, this seems highly improbable.

The proportions of total plasma apo B and cholesterol that are associated with apo(a) are approximately 10% and 5%, respectively. One might hypothesize that the observed fall of apo B and cholesterol from baseline in the t-PA group might be a result of the reduction of the portions of these variables that are associated with Lp(a). We observed no correlation, however, between the within-patient reductions of Lp(a) and apo B. The absence of a correlation between the decrease in Lp(a) and serum apo B does not necessarily rule out a contribution by the fall in Lp(a) to the fall in apo B, given the limitations of our sample size and the wide range of concentrations of both variables.

Others have reported a fall in cholesterol during the early phase of serious illnesses.19 The basis for this might be the initiation of an acute-phase reaction, with the involvement of mediators of inflammation, coagulation, or fibrinolysis that affect circulating levels of cholesterol.19 The administration of t-PA in patients with unstable angina might initiate an acute-phase reaction and thus have an effect analogous to the decrease in cholesterol and apo B-containing lipoproteins seen in response to acute illnesses. Lp(a) has been shown to increase by 100% 8 days after myocardial infarction or a surgical operation, however, implying that its acute-phase response is like those observed for C-reactive proteins, α1-acid glycoprotein, α2-antitrypsin, and haptoglobin.20 These data taken together suggest that the mechanism underlying our observed reductions in Lp(a) and apo B after t-PA are mechanistically unrelated. We also cannot rule out a potential interactive effect of heparin, although the fact that the placebo group also received heparin suggests that the observed reductions were specifically related to

**Clinical Outcome**

In this small group of patients there was no significant association between baseline Lp(a) concentration and either the number of previous myocardial infarctions or the number of coronary arteries with >50% angiographically assessed stenoses. Similarly, there was no significant relation between either baseline Lp(a) or treatment assignment and the incidence of failure of thrombolysis as demonstrated by the subsequent development of myocardial infarction (0 of 15 t-PA versus one of 11 placebo) or the need for urgent percutaneous transluminal coronary angioplasty (one of 15 t-PA versus one of 11 placebo). Two of the three subjects with unfavorable clinical outcomes had Lp(a) concentrations > 15 mg/dl. It is of interest that the presence of intracoronary thrombus was significantly reduced in t-PA recipients from the overall study (from 92% placebo to 50% t-PA), with a similar trend observed in our 26 subjects (10 of 11 placebo versus eight of 15 t-PA, NS). The failure to observe differences at nominal levels of statistical significance was undoubtedly a reflection of the small sample size.

**Discussion**

The principal novel finding of the study is that intravenous t-PA given as a bolus followed by a 10-hour infusion in patients with unstable angina produces an acute, substantial reduction of Lp(a) within 12 hours. These levels return toward baseline by 72 hours. Apo B and total cholesterol also appear to be affected, but triglycerides and apo A-I are not. Acute in vivo changes in these biochemical variables have not previously been reported.

The t-PA-induced fall in Lp(a) suggests that levels can be rapidly modulated. In addition to its lipid-binding component, Lp(a) has lysine-binding structures that interact with fibrinogen and fibrin in the presence of plasmin.11 We thus hypothesize that the acute reduction of circulating Lp(a) during t-PA administration might be related to a plasmin-mediated increase in binding of Lp(a) to intravascular fibrinogen, fibrin, or other receptive site. It could be argued that the acute decline in Lp(a) might be artificial, related to the confounding effects of t-PA in the Lp(a) assay. However, given the short plasma half-life of t-PA (90 seconds) and the persistence of the reduced Lp(a) levels hours after cessation of t-PA, this seems highly improbable.

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t-PA. Interestingly, by 72 hours in our placebo group there was a trend to a higher level of Lp(a), implying a possible acute-phase response.20

Lp(a) has tantalized researchers because of its observed and potential roles in lipid metabolism, coagulation, and thrombosis.1 The apo(a) gene is homologous to the adjacent plasminogen gene on chromosome 6q26-27.21,22 Variation in the size of the apo(a) gene appears to be related directly to variation in the size of apo(a) protein isoforms and inversely to plasma levels of Lp(a).22,23

The basis for physiological variation in Lp(a) concentrations may be related to binding of methylated DNA-binding protein to specific sites within the apo(a) gene.,24 but the effect of such regulation is not likely to acutely affect Lp(a) concentration. Rapid in vivo changes in Lp(a) have not been reported until now. Given that the plasma half-life of Lp(a) is 2–4 days,1 an acute suppression of synthesis at the molecular level would not explain a rapid decrease in Lp(a).

Apo(a) is highly homologous with plasminogen and contains a serine protease domain, one copy of the kringle-5 domain, and a variable number of copies of the kringle-4 domain.21,22 The structural homology to plasminogen notwithstanding, Lp(a) is a “dead protease” because of a crucial amino acid substitution at the activation site.21 In vitro, however, Lp(a) can inhibit both t-PA-mediated25 and streptokinase-mediated fibrinolysis.26,27 The in vivo effect of streptokinase on levels of Lp(a) has not yet been determined.

Others have identified reduced t-PA activity26 and increased t-PA inhibitory activity29 in the plasma of young survivors of acute myocardial infarction. Theoretically, the inhibition of t-PA-induced fibrinolysis might increase the tendency for vascular occlusion in individuals with elevated Lp(a). The evolving fibrin thrombus binds Lp(a), and activation of plasminogen at the fibrin surface by t-PA might further increase Lp(a) binding. The binding of Lp(a) to fibrin would impair plasminogen binding, thus inhibiting thrombolysis. The persisting thrombus with its bound Lp(a) might then be incorporated into the vessel wall, resulting in the accumulation of Lp(a) in the atherosclerotic plaque.30 Analysis of plaques in subjects who had earlier received t-PA might show relatively increased deposition of apo(a).

We found no significant relation between clinical or radiological presentation and Lp(a) at baseline, nor did we find a significant relation between Lp(a) at baseline and the incidence of unfavorable clinical outcomes after t-PA. This might have been because of a small sample size. Alternatively, this might represent a true lack of association between Lp(a) and disease expression. Although this might be somewhat surprising, it is consistent with preliminary results from another group that showed no association between elevated Lp(a) level and unsuccessful thrombolysis31; these researchers, however, used prourokinase rather than t-PA.

In summary, we demonstrate an acute and substantial reduction in Lp(a) and apo B coincident with the administration of t-PA and its return to baseline values 72 hours after the initial t-PA bolus. We speculate that the reductions are not mechanistically related. The fact that Lp(a) can vary substantially and quickly suggests that its levels are not immutable.1 The rapid t-PA-induced reduction in Lp(a) is not likely to be mediated at the level of synthesis of proteins or lipoproteins.

Instead, there might be considerable capacity within the intravascular space for uptake of circulating Lp(a), which is enhanced by t-PA. At present, most modalities that lower Lp(a) affect either its hepatic synthesis (niacin or niacinin2,3) or structural integrity within the plasma (high-dose N-acetylcysteine4). These treatments may not be practical for the general population. These data suggest that Lp(a) concentration is not as static in vivo as had been believed and might be acutely modifiable through some mechanism that induces its removal from the freely circulating state.

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