Lipoprotein(a) in Restenosis After Percutaneous Transluminal Coronary Angioplasty and Coronary Artery Disease

T. Cooke, FIMLS; R. Sheahan, MB, MRCPI; D. Foley, MB, MRCPI; M. Reilly, PhD; G. D’Arcy†, BSc; W. Jauch, MB, MRCPI; M. Gibney, MSc, MA, PhD; G. Gearty, MB, FRCPI; P. Crean, MSc, FRCPI; M. Walsh, MD, FRCPI

Background The purpose of the study was to investigate the relation of lipoprotein(a) and serum lipid parameters to restenosis after percutaneous transluminal coronary angioplasty (PTCA) and to assess the association of these same biochemical markers to coronary artery disease (CAD) in individuals with angiographically defined normal and diseased coronary arteries.

Methods and Results Sixty-two patients with successful PTCA had follow-up angiography at 35±10 weeks. Restenosis occurred in 21 male patients (46%) and 6 female patients (38%). Elevated apolipoprotein B (P<.01) and decreased high-density lipoprotein-2 cholesterol (P<.02) were found to be independently associated with restenosis after angioplasty, whereas lipoprotein(a) was not.

The role of percutaneous transluminal coronary angioplasty (PTCA) in the treatment of myocardial ischemia is well established. Although the primary success rate is very high (>90%), restenosis of the treated artery remains the major long-term complication, occurring in 30% to 40% of lesions.1,2 Newer imaging techniques and a variety of pharmacological agents have failed to reduce the incidence of restenosis.3-4 The association between conventional blood lipid parameters and apolipoproteins in the development and progression of coronary artery disease is now widely accepted.5-9 However, the relevance of these blood lipids with respect to vascular restenosis is conflicting.10,11

In recent years, there has been an upsurge of interest in lipoprotein(a) [Lp(a)] and its association with vascular disease.12-15 Lp(a) is a low-density lipoprotein (LDL)-like particle that has apolipoprotein(a) attached to the apolipoprotein B component via disulfide linkage.16-18 Apolipoprotein(a) [apo(a)] has been shown to structurally resemble plasminogen of the fibrinolytic system.19,20 In vitro studies demonstrate that Lp(a) competitively inhibits the binding of plasminogen to receptors on endothelial and monocytoid cells.21 Associations between elevated serum concentrations of Lp(a) and coronary artery disease (CAD),22,23 myocardial infarction,24 cerebrovascular disease,25 peripheral vascular disease,26 and stenosis of coronary artery bypass grafts27 have been established. Histologically, apo(a) has been localized within stenoses of coronary artery bypass grafts.28 It has been shown that Lp(a) concentrations in serum and arterial wall plaques are correlated.29 The role of serum Lp(a) concentrations in restenosis after coronary angioplasty remains unclear.10

Decreased concentrations of total high-density lipoprotein cholesterol (HDL-C), HDL2, and HDL3 cholesterol are associated with increased risk of coronary heart disease,30 but the association between the HDL-C subfractions and restenosis has not yet been established. We undertook this study to evaluate prospectively the association between Lp(a), HDL-C subsets, blood lipids, and restenosis after PTCA. We also examined the relation of these same parameters to the presence of CAD in an angiographically defined disease and control population.

Patients

One hundred five consenting patients undergoing PTCA were compared with 46 subjects who had no evidence of CAD on angiography. Elevated lipoprotein(a) (P<.001) and reduced apolipoprotein A1 to B ratio (P<.001) were found to be strong independent risk factors for the presence of CAD when adjustment was made for age (P=.005), male sex (P<.01), smoking (P<.005), and hypertension (P=.06).

Conclusions Serum lipoprotein(a) levels are not associated with restenosis after PTCA, but elevated levels are strongly associated with CAD. Low-serum, high-density lipoprotein-2 cholesterol concentration and elevated apolipoprotein B concentration were found to be associated with restenosis after PTCA. (Circulation. 1994;89:1593-1598.)

Key Words • apolipoproteins • angioplasty • lipoproteins

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From the Department of Biological Sciences (T.C., G.D.A.) and the Department of Mathematics (M.R.), Dublin Institute of Technology; the Department of Clinical Medicine (M.G.), Trinity College, Dublin; and the Department of Cardiology (R.S., D.F., W.J., G.G., P.C., M.W.), St James’s Hospital, Dublin.

†Deceased.

Correspondence to Thomas Cooke, Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland.
fast, 20 mL of clotted blood was obtained before the procedure.

All patients (N=66) with a successful angioplasty (reduction of diameter stenosis to <50% without major in-hospital complications) were scheduled to have a restudy angiogram performed 6 months after the procedure or earlier, as the clinical situation demanded. Follow-up angiography was performed in 62 patients (94%). Four patients did not have a restudy (1 died, 3 refused restudy) and were therefore excluded from further analysis. Thirty-three patients had a repeat blood sample taken at the time of the restudy angiogram.

Forty-six patients with normal coronary arteriograms (no visible lesions) that were completed as part of the assessment of atypical chest pain or valvular heart disease were recruited as control subjects. After an overnight fast, 20 mL of clotted blood was taken 24 hours after angiography. Information on other risk factors including age, hypertension, smoking status, family history of ischemic heart disease, alcohol consumption, and drug therapy was obtained for all subjects in the study. All the female cases and 17 (80%) of the female control subjects were postmenopausal. One of the female cases was on hormone replacement therapy (HRT) (Trisequens, combined cyclic estrogen/progestagen preparation).

Subjects were defined as smokers if they were current or ex-smokers. Alcohol intake refers to any alcohol consumed. Subjects were defined as having a family history if a first-degree relative had a history of CAD. Hypertension was defined as a diastolic blood pressure ≥95 mm Hg.

Coronary Arteriography

Coronary cineangiograms were viewed independently by two experienced cardiologists blinded to the procedural outcome and results of serum lipid analysis. The percent diameter stenosis was measured in matched multiple projections before and after dilatation and at follow-up angiography. For the purpose of this study, restenosis was defined as a recurrence of a lesion of ≥50% at the site of the previous angioplasty.

Biochemical Methods

Plasma total cholesterol and triglycerides were measured using automated enzymatic procedures (CHOD-PAP, Boehringer-Mannheim and Triglycerides Enzymatic PAP 150, bioMerieux) on a Centrifichem 400 analyzer. Plasma total HDL, HDL2, and HDL3 cholesterol were determined after selective precipitation with polyethylene glycol (Quantolip HDL (HDL2/HDL3), Immuno Diagnostics, Vienna, Austria). LDL cholesterol (LDL-C) was measured after selective precipitation with dextran sulfate (Quantolip-LDL, Immuno Diagnostics). Apo A1 and B (Apo A1, Apo B) concentrations were analyzed using an automated immunoturbidimetric method. Antibodies and calibrators were obtained from the International Immunology Corporation, Murietta, Calif. Lp(a) concentrations were assessed using an ELISA technique (TintElize Lp(a), Biopool). Precinorm L (Boehringer-Mannheim) and Lp(a) reference standard (Immuno Diagnostics) were used as control materials.

Plasma total cholesterol, triglycerides, HDL, HDL2, HDL3, and LDL cholesterol were analyzed on the day of venipuncture. Serum for apo A1 and B and Lp(a) was stored at −20°C until analysis. Mean storage time of samples for apo A1 and B and Lp(a) analysis was 43.6 days. All patient and control samples were analyzed in duplicate with appropriate quality control procedures operating. The ratios of total cholesterol to total HDL-C, total HDL to LDL cholesterol, and apo A1 to B were also examined.

Statistical Methods

All continuous variables were compared using two-sample t tests, except Lp(a), which has a skewed distribution, so a Wilcoxon test was used. Categorical variables were compared using the χ² statistic and Fisher exact tests. Multivariate analysis was done using the logistic regression model. The analysis was performed using the STATA statistical package.

Results

Restenosis Study

Of the 62 patients (46 male, 16 female) who underwent PTCA and had a follow-up angiogram at 35±10 weeks, 21 male patients (46%) and 6 female patients (38%) restenosed. An exploratory univariate analysis stratified by sex was done to identify risk factors that may be associated with restenosis (Table 1). Only one subject was on HRT, and since inclusion of this case had negligible effect on the results, she was included in the final analysis. Female patients who restenosed differed significantly from those who did not with respect to HDL2-C, triglycerides, apo B, and apo A1 to B ratio. For male patients, a significant difference was found only for the percent occlusion of the treated site before angioplasty.

A multivariate analysis that adjusted for age and sex indicated that higher levels of apolipoprotein B and lower levels of HDL2-C are independently associated with restenosis. The odds ratios and associated confidence intervals are presented in Table 2.

Of the 33 patients who had a restudy blood sample taken, restenosis occurred in 16. No significant difference was found in any of the biochemical markers between the restenosed and nonrestenosed groups in blood samples taken at restudy, neither was there a significant change in these markers from baseline in relation to restenosis. However, a marginal change in HDL3 was detected (restenosis group, −0.16 mmol/L; nonrestenosis group, +0.11 mmol/L; one-sided P value <.06). The correlation coefficient for Lp(a) at baseline and restudy was .944.

Coronary Artery Disease Versus Control Study

Univariate analysis, stratified by sex, was performed to compare the measured risk factors in the patients with documented CAD and control subjects (Table 3). All the female cases were postmenopausal with one case on HRT, while four of the control subjects were premenopausal. The data were analyzed with and without these subjects, but we found that their inclusion had negligible effect on the study results and so they were included in the final analysis. Both male and female cases and control subjects differ significantly with respect to age, smoking status, Lp(a), and apo B. In addition, female cases and control subjects differ significantly with respect to hypertension, total cholesterol,
TABLE 1. Results of Univariate Analysis Stratified by Sex for Restenosed and Nonrestenosed Patients

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Men (N=46)</th>
<th>Women (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>51.9±10.2 (n=21)</td>
<td>55.4±9.3 (n=25)</td>
</tr>
<tr>
<td>Smoking, n</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Family history, n</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Alcohol, n</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Stenosis before angioplasty, %</td>
<td>91 (79-100)</td>
<td>88 (76-100)*</td>
</tr>
<tr>
<td>Stenosis after angioplasty, %</td>
<td>27 (12-49)</td>
<td>25 (7-40)</td>
</tr>
<tr>
<td>Change in % stenosis</td>
<td>65 (42-77)</td>
<td>66 (47-90)</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>253 (11-1118)</td>
<td>174 (6-1415)</td>
</tr>
<tr>
<td>Chol mmol/L</td>
<td>6.1±1.7</td>
<td>5.7±0.9</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>4.3±1.5</td>
<td>3.9±0.9</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.90±0.23</td>
<td>0.94±0.24</td>
</tr>
<tr>
<td>HDL2-C, mmol/L</td>
<td>0.20±0.08</td>
<td>0.23±0.10</td>
</tr>
<tr>
<td>HDL3-C, mmol/L</td>
<td>0.69±0.16</td>
<td>0.72±0.19</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.78±0.82</td>
<td>1.87±0.97</td>
</tr>
<tr>
<td>Apo A1, mg/dL</td>
<td>103±22</td>
<td>110±29</td>
</tr>
<tr>
<td>Apo A1/B</td>
<td>99±27</td>
<td>88±13§</td>
</tr>
<tr>
<td>Apo A1/B</td>
<td>1.12±0.38</td>
<td>1.26±0.33</td>
</tr>
<tr>
<td>Chol/HDL-C</td>
<td>7.14±2.68</td>
<td>6.51±2.11</td>
</tr>
<tr>
<td>HDL-C/LDL-C</td>
<td>0.23±0.07</td>
<td>0.25±0.08</td>
</tr>
</tbody>
</table>

Lp(a) indicates lipoprotein(a); Chol, cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; and Apo, apolipoprotein.

Mean±SD is represented for all continuous variables except Lp(a), percent occlusion before and after angioplasty, and percent change in occlusion after angioplasty, for which the median and range are reported.

*P<.05, †P<.01, §P<.001, ‡P=.1.

LDL-C, and HDL3-C, whereas for male cases, there were significant differences for apo A1 and apo A1/B ratio.

A multivariate logistic regression analysis demonstrated that Lp(a) and apo A1 to B ratio are independently associated with CAD, adjusted for age, sex, and smoking status. The odds ratios and associated confidence intervals are presented in Table 4.

Discussion

Our study examined prospectively the relation between serum Lp(a), lipids, apolipoproteins, HDL-C subsets, nonlipid risk factors with restenosis after PTCA and, retrospectively, the association between these same parameters and the presence of CAD in angiographically defined control and disease populations.

Restenosis Study

The study focused on the association between the biochemical and nonbiochemical risk factors and restenosis after coronary angioplasty. Restenosis is primarily a result of myointimal hyperplasia caused by the release of growth factors from platelets and arterial cells after the controlled injury of angioplasty.43 It has been shown that Lp(a) has a high degree of homology to plasminogen,19 competitively inhibits binding of plasminogen to plasminogen receptors on endothelial and mononuclear cells,21 and inhibits plasminogen activation.34 Our hypothesis was that patients with elevated Lp(a) concentrations in blood may have a greater tendency to thrombosis after platelet activation initiated by the arterial injury of angioplasty. This may result in increased growth factor release and a propensity to coronary restenosis. A univariate analysis stratified by sex showed no significant difference in the median plasma Lp(a) concentration in male (253 versus 174 mg/L) and female (840 versus 411 mg/L) patients with and without restenosis. This contrasts with a retrospective study that found elevated Lp(a) concentrations in restenosed patients after angioplasty.35 However, the authors acknowledge the possibility of a selection bias in that study. It is not clear from their study what percentage of the total angioplasty population had both a follow-up
coronary angiogram and blood sample taken for Lp(a) analysis. Shah and Amin\textsuperscript{36} found no association between Lp(a) and restenosis after angioplasty. In that study, coronary arteriography was not performed in all patients, therefore asymptomatic restenosis may have been missed in some subjects. Ours was a prospective study with an angiographic follow-up of 94%.

Low concentrations of HDL2-C and HDL3-C are associated with premature coronary artery disease.30,37 To our knowledge, no information exists on the association of HDL-C subfractions with restenosis. Shah and Amin\textsuperscript{36} did find a significant association with low total HDL-C concentrations and restenosis after angioplasty. Our study indicates that elevated apo B and decreased HDL2-C are independently associated with restenosis.

While our method for HDL-C subfractions used selective precipitation rather than ultracentrifugation, it has been shown by Widhalm and Pakosta\textsuperscript{38} that these give comparable results. In patients who had a blood sample taken at restudy (n=33), none of the biochemical parameters in the restudy blood or change from baseline were found to be predictive of restenosis.

The association of aberrations in blood lipids and intimal hyperplasia is not fully understood; however, it has been demonstrated that oxidatively modified LDL may be important in the recruitment and retention of monocytes and macrophages during atherogenesis.39 Other investigators suggest that lipoproteins are not mitogenic themselves but may act as substrates for maximal growth of the cells once the signal for growth is initiated.40 Manipulation of lipid metabolism using \(w\)-3 fatty acid supplementation and lovastatin have shown conflicting results with regard to reducing the incidence of restenosis.41

While acknowledging a high angiographic follow-up, we appreciate that the sample size is relatively small, and a larger study would be required to support these findings.

### Coronary Artery Disease Versus Control Study

In this part of the study, we demonstrated a significant independent association between serum Lp(a) concentrations, apo A1 to B ratio, and the presence of CAD. Similar associations with respect to Lp(a) were found in other studies\textsuperscript{15,41}; however, Hearn et al\textsuperscript{41} found no association with respect to apo A1 to B ratio. One
prospective study established apo(a) as a significant independent risk factor but found apo A1 a stronger negative independent risk factor with respect to CAD in men. Armstrong et al. studied the association between Lp(a) and other risk factors for predicting CAD. Lp(a) was found to increase the odds ratio to 1.47 at the lowest tertile of LDL. However, at the middle or upper tertile, the odds ratio increased substantially to 3.86 and 4.73, respectively. Thus, whereas Lp(a) exerts an effect at low LDL levels, its effect is greatly augmented at higher LDL levels. Armstrong et al. suggest that a possible reason for lack of atherogenicity of Lp(a) in the black population might be due to correspondingly low LDL-C levels. Patients with familial hypercholesterolemia and CAD have elevated Lp(a) concentrations that are independent of other risk factors.

It has been suggested that apo(a) concentration could be substituted for parental history in a logistic regression model. Serum Lp(a) levels are largely determined by alleles at the hypervariable apo(a) gene locus, which gives rise to multiple protein isoforms in blood. The smaller-molecular-weight isoforms are associated with elevated plasma concentrations of Lp(a). The presence of the smaller-molecular-weight isoforms in a Chinese population was significantly more frequent in patients with CAD than in control subjects, supporting the hypothesis that alleles at the apo(a) locus determine the risk for CAD through their effect on serum Lp(a) levels, thus suggesting a role for serum Lp(a) as a primary genetic risk factor. Using univariate analysis stratified by sex, we found serum Lp(a), apo B, age, and smoking status to be significantly different between cases and control subjects in both male and female patients. As might be expected, women with disease were older than their male counterparts and, interestingly, they had higher Lp(a) concentrations. Schriewer et al., in 1984, demonstrated a pronounced positive correlation of age to Lp(a) values in women, with a sudden increase in values for 40-to-50-year-olds, and suggested that there may be a menopausal effect. Similarly, Slunga et al., in the Northern Sweden Monica Project, showed menopausal status to be the strongest independent predictor of Lp(a) level in women. However, in the Framingham Offspring Study, 1394 women were studied, and no significant difference was found in Lp(a) concentrations after controlling for age in premenopausal versus postmenopausal women. With regard to hormone replacement therapy in women, it has been shown that progesterone alone or in combination with estrogen lowers serum Lp(a) concentrations significantly in postmenopausal women. In our female population, four of the control subjects were premenopausal, with one of the cases on HRT. Because of the contradictory nature of the literature with regard to postmenopausal status and serum Lp(a) concentrations, further analysis of the data was undertaken excluding these five female subjects. This did not alter the outcome. All of the female subjects in the restenosis study were postmenopausal, so our results for women can only be generalized to those who are postmenopausal.

There is evidence that the serum level of the apolipoproteins A1 and B and their ratios are better discriminators of CAD than conventional plasma lipids and lipoproteins. Using a multivariate analysis, we demonstrated that the apo A1 to B ratio was an independent predictor of CAD but was not as strong as Lp(a). Among the nonlipid risk factors, age, male sex, and smoking were also found to be independently associated with CAD, with odds ratios of 1.07, 3.96, and 4.87, respectively. Lp(a) concentration correlated only weakly with total cholesterol and LDL, whereas no association was found with the severity of disease.

In summary, Lp(a) was not found to be associated with restenosis after PTCA but was highly correlated with the presence of CAD. Elevated levels of apo B and reduced HDL2-C are independently associated with restenosis, and strategies aimed at their modification may result in reducing the Achilles' heel of this procedure.

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In Memoriam
We deeply regret the death of our friend and colleague, Glenn D'Arcy, one of the authors of this work. His academic abilities and analytical expertise were pivotal to the successful completion of this project.

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