Lipoprotein (a) as an independent risk factor for myocardial infarction in patients with common hypercholesterolaemia

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Abstract

Aims: To examine whether lipoprotein (a) (Lp(a)) increases the risk of myocardial infarction (MI) in patients with common hypercholesterolaemia.

Methods: 15 middle aged men with common hypercholesterolaemia (mean serum low density lipoprotein (LDL) cholesterol 4.94 mmol/l, SD 1.0) and a history of MI were selected consecutively from referrals to a lipid clinic. A control group that had not sustained an MI and with similar age, sex, cigarette smoking and blood pressure characteristics was also selected from the same clinic. Serum cholesterol, triglyceride, LDL cholesterol, high density lipoprotein cholesterol, apolipoproteins AI and B and Lp(a) were measured in both groups. Lp(a) was assayed by immunoturbidity.

Results: The serum concentration of Lp(a) was significantly higher in patients with MI (geometric mean 0.64 (95% confidence interval 0.36 to 1.14) v 0.30 (0.21 to 0.42) g/l, $p = 0.02$), but there were no significant differences in other variables. Stepwise logistic regression analysis showed that Lp(a) was the only significant predictor of MI ($p < 0.02$). The odds ratio of MI (adjusted for age, smoking, blood pressure and apolipoprotein B) for an Lp(a) of >0.57 g/l was 16.5, 95% confidence interval 2.3 to 125.4 ($p = 0.001$).

Conclusion: In middle aged men with common hypercholesterolaemia the serum concentration of Lp(a) is a powerful and independent risk factor for MI. Lp(a) should probably be routinely measured in all patients referred to a lipid clinic.

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lesterolaemia being the most frequent reason for referral to lipid clinics.⁵

Among patients with common hypercholesterolaemia CHD takes many forms and varies in degree of severity.³ This is probably due to genetic factors, and an important one may be the serum concentration of lipoprotein(a) (Lp(a)),⁶ as has been suggested in the less common disorder familial hypercholesterolaemia.⁷⁻⁸ Lp(a) is structurally related to LDL and consists of a highly glycosylated subunit, apo(a), linked by disulphide bridges to the LDL particle.⁶ The serum concentration is chiefly under genetic control.⁹ Several studies have indicated that Lp(a) has both atherogenic and thrombogenic properties¹⁰⁻¹³; the latter is due to sequence homology with plasminogen¹⁴ and may be critical in increasing the risk of myocardial infarction (MI).¹⁵ There is dispute, however, as to whether the risk of CHD conferred by raised Lp(a) is independent of LDL or apolipoprotein B.¹⁵⁻¹⁷

Methods

Fifteen men with common hypercholesterolaemia who had sustained an MI within the previous two years were selected from consecutive referrals to the St Thomas's Hospital lipid clinic. Common hypercholesterolaemia was defined as: a serum concentration of LDL cholesterol between 3.5 and 7.2 mmol/l and a triglyceride of <3.8 mmol/l; together with absence of premature CHD (age 40 years or under), or tendon xanthomata in the index patient, or first or second degree relative; and a plasma cholesterol of <8.0 mmol/l in all relatives screened for hyperlipidaemia.^{3 18 19} These criteria were chosen with the intention of excluding familial hypercholesterolaemia and familial combined hyperlipidaemia. MI was defined according to well recognised plasma enzyme and electrocardiographic changes: serial increases in creatine kinase, aspartate transaminase, and hydroxybutyric dehydrogenase activities, with or without an ST segment increase and development of T wave inversion and Q waves, for acute events. Where these data were not available, pathological Q waves on the electrocardiogram was used as major criteria for the retrospective diagnosis of MI.²⁰ Controls were selected consecutively from men without a previous history of MI who had been referred to the lipid clinic at the same time as the men with the primary diagnosis of common hypercholesterolaemia. Resting 12 lead ECGs were within normal limits in controls.

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An increase in serum lipids and lipoproteins is a well recognised risk factor for coronary heart disease (CHD).^{1 2} The most common cause of increased serum cholesterol in populations at high risk of coronary heart disease (CHD) is common, or polygenic, hypercholesterolaemia.³ The disorder is characterised by a modest increase in low density lipoprotein (LDL) cholesterol due to a combination of genetic and environmental factors that disturb the transport of apolipoprotein B-100 in plasma.⁴ Recent preventive strategies for CHD^{1 2} have resulted in common hypercho-

Table 1 Clinical and demographic characteristics of the patients studied

Characteristic	With myocardial infarction	Without myocardial infarction	p Value for difference
Number	15	15	>0.1
Age (y)	52.6 (2.0)	51.3 (1.7)	>0.1
Body mass index (kg/m ²)	26.0 (0.6)	27.2 (0.7)	>0.1
Smoker/ex-smoker (%)	87	93	>0.1
Regular alcohol (%)	73	67	>0.1
Family history CHD (%)	20	27	>0.1
History of hypertension (%)	7	13	>0.1
Angina pectoris (%)	86	53	>0.1
Anti-anginal drugs (%)	73	60	>0.1
Systolic blood pressure (mm Hg)	128 (2.6)	130 (2.0)	>0.1
Diastolic blood pressure (mm Hg)	83 (1.8)	82 (1.3)	>0.1

Mean (SEM) for continuous variables

Patients with secondary hypercholesterolaemia—for example, diabetes mellitus or hypothyroidism—or taking anti-lipaeic medication were excluded.

Details were taken of angina pectoris, medication, smoking habit, alcohol consumption, family history of CHD and personal history of hypertension. Body weight (kg), height (m) and arterial blood pressure (supine Korotkov V) were measured. Body mass index (kg/m²) was calculated. After a 12 hour fast venous blood was obtained (plain glass bottle) with minimal stasis and with the patient recumbent for serum lipid and lipoprotein analyses. All patients had been sustaining diet with no lipid restrictions. MI was confirmed from clinical records. Lp(a) was also measured in a group of healthy, middle aged men working in local government offices and known to be free of CHD and to have a "desirable" serum concentration of LDL cholesterol (<3.5 mmol/l).^{2, 19}

LABORATORY ANALYSES

Serum cholesterol, triglyceride, and high density lipoprotein (HDL) cholesterol were assayed within four days of blood being taken. Enzyme kits were used to measure both cholesterol (Boehringer, Lewes, England) and triglycerides (Wako Chemicals, Neuss, Germany). HDL cholesterol was measured after precipitation of apolipoprotein lipoprotein B with manganese chloride and heparin, excess manganese being chelated with Na₂EDTA before cholesterol assay. Aliquots of serum for apoprotein measurements were stored at -70°C and thawed rapidly at 37°C immediately before analysis. Apolipoprotein AI (ApoAI) and apolipoprotein B (apoB) were measured using the method of Mount *et al.*²¹ Lp(a) was assayed by an immunoturbidimetric kit method (Immuno Ltd., Sevenoaks,

England) (Kearney EM, *et al.* An indirect immunoturbidimetry assay for Lp(a). 55th Meeting of the European Atherosclerosis Society, May 1990). To prevent artefact due to a "matrix effect" standards and serum samples were diluted in phosphate buffered saline supplemented with 7% bovine serum albumin.²² Linear regression analysis showed a very good agreement between our immunoturbidimetric assay (IT) for Lp(a) and an enzyme linked immunosorbent assay (ELISA): IT = 0.76 ELISA + 0.16 g/l; r = 0.88; n = 80. Interassay coefficients of variation for all methods were less than 3.5% at "high" and "low" control values. LDL cholesterol was calculated by the Friedewald formula.²³

STATISTICAL METHODS

Continuous variables were compared between the two groups of patients using the unpaired *t* test, Lp(a) values being logarithmically transformed because of their positively skewed distribution. Discrete variables were compared by Fisher's exact test. Predictors of MI were examined using a forwards stepwise logistic regression analysis. The risk of MI associated with an Lp(a) of >0.57 g/l compared with that associated with an Lp(a) of ≤0.57 g/l⁸ was calculated as the odds ratio with 95% confidence interval²⁴; the corresponding relative risk was also calculated assuming that 10% of the hypercholesterolaemic population without CHD has a serum Lp(a) of >0.57 g/l.²⁵ The odds ratio was adjusted for age, smoking, mean arterial blood pressure and serum apoB using a multiple logistic method; specific adjustment for apoB was made because of evidence suggesting that it is a better predictor of MI than Lp(a).¹⁷ The cutoff value of 0.57 g/l for Lp(a) was chosen for these analyses because it had previously been shown to discriminate well between familial hypercholesterolaemic patients with and without MI.⁸

Results

Table 1 shows the clinical and demographic characteristics of the patients with and without MI. There were no statistically significant differences in the characteristics between the groups, but anginal symptoms and use of anti-anginal drugs were more common in the patients with MI.

Table 2 shows the serum lipid, lipoprotein, and apolipoprotein concentrations in cases and controls. Hypercholesterolaemia was due to an increase in LDL cholesterol, consistent with polygenic or common hypercholesterolaemia. There were no significant group differences in serum cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, apo AI, apo B, or in LDL:HDL cholesterol and apo B:apo AI ratios. The geometric mean of the serum concentration of Lp(a) was, however, twofold higher in the cases than in the controls (p = 0.02). The figure shows the serum concentrations of Lp(a) in the two groups of patients and in normocholesterolaemic

Table 2 Serum lipids, lipoproteins, and apolipoproteins in hypercholesterolaemic patients with and without MI

Lipids, lipoproteins, and apolipoproteins*	With myocardial infarction (n=15)	Without myocardial infarction (n=15)	p Value for difference
Cholesterol (mmol/l)	7.02 (0.23)	6.89 (0.15)	>0.1
LDL cholesterol (mmol/l)	4.94 (0.24)	4.59 (0.16)	>0.1
HDL cholesterol (mmol/l)	1.12 (0.05)	1.17 (0.08)	>0.1
Triglyceride (mmol/l)	2.13 (0.20)	2.33 (0.24)	>0.1
Apolipoprotein AI (g/l)	1.26 (0.05)	1.38 (0.05)	>0.1
Apolipoprotein B (g/l)	1.41 (0.06)	1.38 (0.05)	>0.1
Lipoprotein (a) (g/l)†	0.64 (0.36-1.14)	0.30 (0.21-0.42)	=0.02
LDL/HDL cholesterol	4.57 (0.32)	4.21 (0.36)	>0.1
Apo B/Apo AI	1.15 (0.07)	1.03 (0.07)	>0.1

*Values are mean (SEM); †geometric mean (95% confidence interval)

Serum concentrations of lipoprotein(a) in hypercholesterolaemic patients with and without MI and in normocholesterolaemic subjects without MI. Bars are geometric means. $n = 15$ in each group.

laemic subjects without MI: compared with normocholesterolaemic subjects, Lp(a) was significantly higher in the hypercholesterolaemic patients with ($p < 0.001$) and without MI ($p < 0.02$).

Using the forwards stepwise procedure for selecting explanatory variables, only log_e (Lp(a)) was a significant predictor of MI ($p < 0.02$):

$$\log_e(p/1-p) = 1.10 \times \log_e(\text{Lp(a)}) + 0.91$$

where p = probability of MI.

The unadjusted odds ratio of MI for an Lp(a) concentration of >0.57 g/l compared with an Lp(a) of ≤ 0.57 g/l was 13.0, 95% confidence interval (CI) 2.1 to 81.2 ($p = 0.002$). The equivalent relative risk, assuming that 10% of the hypercholesterolaemic population without CHD has a serum Lp(a) of >0.57 g/l, was 5.9, 95% CI 1.8 to 9.0. Adjusting for age, smoking habit, blood pressure and apoB, the odds ratio increased to 16.7, 95% CI 2.3 to 125.4 ($p = 0.001$).

Discussion

In middle aged men with common hypercholesterolaemia an increase in serum Lp(a) was associated with a greatly increased risk of MI. The association was independent of established cardiovascular risk factors, such as age, smoking habit, blood pressure and the serum concentrations of LDL and HDL cholesterol.^{1,2} To establish further, however, that Lp(a) is causally related to MI will require a prospective study and preferably an intervention trial.

Our findings are consistent with those of previous retrospective reports showing that

Lp(a) is a risk factor for MI.^{6,15} The degree of independent risk attributed to Lp(a) has not, however, been formally tested in other case-control studies;¹⁵ nor have these focused on patients with common hypercholesterolaemia, the most common metabolic disorder seen in lipid clinics. Rhoads *et al* suggested that the odds ratio attributed to an increase in Lp(a) varied from 1.6 to 3.6, the risk being higher in younger subjects.¹⁵ The greater odds ratio found in the present study might be due to use of a higher "cutoff" for Lp(a), a smaller sample size, or selection of patients for increased LDL cholesterol. As shown in familial hypercholesterolaemia risk of CHD attributed to Lp(a) may depend on the serum concentration of LDL cholesterol.^{7,8} Durrington *et al*, however, showed that in patients with hypercholesterolaemia a family history of premature CHD was as good a predictor of MI as apo(a), apoB being the best predictor of MI in discriminant analysis.¹⁷ Discrepancies between their study and ours may have been due to differences in study design, selection of patients, immunochemical assay for Lp(a) and accuracy of eliciting a family history of premature CHD.

That increased serum Lp(a) is part of the acute phase response to underlying atherosclerosis is highly improbable.²⁶ Lp(a) is well recognised to have both atherogenic and thrombogenic properties.^{6,25} Increased susceptibility to arterial thrombosis may have as its basis the sequence homology of the apo(a) subunit with plasminogen.¹⁴ Inhibition of the binding of plasminogen to its activator site on vascular endothelium may explain why Lp(a) increased risk of MI in the present study.^{10,11} Hypercholesterolaemia may also induce a procoagulant state by increasing platelet aggregation, factor VII coagulant activity, and plasma fibrinogen.^{25,27} The association between raised serum Lp(a) and these events is at present unclear.

The metabolic determinants of increased serum concentrations Lp(a) are unknown. Lp(a) is not affected by age, sex, diet or drugs that increase the catabolism of LDL apoB.^{6,25} By contrast, drugs that inhibit hepatic production of apoB tend to lower serum Lp(a).²⁸ Accordingly, the plasma concentration of Lp(a) is determined by its rate of synthesis rather than its rate of catabolism.^{29,30} Given the significant differences in Lp(a) between hypercholesterolaemic patients and normocholesterolaemic subjects found in the present study, it is possible that a transport defect of apoB might have accounted for increased concentrations. This would entail differences in hepatic production of apoB and we cannot entirely exclude a greater prevalence of familial combined hyperlipidaemia in our patients with MI. As indicated elsewhere,³ it is more likely that genetic factors accounted for the differences in Lp(a) between patient groups^{7,8}; patients with MI may, for example, have a preponderance of the Lps¹ and Lps² alleles, size polymorphisms that code for higher serum Lp(a) concentrations.⁹

We conclude that measurement of serum Lp(a) may allow clinicians to identify patients with common hypercholesterolaemia at increased risk of MI. Focusing management on patients with the worst cardiovascular prognosis will optimise use of resources in lipid clinics. Increased Lp(a) concentrations may potentially be treated with ω 3-fatty acids, niacin, statins or neomycin, but the efficacy of these treatments is still controversial³¹; conventional fat modified diets do not seem to lower Lp(a).³² Whether a reduction in raised plasma Lp(a) benefits the course of CHD and mitigates the risk of MI also needs to be proved. Moreover, development of agents specifically targeted at Lp(a) are awaited.³¹ In the interim, measurement of Lp(a) in patients attending lipid clinics instead may be regarded as useful in indicating to clinicians the need for intensive treatment for other cardiovascular risk factors other than Lp(a) per se.²⁸

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