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Original paper

ROLE OF LIPOPROTEIN(a) IN THE DEVELOPMENT OF CORONARY HEART DISEASE IN PATIENTS WITH ESSENTIAL HYPERTENSION

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Summary: Lipoprotein(a) [Lp(a)] is an important and independent cardiovascular risk factor, but its role in the development of coronary heart disease (CHD) in hypertensives have had conflicting results. In order to study the possible role of Lp(a) in the development of coronary heart disease in hypertensive patients, we evaluated Lp(a) levels in 45 (younger than 50 years) CHD hypertensive patients, 45 patients with essential hypertension without CHD and 64 healthy controls. Lp(a) was measured by nephelometric assays in fresh serum samples. The levels of Lp(a) were significantly greater in CHD hypertensive patients (0.33 ± 0.17 g/L) than in controls (0.18 ± 0.08 g/L) or patients with essential hypertension (0.20 ± 0.05 g/L). The levels of Lp(a) were increased more than 0.30 g/L in 46.6% of CHD hypertensive patiens, in 17.7% of hypertensive patiens and in 8.8% of controls. CHD hypertensive patients had also greater levels of total cholesterol, LDL-cholesterol and triglycerides than hypertensive patients and controls. HDL-cholesterol levels were significantly lower in CHD hypertensive patients. This study indicates that high Lp(a) levels can play a major role in the development of CHD in patients with essential hypertension. These findings suggest the great importance of identifying, among hypertensive patients, subjects with higher levels of Lp(a), who belong to a group with cardiovascular risk on the basis of their hypertension.

Key words: lipoprotein(a), coronary heart disease, essential hypertension

Introduction

Coronary heart disease (CHD) is of multifactorial origin. In addition to traditional risk factors, which include age, male gender, smoking, diabetes mellitus, dyslipidemia, and hypertension, a series of novel risk factors have been identified in prospective population studies, for example, lipoprotein(a) (1). Lipoprotein(a) [Lp(a)] is a cholesterol-rich lipoprotein with structural similarities to low-density lipoproteins (LDL), but contains apolipoprotein(a), a glycoprotein with sequence homology to plasminogen and bound by a disulfide bond to apoB100. The structure, metabolism and genetics of lipoprotein(a) have been recently reviewed (2, 3). The physiological function of Lp(a) as well as the precise mechanism by which high plasma levels of Lp(a) increase risk are unknown even four decades after its detection by Berg (4). Lp(a) levels are mostly determined genetically and are inversely related to the relative molecular masses of apo(a) isoforms (5). Lp(a) has been described in the past as a bridge between atherosclerosis and thrombosis, because of its antifibrinolytic actions and structural similarity to plasminogen (6, 7).

The role of excess Lp(a) in atherosclerosis remains somewhat controversial. An association between Lp(a) excess and CHD was initially suggested by cross-sectional and retrospective epidemiologic studies (8 10). Prospective studies that evaluated Lp(a) as a predictor of cardiovascular events have had conflicting results. Some studies suggested that Lp(a) was an independent risk factor of CHD (11 13), while the others showed no significant association (14, 15).

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results (16, 17). Some authors found normal Lp(a) levels in hypertensive patients (18), whereas the others documented elevated Lp(a) levels (19). Sechi et al. (20) found that Lp(a) levels were the best predictor of target-organ damage. This relation was independent of the level of blood pressure. Gazzaruso et al. (21) found that Lp(a) levels and apo(a) phenotypes in CHD-free patients with newly diagnosed essential hypertension were similar to those in controls, but the family history of CHD was strongly associated with high Lp(a) levels and apo(a) isoforms.

In order to study the possible role of Lp(a) in the development of CHD in hypertensive patients, we evaluated Lp(a) levels in a group of essential hypertensive patients and compared the hypertensives who had CHD with those who had not CHD.

Material and Methods

Patients and Study Design

Lp(a) levels were evaluated in patients with essential hypertension. The hypertensives were divided in two subgroups according to whether they had CHD or not (45 patients with essential hypertension without CHD and 45 hypertensive patients with CHD). To compare Lp(a) plasma levels we recruited a group of 64 healthy controls matched for age and sex. The criterion for inclusion in the patients group was essential hypertension defined either by the need for chronic antihypertensive treatment, or, in untreated subjects, by a diastolic blood pressure (DBP) greater than 80 mmHg or a systolic blood pressure (SBP) greater than 130 mmHg, or both on three different occasions at least 1 week apart. Blood pressure was measured by a mercury sphygmomanometer in standard conditions, and diagnosis of hypertension was established by standard criteria. A mean duration of hypertension was 10 ± 2.4 years. CHD was defined as documented previous myocardial infarction (diagnosed by elevation of cardiac enzyme levels and diagnostic change in their electrocardiogram); coronary artery disease documented by angiography (stenosis of > 70% in at least one coronary artery); coronary artery bypass grafting; or a history of angina pectoris together with a positive exercise test. In the other patients their medical history and an exercise stress test excluded the possibility of CHD. Exclusion criteria were: patients age >50 years, severe obesity, diabetes mellitus, pregnancy, stroke, renal failure with creatinine clearance of <30 mL/min, urinary protein excretion >3.0 g/d and use of cyclic hormonal therapy, lipid-lowering therapy and anticoagulant therapy. Approval from the appropriate local Ethics Committee was obtained and subjects gave their written informed consent.

Laboratory measurements

The following lipids were measured: total cholesterol [TC (mmol/L)]; high-density lipoprotein cholesterol [HDLc (mmol/L)]; low-density lipoprotein cholesterol [LDLc (mmol/L)]; triglycerides [TG (mmol/L)] and lipoprotein(a) [Lp(a) (g/L)]. Blood was taken from the antecubital vein in seated patients who had fasted for 12 h. For the quantification of Lp(a) we used plasma obtained by addition of EDTA and low-speed centrifugation at 4 °C for 15 min. Serum cholesterol and triglyceride levels were determined by automated enzymatic methods using the Abbott autoanalyzer. Levels of HDL cholesterol were enzymatically determined in the supernatant after precipitation of other lipoproteins with phosphotungstate/magnesium chloride. Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald's formula (22). Measurement of Lp(a) was determined in freshly isolated sera using a rate nephelometric assay (Behring-Laser nephelometer Modul I). For the nephelometric measurement of Lp(a), polystyrene microbodies coated with antibodies to human Lp(a) were agglutinated when mixed with serum containing Lp(a). Elevated Lp(a) were defined as ≥ 0.30 g/L.

Statistical analysis

Statistical analysis was perfomed using the STATISTICA 4.5 program (Stat soft, Tulsa, OK USA). All values are expressed as mean ± SD. The Student's t-test was used for comparison between two groups and analysis of variance for comparison of more than two groups. Pearson's χ^2 test was uased to compare frequency distributions. The Kruskal-Wallis test was used to compare variables with skewed frequency distribution. Results were considered statistically significant at $p < 0.05$.

Results

The features of hypertensive patients with coronary heart disease (CHD), hypertensive and controls, are shown in *Table I*. The two groups of hypertensive patients had no significant differences in age, sex, body mass index (BMI), duration of hypertension, SBP and DBP. Regarding lipid profile, several differences were observed between hypertensive and control subjects *(Table II)*. Serum levels of Lp(a) were significantly greater in hypertensive patients with CHD (0.33 ± 0.17 g/L) than in controls (0.18 0.08 g/L $p<0.001$) or hypertensive patients without CHD (0.20 ± 0.05 g/L $p<0.001$). Serum levels of Lp(a) were increased more than 0.30 g/L in 46.6% of CHD hypertensive patiens, 17.7% of hypertensive patiens and 8.8% of controls. The Lp(a) levels increased more than 0.50 g/L we found in 15.5% of CHD hypertensive patiens and 6.6% of hypertensive patients. No differences in serum Lp(a) levels, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were observed between hypertensive patients without CHD and controls. Hypertensive CHD patients had also greater levels of total cholesterol, LDL-cholesterol and triglycerides than hypertensive patients and controls. Plasma HDL cholesterol were significantly lower in hypertensive CHD patients.

Table I Features of hypertensive patients with coronary heart disease (CHD), hypertensive patients and controls

	Hypertensive CHD patients (n=45)	Hypertensive patients (n=45)	Controls (n=64)
Age (years)	44.30 ± 4.28	45.29 ± 4.63	44.75 ± 6.85
Sex (M/F)	30/15	29/16	28/36
BMI (kg/m^2)	24.57 ± 3.7	23.25 ± 4.5	23.75 ± 3.6
Hypertension duration (years)	10 ± 2.4	11 ± 3.7	
Age of CHD diagnosis (years)	42 ± 4.26		
Systolic BP (mmHg)	175 ± 9.26***	170 ± 8.30	115 ± 4.18
Diastolic BP (mmHg)	102 ± 6.86***	100 ± 5.89	70 ± 4.85

Values are expressed as means ± SD; BMI=body mass index; *P < 0.05, **P < 0.01, ***P < 0.001 versus controls.

Table II Lipid profiles of hypertensive patients with coronary heart disease (CHD), hypertensive patients and controls

	Hypertensive CHD patients (n=45)	Hypertensive patients (n=45)	Controls (n=64)
Cholesterol (mmol/L)	7.50 ± 1.07***	5.72 ± 1.09	5.10 ± 0.60
LDL-c (mmol/L)	5.25 ± 0.64***	3.75 ± 0.98	3.21 ± 0.92
HDL-c (mmol/L)	1.17 ± 0.35**	1.16 ± 0.25	1.27 ± 0.50
Triglycerides (mmol/L)	2.63 ± 1.71***	1.65 ± 0.26	1.42 ± 0.35
Lp(a) (g/L)	0.33 ± 0.17***	0.20 ± 0.05	0.18 ± 0.08
Lp(a) > 0.30 g/L (%)	46.66***	17.77	8.88
Lp(a) > 0.50 g/L (%)	15.55***	6.66	0.00

Values are expressed as means ± SD; Lp(a)=lipoprotein(a); LDL=low-density lipoprotein; HDL=high-density lipoprotein.
*P < 0.05, **P < 0.01, ***P < 0.001 versus controls.

Discussion

In this study the presence of arterial hypertension is associated with increased Lp(a) levels. We hypothesize that hypertensive patients with high Lp(a) levels might have an earlier development and a more rapid progression of atherosclerotic lesions. We showed a possible relationship between Lp(a) and development of CHD among hypertensive patients with CHD younger than 50 years. The main finding of this study is that Lp(a) could play a considerable role in the development of CHD in hypertensive patients especially in those with concomitant abnormal lipid profiles. We recently found that Lp(a) plasma levels in CHD-free patients with essential hypertension were similar to those in controls. We showed that Lp(a) are not strongly linked to essential hypertension in a group of hypertensive patients without CHD. It is very important to know whether or not Lp(a) levels play a role in the development of CHD in hypertensives, who already have a high cardiovascular risk. Nevertheless, in essential hypertensive patients the cardiovascular risk related to Lp(a) (genetically inherited) is added to the CHD risk caused by hypertension and other risk factors associated with hypertension. Because hypertension and abnormal lipid metabolism are consistently found in hypertensive patients, such an interaction might accelerate atherosclerosis and its important consequences.

There is a strong link between high blood pressure and coronary heart disease (CHD) (23). This close association does not mean that hypertension is the only cause of CHD in hypertensives, since several other cardiovascular risk factors are more prevalent among hypertensive patients than they are among normotensive subjects (24).

Lp(a) exscess may promote premature atherosclerosis by the following mechanisms: inhibition of clot lysis by Lp(a) leading to a thrombogenic state (25 28), increased binding to proteoglycans (29) or to the very low density lipoprotein (VLDL) receptor (30). Lp(a) also promotes increased uptake by macrophages, direct chemoattraction of monocytes (31) with induction of monocyte chemotactic activity in endothelial cells (32) and promotion of smooth muscle cell proliferation by blocking the plasmin-dependent activation of transforming growth factor-b (TGF-b) (33). Oxidized Lp(a) may also contribute directly to accumulation of lipids in macrophages (34).

While these several mechanisms appear to suggest that Lp(a) can act independently to promote atherosclerosis, further considerations suggest depend-

ence on other risk factors. The combination of high levels of Lp(a) with other cardiovascular risk factors, when cholesterol or LDL-cholesterol is elevated in particular low HDL, strongly increases the risk for coronary heart disease (35, 36). Solymoss et al. (37) reported a marked increase in risk of angiographically defined CHD among women under the age of 60 associated with high Lp(a) together with a ratio of total: HDL cholesterol level above 5.8. In a prospective population study of the Prospective Cardiovascular Muenster Study, Von Eckardstein et al. (38) reported that lipoprotein(a) increases coronary risk, especially in men with high LDL cholesterol, low HDL cholesterol, hypertension and high global cardiovascular risk.

Therefore and because Lp(a) increases the risk of coronary events strongly depending on the presence of additional coronary risk factors, it is imperative to strictly control additional risk factors in individuals with elevated Lp(a) (39, 40).

This study indicates that high Lp(a) levels can play a major role in the development of CHD in patients with essential hypertension. These findings suggest the great importance of identifying, among hypertensive patients, subjects with higher levels of Lp(a), which is added to the cardiovascular risk related to their hypertension. Further studies are required to elicit cause and effect in the relationship between arterial hypertension and Lp(a) and other lipoprotein disorders in hypertensive patients. In addition, our findings might have implications not only on the prognosis but also on the management of hypertensive patients.

ULOGA LIPOPROTEINA(a) U RAZVOJU KORONARNE BOLESTI U PACIJENATA SA ESENCIJALNOM HIPERTENZIJOM

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Kratak sadržaj: Uloga liipoproteina(a) [Lp(a)] kao nezavisnog faktora rizika za razvoj koronarne bolesti u pacijenata sa esencijalnom hipertenzijom još uvek je nedovoljno poznata. Određivane su vrednosti Lp(a) u hipertenzivnih pacijenata sa i bez koronarne bolesti a rezultati poređeni sa kontrolnim zdravim osobama. Lp(a) je određivan nefelometrijskom metodom. Dobijene su značajno povišene vrednosti Lp(a) u hipertenzivnih pacijenata koji imaju koronarnu bolest (0,33 ± 0,17 g/L) u poređenju sa pacijentima sa esencijalnom hipertenzijom (0,20 ± 0,05 g/L p<0,001) i zdravim osobama (018 ± 008 g/L). Vrednosti Lp(a) veće od 0,30 g/L imalo je 46,6% hipertenzivnih koronarnih pacijenata, 177% pacijenata sa esencijalnom hipertenzijom i 8,8% u zdravih. Hipertenzivni koronarni pacijenti imali su takođe značajno povišene vrednosti holesterola, LDL-holesterola i triglicerida a značajno niže vrednosti HDL-holesterola. Rezultati ukazuju na značaj identifikacije povišenih vrednosti Lp(a) kao dodatnog faktora rizika za koronarnu bolest u pacijenata sa esencijalnom hipertenzijom.

Ključne reči: lipoprotein(a), koronarna bolest, esencijalna hipertenzija

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