Is Lipoprotein(a) Regulating Prostaglandin I_2-Synthesis Stimulating Plasma Factor?

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SUMMARY

Introduction Lipoprotein(a) – Lp(a) is accepted as an independent risk factor for the development of atherosclerosis. The mechanism, however, and how it exerts its pathogenetic role is still unclear. More than a decade ago a deficiency of prostacyclin synthesis stimulating plasma factor (PF) was claimed to be associated with an increased Lp(a).

Objective The aim of this retrospective analysis was to assess whether elevated Lp(a) is associated with a PF-deficiency and whether certain risk factors may exert influence.

Methods In a total of 185 patients (131 men and 54 women), aged 30-85 years, suffering from clinically manifested atherosclerosis risk factor profile, lipids, lipoproteins and PF under drug intake were evaluated.

Results Patients with absent PF-activity did not differ concerning age, height, weight, body mass index, waist circumference and different lipid and lipoprotein parameters. Mean Lp(a) in patients with absent PF-activity was 18 vs. 94 mg/dl ($p<0.001$). Laboratory parameters such as C-reactive protein, fibrinogen, protein S, protein C, activated protein C resistance and others were not different. In patients with normal (<30 mg/dl) Lp(a) only 4 males (3.4%) and 3 females (4.8%) had PF-deficiency, while the Lp(a) cut-off of 30 mg/dl the prevalence was 61.1% males and 64.4.% females.

Conclusion These findings indicate that the association of PF-deficiency with increased Lp(a), at least in part, could contribute to the pathogenesis of atherosclerosis in these patients.

Keywords: lipoprotein (a); prostaglandin I_2; atherosclerosis; plasma factor activity

INTRODUCTION

Lipoprotein(a) – Lp(a), originally described by Berg [1] in the 1960s, did not gain clinical interest for a long time. In the late 1980s Dahlen [2] found a correlation with coronary heart disease (CHD) and Scanu [3] a potential link between lipoprotein metabolism, atherosclerosis and fibrinolysis. The elevated Lp(a) of >30 mg/dl is widely accepted as an independent risk factor for the development of atherosclerosis. Also long ago, McIntyre reported on a factor present in plasma that stimulated prostacyclin (prostaglandin (PG) I_2) synthesis. It was called prostacyclin synthesis stimulating factor [4]. Later on it was discovered that this plasma factor (PF) could be increased, decreased or lacking and was associated with a variety of abnormal conditions. The lacking PF-activity was described by us as an inborn, non-familial, total or acquired or persistent or temporary defect [5, 6]. For the first time Kritz et al. described a deficiency of prostacyclin synthesis stimulating PF in patients with increased Lp(a) [7].

OBJECTIVE

The aim of this retrospective analysis was to elucidate in a larger number of patients whether there was a relationship between Lp(a) and PF-activity in patients with proven CHD.

METHODS

Patients admitted to the Rehabilitation Center in Bad Tatzmannsdorf, Austria, were examined. They were sent to the Center to undergo rehabilitation and secondary prevention training. On the day of admission the patients' history was taken, risk factors for the development of atherosclerosis, drugs the patients were currently on were assessed (Table 1) and blood was drawn after an at least 12 hours of overnight fasting period (characteristics are summarized in Table 2).

Table 1. Pharmacological agents applied in patients (%)

Medication	Men	Women
A-blocker	0.0	0.0
B-blocker*	77.9	61.1
Ca^{2+} channel blocker*	6.1	16.7
Angiotensin antagonist*	10.7	22.2
ACE-inhibitor*	56.5	27.8
NO-vasodilators	10.7	9.3
Diuretic	27.5	38.9
Clopidogrel*	33.6	18.5
Acetylsalicylic acid*	80.2	63.0
Statin	69.5	61.1
Colestyramin	0.8	0.0
Fibrate	1.5	3.7

* significant differences of intake ($p<0.01$)

Table 2. Patients' characteristics

General features	Men (n=131)	Women (n=54)
Percentage (%)	70.8	29.2
Age (years)*	54.2±10.2	56.9±10.1
Height (m)*	1.75±0.07	1.61±0.06
Weight (kg)*	87.1±14.4	72.4±14.3
Body Mass Index (kg/m^2)	28.5±4.2	28.1±5.5
Waist circumference (cm)*	102.9±10.9	98.0±14.4
Cardiovascular history (%)	90.8	83.3
Positive family history (n)	61	24

* significant differences ($p<0.01$)

Lipids, lipoproteins and Lp(a) were routinely assessed (data are summarized in Table 3). The risk factor profile is presented in Table 4. Plasma for the determination of PF-activity was preserved after separation from cells by centrifugation at less than -20°C.

Determination of prostacyclin synthesis stimulating PF

Preparation of rat aortic tissue samples and incubation with patients' plasma

Activity of PF was assessed using rat aortic tissues of six-month-old male Wistar rats which were killed by decapitation. Preceding sacrifice, the animals had been treated with 100 units of heparin in order to avoid activation of the clotting system and subsequently thrombin which could increase PGI_2-generation. After decapitation, the animals were dissected in an ice-cold water bath, and the thoracic and abdominal aortic segments were swiftly removed. The excised aorta was then cut into small aortic rings weighing 2 to 7 mg each and stored immediately at -70°C until the final determination of PF.

Radioimmunoassay employed to measure PF

Obtained rat aortic tissue rings were then incubated in the patients' platelet poor plasma (PPP) or 300 µl Tris-HCl buffer for 3 minutes at 37°C, respectively. A 100 µl aliquot of the incubation fluid was then removed to assess the PGI_2 production of the rat aortic tissue in the buffer solution or plasma, respectively. As PGI_2 decays quickly, the stable metabolite 6-oxo-$PGF_{1\alpha}$ of the spontaneous decay was measured instead of using a specific radioimmunoassay [8]. A double antibody (OTOP 15/16) was used for the separation of free and bound antigen. Presence of PF was defined as an increase of at least 10 per cent in PGI_2 production (6-oxo-$PGF_{1\alpha}$/mg tissue wet weight) of tissue samples after incubation with plasma, compared to synthesis in buffer solution.

Statistical analysis

The results of the investigation are presented in medians. The 10th and 90th percentile were taken as the measure of statistical distribution. This was necessary since sometimes Lp(a) and C-reactive protein (CRP) could not be determined by laboratory or were below a certain level (Lp(a)<12 mg/dl; CRP<0.5 mg/dl). Without exact values for these variables the assessment of means or standard deviations for the whole sample was not possible, while medians and percentiles still could be computed. For the presentation of general features like height or body weight, means and standard deviations were calculated.

As standard deviations and means could not be worked out for all the laboratory parameters, the Mann-Whitney U-test was used instead of the Student's t-test to compute the levels of significance of differences in values between comparison groups. For the nominal data like presence or absence of hypertension, Chi-standard test was employed to examine whether differences in frequencies were significant between the groups under observation. If absolute numbers in the cells of cross-tabulations were below five, the Fisher's exact test was taken to assess the levels of significance.

To assess whether the differences in PF-activity after incubation in patients' plasma – expressed in percentage (%) of the production of PGI_2 by rat aortic tissue – correlated with the increase in Lp(a), Spearman's Rank Correlation coefficient (r_s) was used. This was done since its use permitted assessment of the correlation coefficient even if the relation was not linear. To assess the fitting of various standard curves to dot clouds, r^2 was calculated by linear and nonlinear regression models.

Statistical analysis was performed using SPSS and Excel.

RESULTS

Although intake of some drugs was significantly different between females and males (Table 1), no influence of any of these could be related either to Lp(a) or PF.

Despite the sex related differences (height, body weight and waist circumference), females were older ($p<0.01$) and amounted to 29.2% (Table 2) of the total number examined. Although medians for Lp(a) were higher in females (Table 3), due to a high variation, the difference was not significant. Relevant non-lipid parameters claimed as risk factors (e.g. CRP) or considered to play at least a contributory role in the development of atherosclerosis (Tables 4 and 5) did not significantly differ between females and males.

Lp(a) showed most significant relation of all the tested parameters with PF and was significant ($p<0.01$) for both genders. Median Lp(a) in PF-deficient men was 94 mg/dl, while in men with intact PF Lp(a) amounted only to

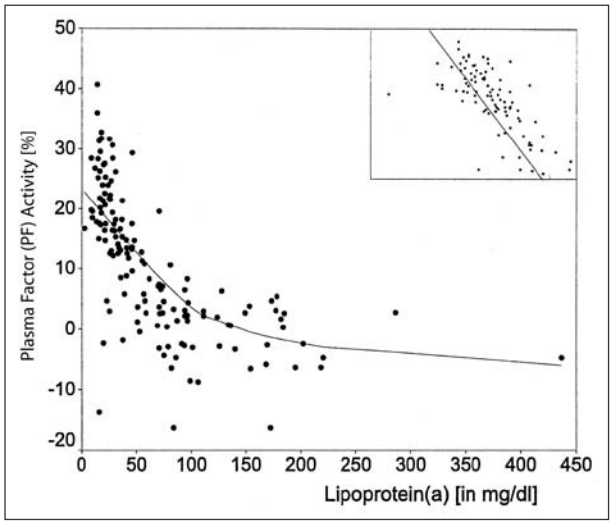
Table 3. Lipid levels

Lipid values	Men			Women		
	10%	Median	90%	10%	Median	90%
Lp(a) (mg/dl)	11.0	31.0	121.8	12.0	57.3	179.5
Triglycerides (mg/dl)	89.4	160.0	307.0	83.0	143.5	264.0
Total cholesterol (mg/dl)*	137.0	182.0	249.0	155.0	210.0	278.0
HDL-cholesterol (mg/dl)*	31.2	42.0	57.8	34.5	48.0	75.0
LDL-cholesterol (mg/dl)*	76.0	114.0	166.0	92.5	121.5	193.5
Total cholesterol/HDL-cholesterol	3.0	4.3	6.7	2.8	4.1	6.3

* significant differences ($p<0.01$)

Table 4. Clinical risk factors for atherosclerosis except HLP (values in %)

Clinical risk factors		Men	Women
Smoking	No	16.8	24.1
	Yes	57.3	44.4
	Ex	26.0	31.5
Diabetes mellitus		35.9	22.2
Hypertension		60.3	64.8



Graph 1. Lp(a) shows a strong inverse relationship to plasma factor (PF) activity (in % vs. buffer incubation)

19.3 mg/dl. For females the respective values were 94 and 18 mg/dl. This difference was statistically constant in all examined age groups. Apparently, PF deficiency was not linked to a certain cut-off of Lp(a), only the prevalence of PF deficiency was significantly increasing with increasing Lp(a) levels (Graph 1). Rarely (3.4% in males and 4.8% in females), however, there was a PF-deficiency even in individuals with normal Lp(a) (<30 mg/dl). In-vitro addition of various amounts of Lp(a) ranging from 50-500 mg/dl did not reveal any change in PF-activity indicating the need for an in-vivo cofactor (unpublished data).

DISCUSSION

Although known for about two decades now [9, 10] and despite some remarkable correlations of PF-activity to various clinical disease entities associated with increased or decreased bleeding tendency, research of a potential underlying mechanism did not progress and was recently almost abandoned. Exact biochemical definition of PF is still not available. A decreased local availability of PGI_2 at the blood vessel wall interaction level either due to decreased PGI_2-synthesis or diminished PF-activity, as well as faster decay may act as an important determinant of haemostatic imbalance. The apo(a) moiety of Lp(a), a unique feature of this lipoprotein, could be involved in the interaction with PF as well. Due to its moiety it inhibits fibrinolysis binding to the lysine sites of fibrin. These binding sites or other parts of the apo(a) moiety may remove substances responsible for PF-activity.

It is not known whether some constituent of the Lp(a) particle might negatively interfere with PGI_2-synthesis, which is stimulated by PF [11]. This inhibition might be effected via various oxidized polyunsaturated fatty acids which the PGI_2-synthase is known to be susceptible to and may be carried by Lp(a). As the PF-activity is unrelated to other lipoproteins (HDL, LDL, VLDL) their influence is unlikely. As HDL has the capacity to stabilize PGI_2, this is of particular importance.

Limitation of the study

As LDL-apheresis is the only efficient approach to lower Lp(a) temporarily, it remains to be established whether a drop in Lp(a) is associated with a change in PF-activity. The extent to which Lp(a) was oxidized (modified) was not assessed.

CONCLUSION

Although the exact biochemical background still remains to be elucidated, these findings indicate that the PGI_2-synthesis stimulating PF might be a so far undervalued property being at least influenced by plasma Lp(a) and thus a relevant coregulator of haemostatic balance.

NOTE

Part of these data is derived from the MD thesis of Ernst Ruppert presented at the Medical University of Vienna.

Table 5. Relevant non-lipid parameters which might be involved in atherosclerosis

Possible risk factors	Men			Women		
	10%	Median	90%	10%	Median	90%
CRP (mg/dl)	0.07	0.33	1.62	0.08	0.47	1.55
Fibrinogen (mg/dl)	237.20	354.0	570.40	253.5	410.5	517.0
TSH (µU/ml)	0.53	1.48	3.92	0.70	1.83	4.20
Platelets (g/l)	162.60	226.0	343.60	176.5	249.0	341.5
Packed cell volume (%)	38.0	43.0	46.8	36.5	40.0	44.0

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Да ли липопротеин(а) регулише синтезу простагландина I_2 стимулишући фактор плазме?

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КРАТАК САДРЖАЈ

Увод Опште је прихваћено да је липопротеин(а) – *Lp(a)* независан фактор ризика у настанку атеросклерозе. Међутим, остаје нејасан механизам испољавања његове патогенетске улоге. Пре више од десет година тврдило се да је дефицијенција синтезе простациклина у стимулацији фактора плазме (ФП) повезана с повишеним вредностима *Lp(a)*.

Циљ рада Циљ ове ретроспективне анализе био је да се утврди да ли су повишене вредности *Lp(a)* повезане са дефицијенцијом ФП и да ли постоје одређени фактори ризика који испољавају такав утицај.

Методе рада Испитано је 185 болесника (131 мушкарац и 54 жене), старости између 30 и 85 година, оболелих од клинички манифестних фактора ризика за развој атеросклерозе, вредности липида, липопротеина и ФП који су медикаментно лечени.

Резултати Болесници без активности ФП нису се разликовали према старосном добу, висини, тежини, индексу телесне масе, обиму струка и параметрима различитих липида и липопротеина. Средња вредност *Lp(a)* болесника без активности ФП била је 18 *mg/dl*, а оних са активношћу овог фактора 94 *mg/dl* ($p<0,001$). Није било разлике у односу на лабораторијске параметре, као што су *C*-реактивни протеин, фибриноген, протеин *S*, протеин *C*, резистенција активног протеина *C* и осталих фактора. Код испитаника с нормалним вредностима *Lp(a)* (<30 *mg/dl*) само четири мушкараца (3,4%) и три жене (4,8%) имале су дефицијенцију ФП, док су граничне вредности *Lp(a)* изнад 30 *mg/dl* установљене код 61,1% мушкараца и 64,4% жена.

Закључак Добијени резултати показују да повезаност дефицијенције ФП и *Lp(a)*, барем делимично, код ових болесника вероватно доприноси патогенези атеросклерозе.

Кључне речи: липопротеин(а); простагландин I_2; атеросклероза; активност фактора плазме

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