Cardiol Young 2001; 11: 25–29

ISSN 1047-9511

Original Article

Levels of lipoprotein (a) in pulmonary arterial hypertension

Raul D. Santos[1], Antonio Foronda[1], José, A. F. Ramires[1] and Raul C. Maranhão[1,2]

[1]*Heart Institute (InCor) of the Medical School Hospital;* [2]*Faculty of Pharmaceutical Sciences University of Sao Paulo- Sao Paul, Brazil*

Abstract We compared the levels of lipoprotein (a) in 48 Caucasian patients with pulmonary arterial hypertension, comprising 32 females and 16 males, aged 28.0 ± 12.0 years, with a range from 4 through 52 years, with 48 normal Caucasian subjects matched for age and sex. Pulmonary hypertension was secondary in 41 patients with Eisenmenger's syndrome, these comprising 27 females and 14 males aged 27.0 ± 12.0 years, with a range from 4 through 51 years, and primary in the other 7 patients, 5 females and 2 males, whose age was 30.0 ± 14.0 years, with a range from 9 through 52 years. Lipoprotein (a) was measured using an immunoprecipitation and turbidimetric assay after a 12 hour fast. Levels of the protein, expressed as the median (% 25; % 75), were higher in those with Eisenmenger's syndrome than in normal controls ($p=0.003$). In addition, there was a greater prevalence of levels of lipoprotein greater than 30.0 mg/dl in those with secondary pulmonary arterial hypertension patients than in our normal population ($p = 0.03$). We have found no differences, however, in the levels of lipoprotein(a) in those who had primary pulmonary arterial hypertension when compared with their matched controls, albeit that the number of patients studied was small. We conclude that increased levels of lipoprotein (a) may be secondary to pulmonary arterial hypertension as a marker of tissue damage or may be genetically determined. In either way, the increase in lipoprotein (a) could be an additional factor predisposing to the vascular alterations known to occur in this disease.

Keywords: Lipoprotein (a); pulmonary hypertension; congenital cardiac malformations

LIPOPROTEIN(A) IS A CHOLESTEROL-RICH PLASMA lipoprotein that has been linked to atherogenesis in Caucasian and oriental individuals.[1] It is a low-density lipoprotein-like particle which possesses an additional apolipoprotein, namely apolipoprotein (a), which is attached to apolipoprotein B100 by disulphide bonds. Its physiological function is still undetermined, although apolipoprotein (a) is known to be homologous to plasminogen, the zymogen of plasmin, with which it competes for specific receptors. As a consequence, increased levels of lipoprotein (a) may inhibit fibrinolysis and predispose to thrombosis. It has also been shown that lipoprotein (a) stimulates proliferation of human smooth muscle cells.[2] The lipoprotein is synthesised by the liver, and the extent of its concentration in the plasma is chiefly genetically determined.[1,3] In Caucasian individuals, the distribution of its levels in the plasma is fairly scattered, but about three-quarters of the population have levels below 30.0 mg/dl. Levels above these values are considered pathogenic,[1,4] since they have been associated with an increased risk of atherosclerosis. Moreover, experimental studies have shown that, at these levels, binding of plasminogen is reduced, and consequently cellular fibrinolysis is diminished.[5] One of its peculiar characteristics is that it acts like an acute phase protein,[6] and is transiently increased after major surgery, acute myocardial infarction, and unstable angina.[7]

Vascular damage is one of the main characteristics of pulmonary arterial hypertension, be it primary, or secondary to congenital cardiac disease as seen in Eisenmenger's syndrome.[8] Thrombosis,

Correspondence to: Raul C. Maranhão, Av. Dr. En,as C. Aguiar 44, 05403–000 Sao Paulo-SP, Brazil. Tel/Fax: 55 11 30630779; E-mail: rmaranas@usp.br

Accepted for publication 21 June 2000

and proliferation of smooth muscle cells, are the main pathological findings.[9,10] It seems that, given a genetic susceptibility, shear stress and inflammation are the principal pathogenic factors involved in remodelling of the pulmonary vasculature.[10] With this in mind, we sought to establish whether levels of the lipoprotein would be increased in the setting of pulmonary arterial hypertension.

Material and methods

Subjects studied

We studied 48 Caucasian patients with pulmonary arterial hypertension, comprising 32 females and 16 males who were aged 28.0 ± 12.0 years, with a range from 4 through 52 years. All were patients at the clinic of the Heart Institute (InCor) of the University of São Paulo Medical School Hospital. Of these, 7 had primary pulmonary hypertension, 5 females and 2 males, and were aged 30.0 ± 14.0 years, with a range from 9 through 52 years, while 41 had Eisenmenger's syndrome. The latter group comprised 27 females and 14 males, aged 27.0 ± 12.0 years, with a range from 4 through 51 years. We compared these patients with 48 healthy Caucasian individuals matched for age and sex. The control population was aged 28.0 ± 11.0 years, with a range from 5 through 50 years.

Presence of pulmonary arterial hypertension was established by clinical, echocardiographic, and cardiac catheterization investigations showing the mean pulmonary arterial pressure to be greater than 25.0 mmHg[11] after oxygen and tolazoline tests.[12] Hypertension was considered to be primary according to internationally accepted criterions after other causes of pulmonary arterial hypertension had been excluded.[13] In those with primary disease, the average mean pulmonary arterial pressure was 46.0 ± 16.0 mmhg. Eisenmenger's syndrome had occurred in patients with congenital cardiac malformations producing increased pulmonary flow in the absence of previous surgical treatment, all patients presenting with cyanosis, dyspnoea at minimal effort, and polycythaemia.[13] In these patients, the average mean pulmonary arterial pressure was 70.0 ± 22.0 mmhg. We excluded patients with renal failure, those with proteinuria greater than 1.0 g/l, and those with diabetes mellitus or acute inflammatory or infectious diseases, since all these conditions are known to produce increased levels of lipoprotein (a).[1]

Laboratory determinations

Lipoprotein (a) was measured after 12 hours fasting using an immunoprecipitation and turbidimetric assay (Incstar Corp. Stillwater, USA) in a COBAS MIRA analyser (Roche Laboratories, Basel, Switzerland). This assay contains a monoespecific antibody for lipoprotein (a) which detects values in the range from 4.9 through 81.0 mg/dl. The analyser uses the delta absorbance of 5 standards to develop a calibration curve with four-parameter logit function. Samples above the upper ranges of the test were flagged above the test range by the analyser, diluted 1 to 2, and reassayed. These results were multiplied by a correction factor of 2.0. Levels of plasminogen up to 230.0 mg/dl do not interfere with this assay. The intraassay coefficient of variation was 9.3 to 2.6, respectively, for concentrations of lipoprotein (a) of 6.4 and 73.7 mg/dl. Levels of lipids such as total cholesterol and triglycerides in the plasma were determined in the patients and in normal subjects using enzymatic methods (Roche Laboratories, Basel, Switzerland). HDL-cholesterol was determined in those with pulmonary arterial disease by the same method used for total cholesterol after chemical precipitation of the proteins containing apolipoprotein B–100 with magnesium chloride and phosphotungistic acid. LDL-cholesterol was calculated by the Friedwald formula in those patients with levels of triglyceride up to 400 mg/dl.[14] In the patients with pulmonary hypertension, apolipoprotein B was also determined by immunoturbidimetry (Roche Laboratories, Basel, Switzerland). Measures of lipids and apolipoprotein were also performed automatically in a COBAS–MIRA analyzer (Roche Laboratories, Basel, Switzerland). The haematocrit was determined by micro-ultracentrifugation. In the month prior to sampling of blood, no patient was submitted to haemodilution nor given lipid-lowering drugs.

Statistical analysis

Since lipoprotein (a) presents a skewed non-gaussian distribution, its values were expressed as the median (25%; 75%). All other parameters were expressed as mean ± standard deviation. Levels of lipoprotein (a) were compared between the groups by the Mann Whitney test. Lipids in the plasma were compared by Student's t test. The categorical variables, with interest on the prevalence of increased levels of lipoprotein (a) between the groups, were evaluated by Fisher's exact test. In patients with secondary pulmonary arterial hypertension, we also sought to establish whether the levels of lipoprotein (a) were related to the duration of the disease, using age as a surrogate marker, levels of the haematocrit and LDL-cholesterol, or concentrations of apolipoprotein B, using the

Spearman correlation test for these calculations. We also evaluated if the patients with secondary pulmonary arterial hypertension having levels of lipoprotein (a) greater than 30 mg/dL differed from those patients with normal values in terms of duration of disease, gender, and aetiology of pulmonary arterial disease using Student's t test and Fisher's exact test. We accepted significance at the level of 5%.

Our study was approved by the Scientific and Ethical committee of the Heart Institute (InCor) of the University of São Paulo Medical School Hospital, and informed consent was obtained by the participants or their parents.

Results

Levels of lipoprotein (a) were higher in patients with secondary pulmonary arterial hypertension than in normal subjects {24.0 (11.0; 43.0) mg/dL vs. 13.0 (5.0; 24.0) mg/dL, p=0.003}. Furthermore, there was a greater prevalence of levels of lipoprotein (a) greater than 30.0 mg/dl in the patients than in their normal controls {17/41 patients (41%) as opposed to 8/41 controls (19.5 %), p = 0.03}. The distribution of levels of lipoprotein (a) in the two groups is shown in Figure 1. There were no differences in the measured levels between the patients with primary pulmonary hypertension and their controls {27.0 (16.0; 41.0) mg/dL and 25.0 (12.0;36.0) mg/dL respectively, p= 0.6}. Within the group having secondary pulmonary arterial hypertension, levels of the lipoprotein were not correlated with age (r= 0.09), the haematocrit (r= –0.16), LDL-cholesterol (r= 0.17), plasma triglycerides (r= 0.11), or levels of apolipoprotein B (r = 0.3). Levels of total cholesterol and triglycerides were lower in the plasma from patients with secondary pulmonary arterial hypertension than in their controls {151.0 ± 31.0 mg/dl vs. 185.0 ± 40.0 mg/dL, p < 0.0001 and 104.0 ± 55.0 mg/dl vs. 151.0 ± 120.0 mg/dL, p= 0.04}.

We found no differences among the patients having secondary pulmonary artery hypertension with increased or normal levels of lipoprotein (a) with regard to duration of disease (31.0 ± 10 years vs. 25.0 ± 13.0 years, p= 0.12), gender (33% vs. 35% males, p=1.0) or the aetiology of pulmonary arterial hypertension (Table 1).

Discussion

It is known that levels of lipoprotein (a) can be elevated in patients with pulmonary arterial hypertension as a consequence of vascular damage resulting from the ongoing inflammatory proliferative process.[10,15] In this respect, we have also found increased levels in those having systemic lupus erythematosus,[16] as have others in those with thromboangiitis obliterans.[17] As with pulmonary arterial hypertension, both these diseases involve vascular inflammatory proliferation. In the setting of pulmonary arterial hypertension, inflammatory cells such as T and B lymphocytes and macrophages have been found surrounding vascular lesions.[10] These cells secrete vascular growth factors and cytokines such as interleukins, tumour necrosis factor, and transforming growth factor-β. There is evidence that interleukin–6 stimulates the promoter gene for apolipoprotein (a), thus producing increased levels.[18] It is also known that the lipoprotein itself antagonises cytokin and transforming growth factor-β.[19] In males, the levels of lipoprotein (a) have been shown to bear an inverse relation with the levels of this cytokine.[19] The possibility may exist, therefore, that cytokines are involved with the increase in lipoprotein (a) observed

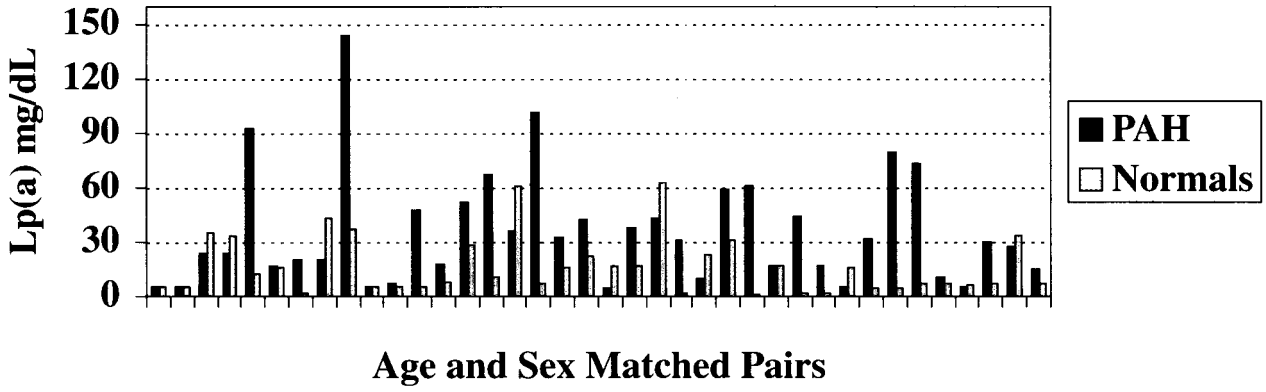

Figure 1.
Lp(a) levels in secondary pulmonary arterial hypertension patients: Eisenmenger's Syndrome and in matched controls.

https://doi.org/10.1017/S1047951100012385 Published online by Cambridge University Press

Table 1: Aetiology of secondary pulmonary arterial hypertension in patients with high and low levels of lipoprotein (a).

	Lipoprotein (a) ⩾ 30 mg/dl N = 17	Lipoprotein (a) < 30 mg/dl N = 24	P
Patency of arterial duct	4	4	0.7
Atrial septal defect	3	4	1.0
Ventricular septal defect	6	7	1.0
Double outlet right ventricle	2	4	1.0
Atrioventricular septal defect	2	2	1.0
Tricuspid atresia	0	1	1.0
Complete transposition	0	1	1.0
Anomalous pulmonary venous connection	0	1	1.0
Common arterial trunk	0	1	1.0

in pulmonary artery hypertension. On the other hand, genetic inheritance linked with the appearance of the disease could also be causative. Subjects with an increased risk for pulmonary arterial hypertension as the result of a congenital cardiac malformation could also have an hereditary trend for high levels of lipoprotein (a). The first hypothesis seems more likely, since lipoprotein (a) may be a marker of vascular damage rather than its cause.[20] In any event, the increased levels of lipoprotein (a) found in the setting of pulmonary arterial hypertension may contribute do the pathological findings of the disease by predisposing to thrombosis,[1] and by increasing the number of smooth muscle cells.[2] The small number of patients we were able to evaluate with primary pulmonary hypertension precludes us making any reliable conclusion regarding the relationship between lipoprotein (a) levels and this disease, and we are now seeking to evaluate a greater number of patients.

The fact that we could not relate high or low levels of the lipoprotein with parameters such as age, the haematocrit, or the aetiology of pulmonary arterial hypertension, does not exclude a relationship with the severity of the disease, since this factor was not assessed in our study, neither by concomitant measurement of pulmonary pressures nor evaluation of inflammatory mediators. In this regard, a recent study has shown that levels of α-tumour necrosis factor are increased in primary pulmonary arterial hypertension, and are directly related to pulmonary arterial pressures.[21]

The reduced levels of total cholesterol and plasma triglycerides found in those with secondary pulmonary arterial pressure when compared to normal subjects is certainly secondary to the severity of the chronic illness, this compromising ingestion of food, absorption of fat, and hepatic metabolism.[22]

In summary, therefore, we have found increased levels of lipoprotein (a) in patients with pulmonary arterial hypertension due to congenital cardiac disease, showing that the prevalence of levels of the lipoprotein greater than 30.0 mg/dl levels are also increased in this disease when compared to normal subjects. Further studies are now needed to correlate the increased levels with pulmonary arterial pressures and with inflammatory markers, and also to search for lipoprotein(a) in the diseased pulmonary vessels, if the relationship is to be clarified between this lipoprotein and pulmonary arterial hypertension.

Acknowledgements

We thank Dr Carmen Vinagre for the technical support.

References

1. Utterrman G. Lipoprotein(a). In: The metabolic and molecular bases of inherited disease. Scriver CL, Beaudet AL, Williams SS, Valle D eds. New York Mc Graw-Hill,1995:1887–1912.
2. Grainger DJ, Kirschenlohr HL, Metcalfe JC, Weissberg PL, Wade DP, Lawn RM. Proliferation of human muscle cells promoted by lipoprotein(a). Science 1993;260:1655–1658.
3. Gaw A, Hobbs HH. Molecular genetics of lipoprotein(a):New pieces to the puzzle. Curr Op Lipidol 1994;5:149–155.
4. Kostner GM, Avogaro P, Cazzolato G, Morth E, Bittolo-Bon G, Quina GB. Lipoprotein(a) Lp(a) and the risk for myocardial infarction. Atherosclerosis 1981;38:51–61.
5. Scott J. Thrombogenesis linked to atherogenesis at last? Nature 1989;341:22–23.
6. Maeda S, Abe A, Seishima M, Makino K, Noma A, Kawade M. Transient changes of serum lipoprotein(a) as an acute phase protein. Atherosclerosis 1989;78:145–150.
7. Oshima S, Uchida K, Yasu T, Uno K, Nonogi H, Haze K. Transient increase of plasma lipoprotein(a) in patients with unstable angina pectoris. Arterioscler and Thromb 1991;11:1772–1777.
8. Rich S, Brundage BH. Pulmonary hypertension: a cellular basis for understanding the pathophysiology and treatment. J Amer Coll Cardiol 1989;14:545–550.
9. Chaouat A, Weitzenblum E, Higenbottam T. The role of thrombosis in severe pulmonary hypertension . Eur Respir J 1996;9:356–363.

10. Voelkel NF, Tuder RM. Cellular and molecular mechanisms in the pathogenesis of severe pulmonary hypertension. Eur Respir J 1995;8:2129–2138
11. Greenberg HE, Scharf FM. Pulmonary hypertension: pathophysiology and clinical disorders. In: Baum GL, Wolinsky E; eds. Textbook of Pulmonary Diseases. Boston : Little, Brown and Co;1994:1285–1304.
12. Rich S, Braunwald E, Grossman W. Pulmonary Hypertension . In Heart Disease. Braunwald E ed. Philadelphia, W.B. Saunders Co; 1997;780–806.
13. Rich S, Dantzker DR, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Koerner SK. Primary Pulmonary Hypertension . A National prospective study Ann Intern Med 1987;107:216–223.
14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of preparative ultracentrifuge. Clin Chem 1972;18: 499–502.
15. Tuder RM, groves B, Badesch DB, Voelkel NF. Exuberant endothelial cell growth and elements of inflammation are present in plexiform lesions of pulmonary hypertension, Amer J Pathol 1994;144:275–85.
16. Borba EF, Santos RD, Bonfa E, Vinagre CG, Pileggi FJ, Cossermelli W, Maranhao RC. Lipoprotein(a) levels in systemic lupus erithematosus. J Rheumatol 1994;21:220–3.
17. Takami S, Kubo M, Yamashita S, Kameda-Takemura K, Kawasaki T, Kanbayashi J, Nakamura Y, Yokoi Y, Ohnishi K, Matsuzawa Y. High levels of serum lipoprotein(a) in patients with ischemic heart disease with normal coronary angiogram and thromboangiitis obliterans. Atherosclerosis 1995;112:253–60.
18. Wade DP, Clarke JG, Lindahl GE, Liu AC, Zysow BR, Meer K, Schwartz K, Lawn RM. 5' Control regions of the apolipoprotein(a) gene and members of the related plasminogen gene family. Proc Natl Acad Sci USA 1993;90:1369–73.
19. Grainger DJ, Kemp PR, Metcalfe JC, Liu AC, Lawn RM, Williams NR, Grace AA, Schofield PM, Chauhan A. The serum concentration of active transforming growth factor-β is severely depressed in advanced atherosclerosis. Nat Med 1995;1:74–79.
20. Kinlay S, Dobson AJ, Heller RF, McElduff P, Alexander H , Dickeson J. Risk of primary and recurrent acute myocardial infarction from lipoprotein(a) in men and women. J Amer Coll Cardiol 1996;28:870–5.
21. Galie N, Grigioni F, Uguccioni L, Cervi V, Di Luzio S, Serafini F, Catanzariti P, Callegari G, Fracchia C, Branzi A. Magnani B. Increased levels of α-TNF in patients with primary pulmonary hypertension. Eur Heart J 1997;18 supl:528.
22. Braunwald E, Colucci WS, Grossman W. Clinical Aspects of heart failure: high –output, heart failure, pulmonary edema. In Heart Disease. Braunwald E ed. Philadelphia, W.B. Saunders Co; 1997;445–470.