

Original Article

Endothelial Prostanoid Secretion in Essential Hypertension

Oravec Stanislav¹, El-Ghawabi Mohamed², Gavornik Peter³, Balazovjeh Ivan³, Kosmalova Viera³, Hirnerova Eva³

¹Laboratory Department and ²Pediatric Department, Al-Jahra Hospital, Al-Jahra, Kuwait

³2nd Medical Department, Teaching Hospital, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

Kuwait Medical Journal 2004, 36 (1): 31-34

ABSTRACT

Background: Patients suffering from arterial hypertension have hyperlipoproteinemia more frequently than normotensive subjects. Low density lipoproteins in the culture of human endothelial cells stimulate thromboxane A₂ (TxA₂) secretion and suppress prostacyclin (PGI₂) secretion. An increase in TxA₂ serum levels and a decrease in PGI₂ serum levels would theoretically lead to increased vasoconstriction causing an increase in peripheral vessel resistance and leading to the development of arterial hypertension.

Objectives: To confirm the modulating effect of hyperlipoproteinemia on endothelial prostanoid secretion in patients with essential hypertension.

Methodology: The study included 100 newly diagnosed hypertensive patients, 63 patients with hyperlipoproteinemia without hypertension, and 30 healthy volunteers. The following blood parameters were evaluated: total cholesterol, triacylglycerols, HDL-cholesterol, 6-keto-PGF₁, Tromboxane B₂ (TxB₂). The patients were then further classified into 3 main groups. Group 1 consisted of patients with essential hypertension, either isolated (EH), or arterial hypertension associated with hyperlipoproteinemia (EH & HLP), Group 2 consisted of patients with

hyperlipoproteinemia without treatment (HLP) and patients with hyperlipoproteinemia treated with statins (treated HLP), and Group 3 was the control group.

LDL-cholesterol was calculated according to Friedewald's formula. 6-keto-PGF₁ and TxB₂ were analyzed by ELISA technique after processing the samples by chromatographic column SEP-PAK C-18 cartridges. Statistical evaluation: Non-parametric Mann-Whitney's test.

Results: The most significant changes were seen in the patient group with essential hypertension. In the arterial hypertension combined with hyperlipoproteinemia subgroup, the secretion increment of TxA₂ was 309% (p < 0.0001). In the subgroup with isolated arterial hypertension, the secretion of PGI₂ was suppressed to 15% (p < 0.0001) of PGI₂-secretion, compared to the control group levels.

Conclusion: Increased serum LDL in patients with hyperlipoproteinemia suppresses endothelial PGI₂-secretion and stimulates TxA₂ secretion. These changes result in altered vessel homeostasis and may cause vasoconstriction and increased peripheral vessel resistance, which in turn can cause arterial hypertension.

KEYWORDS: hyperlipoproteinemia, hypertension, prostanoids

INTRODUCTION

Patients with arterial hypertension have hyperlipoproteinemia with increased plasma LDL-cholesterol concentration more frequently than normotensive subjects^[1]. Plasma lipoproteins modulate secretion of vasoactive mediators from the endothelium, including prostacyclin (PGI₂) and thromboxane A₂ (TxA₂)^[2,3].

Low density lipoproteins (LDL) in the culture of endothelial cells stimulate TxA₂-secretion^[4,5,6]. TxA₂ is a vasoactive mediator with potent vasoconstrictory, aggregatory and atherogenic effect on vessel wall. LDL inhibit PGI₂-secretion thereby decreasing vasodilation, anti-aggregation and anti-atherogenicity^[7,8]. An insufficient PGI₂ secretion favours a predominance of vasoconstrictive factors^[9].

The imbalance in prostanoids secretion creates the humoral conditions conducive to an increase in peripheral vessel resistance and to the development of arterial hypertension. The concept of the regulatory relations among LDL, prostanoids secretion and arterial hypertension is supported by the presence of arterial hypertension in LDL-receptor deficient animals^[10], blood pressure lowering effect of LDL apheresis, and blood pressure lowering effect following treatment with statins in patients suffering from hyperlipoproteinemia and essential hypertension^[11,12,13].

High density lipoproteins (HDL), on the contrary, stimulate endothelial PGI₂-secretion and inhibit TxA₂-secretion^[14,15]. HDL effect on endothelial prostaglandin synthesis antagonizes

Address correspondence to:

Dr. Oravec Stanislav, MD, Ph D., Laboratory Department, Al-Jahra Hospital, Kuwait. Tel. Res: 2642786. E-mail oravecstano@yahoo.com

unfavorable LDL effects. HDL represent a beneficial part of lipoproteins that maintains an optimal homeostasis for the vessel wall.

The results from previous studies on the effects of plasma lipoproteins on endothelial prostanoids secretion *in vitro* conditions^[4,5] served as the theoretical platform to formulate the hypotheses about firstly, the possible modulating effect of lipoproteins on endothelial prostanoids-secretion *in vivo* and secondly, the potential role of prostanoids in the pathogenesis of arterial hypertension.

Increased plasma LDL-concentration in patients with hyperbeta-lipoproteinemia could alter the endothelial secretion of PGI₂ and TxA₂, leading to altered tone of vessel wall. Longstanding and excessive secretion of vasoconstrictors and suppression of vasodilators secretion lead to increased peripheral vessel resistance, causing arterial hypertension.

Essential hypertension being multifactorial, the monitoring of prostanoid effects on vessel tone and peripheral vessel resistance can help us to complete the mosaic picture of patho-mechanisms, which are in force in arterial hypertension.

PATIENTS AND METHODS

The study consisted of 100 newly diagnosed, non-treated persons with essential hypertension; 47 male and 53 female between 24 and 42 years of age. Renal, renovascular and/or other secondary causes of arterial hypertension were excluded and the patients were divided into two groups:

1. subgroup with isolated arterial hypertension [EH], (13 male and 12 female),
2. subgroup with arterial hypertension combined with hyperlipoproteinemia [EH & HLP], (34 male and 41 female).

The second group was divided into two sub-groups. The first [HLP] consisted of 24 normotensive patients with non-treated newly recognized hyperlipoproteinemia (four males and 20 females between 21 and 62 years of age) and the second sub-group, [treated HLP], a group of 39 patients with hyperlipoproteinemia (five males and 34 females between 43 and 66 years of age) treated with statins and who during the period of treatment were normolipemic. The treatment was simvastatin 10-20 mg/day.

Exclusion criteria: Smokers were excluded from this study.

The control group consisted of 30 healthy normotensive and normolipemic volunteers (five male and 25 female) aged between 20-24 years, who had no signs of heart, lungs, liver or kidney problems. After a 12-hour fasting period, the blood was collected by cubital vein puncture in the

morning and the serum was separated. Aliquots of serum were stored immediately at -20 °C till the time of sample analysis. Total cholesterol (TC) triacylglycerols (TG) and HDL cholesterol were analyzed by biochemical kits (Boehringer GFR, Immuno AG Vienna, Austria). LDL cholesterol was calculated by Friedewald's formula: LDL cholesterol = TC - (TG / 2.2 + HDL cholesterol) in mmol/l.

Two prostanoid parameters were analyzed: 1). 6-keto-PGF₁ (a stable metabolite of PGI₂), 2). thromboxane B₂ (TxB₂) (a stable metabolite of TxA₂). ELISA technique (DRG Instruments GmbH, FRG) was used for analysis. The prostanoids were analysed after processing the serum by chromatographic column SEP-PAK C-18 cartridges (Waters, USA) according to generally recommended analytical procedure^[15,16]. Recovery of used extraction procedure was 98%. Statistical evaluation was carried out by the statistical software SPSS Windows Version 10, utilizing non parametric Mann-Whitney's test. The average values of analyzed parameters in all tested patient groups were compared against the values of corresponding parameters of the control group.

RESULTS

- **1st Group:** In the EH patient subgroup, patients with isolated arterial hypertension:
 - the serum lipid concentration was normal
 - the PGI₂ (6 keto-PG F₁) level was reduced upto 580.5 pg/ml (15% of the control value, p < 0.0001)
 - TxA₂ (TxB₂) level was increased upto 5,775.4 mg/ml (80,4% increment, comparing to the control value (p < 0.003) (Table 1).
- In the EH & HLP subgroup, patients with arterial hypertension and HLP:
 - the serum cholesterol level was increased upto 6.49 mmol/l, LDL cholesterol was found to be 4.27 mmol/l. Both of them reached the increased risk level of atherosclerosis development (TC > 6.2 mmol/l, and LDL cholesterol > 3.4 mmol/l).
 - the PGI₂ (6 keto-PGF₁) levels were decreased upto 1,297.8 pg/ml (34,2% of the control value).
 - TxA₂ (TxB₂) levels were increased by more than 309%, compared to the control value (p < 0.0001 for both prostanoid parameters) (Table 1).
- **2nd Group:** Sub-group HLP: Patients with hyperbeta-lipoproteinemia were newly diagnosed patients and not treated:
 - serum cholesterol level was 5.8 mmol/l, which represented intermediary increased risk of atherosclerosis development.
 - LDL cholesterol was 3,65 mmol/l (increased risk of atherosclerosis development).
 - PGI₂ (6 keto-PG F₁) - secretion decreased to 35,2% (1,336.3 mg/ml) (p < 0.0001).

Table 1

Lipid and prostanoid values

	TC (mmol/l)	TG (mmol/l)	HDLchol (mmol/l)	LDLchol (mmol/l)	6-K-PGF1 (pg/ml)	TxB ₂ (pg/ml)
EH (n = 25)	4.61 ± 0.10	1.48 ± 0.20	1.14 ± 0.06	2.86 ± 0.12	580.5 ± 71.3 p < 0.0001	5 775.4 ± 1 190.6 p < 0.003
EH & HLP (n = 75)	6.49 ± 0.09	2.11 ± 0.13	1.27 ± 0.04	4.27 ± 0.09	1 297.8 ± 192.7 p < 0.0001	13 079.9 ± 1 983.2 p < 0.0001
HLP (n = 24)	5.80 ± 0.07	2.30 ± 0.80	1.34 ± 0.07	3.65 ± 0.11	1 336.3 ± 170.4 p < 0.0001	7 679.5 ± 2 128.8 p < 0.006
treated HLP (n = 39)	4.36 ± 0.10	1.00 ± 0.07	1.32 ± 0.05	2.65 ± 0.10	1 515.8 ± 341.0 p < 0.0001	5 714.5 ± 1 547.0 n.s.
Control (n = 30)	4.38 ± 0.12	0.94 ± 0.06	1.50 ± 0.08	2.45 ± 0.12	3 793.8 ± 801.5	3 201.1 ± 630.6

Legend: Presented p < expresses a statistical significance of tested biochemical parameters of patient groups compared to control group values.

- TxA₂ secretion increased to 240% when compared to control values (7,679.5 pg/ml) (p < 0.006) (Table 1).

In the treated HLP sub-group: patient with HLP and on treatment:

- HLP became normal during treatment with statins. Lipids levels were in the reference intervals (TC: 3,8 – 5,2 mmol/l , TG: 0,4 – 2,3 mmol/l).
- PGI₂ (6 keto-PG F_{1 alpha}) secretion decreased to 40% (1,515.8 mg/ml) compared to control values (p < 0.0001)
- TxA₂ (TxB₂) serum level increased upto 178% (5,714.5 mg/ml) (Table 1).

The alterations in PGI₂ secretion in all tested patient groups compared to the control group values were highly significant and reached 99.99% level of significance (p < 0,0001). Statistical significance in altered TxA₂ secretion (except the 2 subgroup of EH [EH & HLP]), did not reach the significance of PGI₂ .

DISCUSSION

Arterial hypertension and hypercholesterolemia represent two cardinal risk factors for the development of vascular diseases. They lead to precocious development of atherosclerosis and angio-organic ischemic syndromes (ischemic heart disease, ischemic cerebrovascular disease, peripheral vaso-occlusive disease) and shorten the life span of those individuals who have arterial hypertension or hyperlipoproteinemia or a combination of both.

It is a known fact that patients with arterial hypertension have hyperlipoproteinemia and increased serum LDL concentration more frequently than normotensive subjects^[1] and vice versa. Combination of more risk factors in the same person cause an increased risk of atherosclerosis development that is directly proportional (Table 1) to the number of risk factors^[17].

Table 2

TxA₂- secretion in patients groups is directly proportional to the increase in LDLserum concentration

	LDLcholesterol (mmol/l)	TxA ₂ (pg/ml)
Control group	2.45	3 201.1 (basal secretion)
Treated HLP	2.65	5 714.5
EH	2.86	5 775.4
HLP	3.65	7 679.5
EH & HLP	4.27	13 079.9

In the present study it was confirmed that increased serum LDL in patients with hyper-beta lipoproteinemia suppresses endothelial secretion of PGI₂ and stimulates the TxA₂ secretion. Stimulation of TxA₂ secretion by LDL is directly proportional to the LDL level in serum (Table 2) and this finding was confirmed in all tested groups of patients and/or volunteers. Hence the hypothesis of active role of prostanoids in the pathogenesis of arterial hypertension was proven. Long-lasting hyperbeta-lipoproteinemia causes alterations in the endothelial secretion of prostanoids and causes the systemic changes in the whole vessel bed^[18]. Subsequent vasoconstriction with increased peripheral vessel resistance can lead to the structural remodeling of the vessel wall and to the development of arterial hypertension.

The most significant alteration in prostanoid secretions were found in both subgroups of EH. In the EH subgroup (EH & HLP), there was a three-fold increase of TxA₂ -secretion up to 13,079.9 pg/ml when compared to those results of control group (p < 0.0001). In this case a multiplication effect of both risk factors (arterial hypertension plus HLP) on endothelial TxA₂-secretion was confirmed. In the EH subgroup with isolated arterial hypertension (EH), the endothelial secretion of PGI₂ was 580,5 pg/ml, i.e., 15% when compared to the

control group values (3,793.8 pg/ml) ($p < 0.0001$). From these findings it can be concluded that in patients with essential hypertension (or isolated arterial hypertension) the additive patho-mechanisms exist, which represent a serious impairment in the synthesis of endothelial protective factor. These patho-mechanisms have a greater deleterious effect on the integrity of the vessel wall than hyperbeta-lipoproteinemia alone. The changes in prostanoid secretion, with injury to vessel wall due to isolated arterial hypertension are more dangerous than isolated hyperlipoproteinemia.

While on treatment, the TxA_2 -secretion was restored to normal in only 33% (in 12 patients) and PGI_2 in only 15% (in six patients) of patients with hyperlipoproteinemia. In spite of treatment, the remaining patients remained at higher risk of precocious development of atherosclerosis.

The above findings highlight the fact that further research is required to find drugs that normalise endothelial dysfunction in patients treated for hyperlipoproteinemia^[19].

REFERENCES

1. Esler M. Hyperadrenergic and 'labile' Hypertension in Textbook of Hypertension. Ed. JD Swales, Blackwell Scientific Publications, Oxford 1994. p. 741-749.
2. Creager MA, Cooke JP, Mendelsohn ME, Gallagher SJ, Coleman SM, Loscalzo J., Dzau VJ. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest* 1990; 86:228-234.
3. Drexler H. Endothelial dysfunction: Clinical implications. *Prog Cardiovasc Dis* 1997; 39:287-324.
4. Oravec S, Demuth K, Myara I, Hornych A. Vplyv plazmatických lipoproteínov na sekréciu prostanoidov endotelovou bunkou 'in vitro' (Effect of plasma lipoproteins on endothelial prostanoid secretion *in vitro*). *Bratisl lek Listy* 1998; 98:19-21.
5. Oravec S, Demuth K, Myara I, Hornych A, Balazovjeh. Aterogénne a antiaterogénne lipoproteíny plazmy modulujú sekréciu prostanoidov endotelovou bunkou 'in vitro'. (Atherogenic and antiatherogenic lipoproteins of human plasma modulate the prostanoid secretion by endothelium *in vitro*). *Bratisl lek Listy* 1998; 99:525-530.
6. Weisser B, Locher R, DeGraaf J, Moser R, Sachinidis A, Vertter W. Low density lipoprotein subfractions increase thromboxane formation in endothelial cells. *Biochem Biophys Res Commun* 1993; 192:1245-1250.
7. Lüscher TF, Vanhoutte PM. Modulator of cardiovascular function. CRC Press, Boca Raton Florida, 1990. p. 1-215.
8. Lüscher TF, Boulanger CM, Dohi Y, Yang Z. Endothelium-derived contracting factors. *Hypertension* 1992; 19:117-130.
9. Hornych A. The deficit of prostacyclin in the pathogenesis of hypertension. *Cor Vasa* 1991; 33:492-505.
10. Trieu VN, Uckun FM. Male-associated hypertension in LDL-R-deficient mice. *Biochem Biophys Res Commun* 1998; 247:277-279.
11. Abetel G, Poget PN, Bannabry JP. Hypotensive effect of an inhibitor of cholesterol synthesis (fluvastatin). A pilot study. *Schweiz Med Wchschr* 1998; 128:272-277.
12. Glorioso N, Troffa C, Filighedou F, et al. Effect of the HMG-CoA reductase inhibitors on blood pressure in patients with essential hypertension and primary hypercholesterolemia. *Hypertension* 1999; 34:1281-1286.
13. Tamai O, Matsuoka H, Itabe H, Wada Y, Kohno K, Imaizumi T. Single LDL apheresis improves endothelium dependent vasodilatation in hypercholesterolemic humans. *Circulation* 1997; 95:76-82.
14. Oravec S, Demuth K, Myara I, Hornych A. Účinok lipoproteínov vysokej hustoty (HDL) a ich subfrakcií HDL2 a HDL3 na sekréciu prostanoidov endotelovou bunkou 'in vitro'. (The effect of high density lipoproteins (HDL) and their HDL2 and HDL3 subfractions on endothelial prostanoid secretion *in vitro*). *Vnitni Lekařství* 1997; 43:491-496.
15. Oravec S, Demuth K, Myara I, Hornych A. The effect of high density lipoprotein subfractions on endothelial eicosinoid secretion. *Thromb Res* 1998; 92:65-71.
16. Oravec S, Ronda N, Carayon A, Milliez J, Kazatchkine MD, Hornych A. Normal human polyspecific immunoglobulin G (intravenous immunoglobulin) modulates endothelial cell function *in vitro*. *Nephrol Dial Transplant* 1995; 10:796-800.
17. Rifai N, Warnick GR. Methods for clinical and laboratory measurement of lipid and lipoprotein risk factors, AACC Press, Washington DC. 1991.
18. Lüscher TF. Local relaxant and constricting factors in the vessel wall. in: Textbook of Hypertension, ed.: J.D.Swales, Blackwell Scientific Publications, Oxford 1994. p. 145-159.
19. Gavorník P. Vaskulárna endotelová dysfunkcia – etiopatogenéza, základné diagnostické metódy a liečebné možnosti (Endothelial dysfunction ethio-pathogenesis, basic diagnostic methods and treatment). *Všeobecná angiológia* 2002; 2:59-66.