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Biochemical Markers of Endothelial Dysfunction in Patients with Endocrine and Essential Hypertension

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Summary

The aim of our study was to evaluate potential differences in the concentration of biochemical markers of endothelial dysfunction between essential hypertension, endocrine hypertension (pheochromocytoma, primary hyperaldosteronism) and control healthy group and to assess a potential relationship between these markers of endothelial dysfunction and vasopressor substances overproduced in endocrine hypertension. We have investigated 21 patients with moderate essential hypertension, 29 patients with primary hyperaldosteronism, 24 subjects with pheochromocytoma and 26 healthy volunteers. Following parameters of endothelial dysfunction were measured, von Willebrand factor (vWf), plasminogen activator (t-PA) and E-selectin (E-sel). Clinical blood pressure was measured according to the European Society of Hypertension recommendations. We found significantly higher levels of the von Willebrand factor in patients with essential hypertension in comparison with a control group (114±20 IU/dl vs 90±47 IU/dl; P=0.04) and patients with primary hyperaldosteronism (114±20 IU/dl vs 99±11 IU/dl; P=0.01). Patients with endocrine hypertension revealed increased levels of vWF compared to the control group, but these differences did not reach statistical significance. Levels of t-PA were increased in patients with pheochromocytoma in comparison with the control group (4.6±1.9 ng/ml vs 3.4±0.9 ng/ml; P=0.01) and with primary hyperaldosteronism (4.6±1.9 ng/ml vs 3.4±1.1 ng/ml; P<0.01). In case of E-selectin we found lower levels in patients with pheochromocytoma in comparison with other groups, but they differed significantly only with primary hyperaldosteronism (40.2±15.0 ng/ml vs 51.3±23.0 ng/ml; P=0.05). Our study did not reveal any convincing evidence of differences in the levels of biochemical markers of endothelial dysfunction between essential and endocrine hypertension. No correlation between the biochemical markers of endothelial dysfunction and vasopressor substances activated in endocrine hypertension was found.

Key words

Essential hypertension • Pheochromocytoma • Primary hyperaldosteronism • Endothelial dysfunction • Adhesive molecules

Introduction

Endothelial dysfunction is considered to be a preclinical stage of atherosclerosis. It is characterized by an imbalance between humoral and cellular factors which influence the function and structure of the endothelial

wall. Traditional risk factors such as arterial hypertension, dyslipidemia, hyperglycemia, smoking, age and obesity may initiate endothelial damage (Libby *et al.* 2002).

Impairment of endothelial barrier is the initial step in the atherosclerotic process and is supposed to allow an increased penetration of lipoprotein particles into the subendothelial space, where LDL particles are oxidized. Presence of these particles can induce production of various chemotactic factors which attract monocytes and other immunocompetitive cells from circulation. This process may initiate a low-grade chronic inflammatory process (Fialová 1995).

Recent advances in science have established a crucial role for inflammation in mediating all stages of atherosclerosis. Endothelial dysfunction is associated with increased basic levels of glycoprotein adhesion molecules of the selectin group, such as E-selectin (Hillis 2003, Huo and Ley 2001, Ballantyne and Entman. 2002). Endothelial stress and/or damage is also closely associated with increased levels of the von Willebrand factor, the tissue activator of plasminogen and the inhibitor of plasminogen activator and C-reactive protein (Lee 2002, Van der Meer *et al.* 2002, Sung *et al.* 2003, Cao *et al.* 2003). These markers of inflammation lead to a higher risk of acute coronary syndromes (Macias *et al.* 2003, Ridker *et al.* 1997)).

Endothelial dysfunction may also lead to an increased peripheral resistance and high blood pressure. No data are available with respect to the biochemical markers of endothelial dysfunction in endocrine hypertension. An uncontrollable release of catecholamines into circulation with subsequent hypertension and high blood pressure variability can participate in an earlier manifestation of atherosclerosis and induction of a more severe form of endothelial dysfunction (Touze *et al.* 1984, Zelinka *et al.* 2004, 2005). Primary hyperaldosteronism was studied as one of the most frequent types of uncontrollable endocrine hypertension with higher risk of fibroproliferative damage caused by aldosterone on the endothelial wall (Mahmud and Feely 2004).

The aim of this study was to investigate, whether prolonged overproduction of vasopressor substances in endocrine hypertension (pheochromocytoma, primary hyperaldosteronism) compared with essential hypertension and healthy controls may lead to an increased activation of biochemical markers of endothelial dysfunction. We also evaluated the potential relationship between the levels of biochemical markers of endothelial dysfunction with levels of blood pressure and the concentration of hormones overproduced in endocrine hypertension.

Subjects and Methods

We investigated four groups of middle-aged patients at our hypertension unit, in the period from October 2000 to December 2004. The groups were as follows:

1. Twenty-one patients with essential hypertension (EH), 16 women and 5 men.

2. Twenty-nine patients with primary hyperaldosteronism (PA), 16 women and 13 men, 13 patients with aldosterone producing adenoma and 16 patients with idiopathic hyperaldosteronism. All patients were examined before specific treatment (surgical or medical).

3. Twenty-four patients with pheochromocytoma (P), 10 women and 14 men. All were examined before adrenalectomy. 22 patients had a benign and two patients had a malign form of pheochromocytoma.

4. Twenty-six healthy subjects as control group, 19 women and 7 men.

All patients were examined during hospitalization, after three weeks of discontinuation of antihypertensive therapy. Only alfa blockers (doxazosin) were admitted. The subjects were not receiving any hypolipidemic therapy nor any oral contraceptive pills or hormone replacement therapy. Patients with coronary heart disease, stroke, diabetes mellitus and dyslipidemia were excluded from the study except patients with pheochromocytoma, in which diabetes mellitus is a part of the disease. Height and weight were measured and body mass index (BMI) calculated and expressed as kg/m^2. Clinical blood presure (BP) was assessed according to the guidelines of European Hypertension Society. Peripheral blood samples were withdrawn after an overnight fast from a cubital vein at 08:00 h into Vacutainer™ vacuum test tubes (Becton Dickinson, Vacutainer Systems, Rutherford, New Jersey, USA). All patients underwent basic laboratory screening such as lipidogram, renal functions and basal glycemia by standard methods.

The diagnosis of primary hyperaldosteronism was made on the basis of usual hormonal procedures (aldosterone, plasma renin activity and aldosterone/renin ratio, suppression test by saline infusion) and

Table 1. Subject characteristics

	Essential hypertension	Primary aldosteronism	Pheochromocytoma	Controls
Total (number)	21	29	24	26
Gender (woman/man)	(16/5)	(16/13)	(10/14)	(19/7)
Age (years)	47 ± 13	50 ± 8	46 ± 14	47 ± 7
Systolic blood pressure (mmHg)	163 ± 20 *	162 ± 19 *	139 ± 23**	121 ± 9
Diastolic blood pressure (mmHg)	103 ± 12 *	102 ± 13 *	85 ± 17**	76 ± 10
Total cholesterol (mmol/l)	5.3 ± 1.0	5.0 ± 0.9	5.5 ± 1.0	5.1 ± 0.6
HDL cholesterol (mmol/l)	1.3 ± 0.3 ***	1.3 ± 0.3 ***	1.6 ± 0.3	1.6 ± 0.3
LDL cholesterol (mmol/l)	3.3 ± 0.8	3.1 ± 0.8	3.3 ± 1.0	3.0 ± 0.6
Triacylglycerol (mmol/l)	1.6 ± 0.7 $^{+}$	1.5 ± 0.7	1.5 ± 0.8	1.2 ± 0.7
Body mass index (kg/m^2)	29 ± 5 *	29 ± 5 *	24 ± 5	25 ± 4
Glycemia (mmol/l)	5.3 ± 0.8	5.0 ± 0.5	7.2 ± 2.0 $^{++}$	5.1 ± 0.6
Creatinin (umol/l)	88 ± 16	90 ± 17	85 ± 15	86 ± 9

P<0.001 EH vs PHEO, EH vs CO, PH vs PHEO and PH vs CO, ** P=0.002 SBP PHEO vs CO and P=0.04 DBP PHEO vs CO, *** P<0.01 EH vs PHEO, EH vs CO and P<0.001 PH vs PHEO and PH vs CO, $^{+}$ P=0.05 EH vs CO, $^{++}$ P<0.001 PHEO vs PH, EH and CO.

morphological methods including adrenal CT scan and adrenal venous sampling.

The diagnosis of pheochromocytoma was made on the basis of urine and plasma catecholamines (HPLC), adrenal CT scan and histological examination.

For soluble adhesion molecules serum samples were stored for a maximum of one month at –80 °C until assay. Blood serum was used to determine the levels of soluble adhesion molecules $_{s}$E-selectin (ELISA using human soluble E-selectin™ kits, R&D Systems Europe, Abingdon, United Kingdom) with precisions within an intraassay variability 5.0 % and interassay CV 8.8 %. Citrate plasma was used to determine the levels of t-PA Ag (ELISA tests using Coaliza t-PA™; Chromogenix AB, Mölndal, Sweden) and von Willebrand factor (Elisa test using Asserachrom™ vWF; Diagnostica STAGO, Asnéres, France).

According to the manufacturer, the expected values are 1-12 ng/ml for t-PA Ag and 111±45 % for vWF Ag in normal plasma. Precisions of the tests were within an assay variability 7.0 % and inter assay variability 4.8 % for t-PA Ag and variability 5.82 % and interassay variability 7.21 % for vWF Ag The sensitivities of the tests were below 0.5 ng/ml for t-PA Ag and 2 % for vWF Ag.

Statistical analysis

Statistical analysis was preformed using Statistica for Windows ver. 6.1 statistical software. Comparisons between the groups of patients were performed by Student´s T-test.

Results

Subject characteristics are summarized in Table 1. We did not find any differences in age and in concentrations of creatinine and total cholesterol. In basic laboratory tests we found significantly higher HDL in patients with pheochromocytoma and the control group as compared to patients with primary hyperaldosteronism and essential hypertension. Levels of triglycerides were significantly higher in patients with essential hypertension as compared to the control group. As expected, patients with pheochromocytoma showed significant increase in basal levels of glycemia in contrast to other forms of hypertension and the control group. In patients with essential hypertension and primary hyperaldosteronism a higher body mass index was found in comparison with patients with pheochromocytoma and the control group.

Levels of systolic and diastolic clinical blood pressure in patients with essential hypertension and primary hyperaldosteronism were significantly higher in comparison to the other two groups.

Concentrations of biochemical markers of endothelial dysfunction are shown in Table 2.

In these markers we found significantly higher

Table 2. Concentrations of biochemical markers of endothelial dysfunction.

	Essential hypertension	Primary aldosteronism	Pheochromocytoma	Controls
vWF (IU/dl)	114 ± 20*	99 ± 19	111 ± 32	90 ± 47
t-PA (ng/ml)	3.9 ± 1.6	3.4 ± 1.1	4.6 ± 1.9 **	3.4 ± 0.9
E-selectin (ng/ml)	45.1 ± 19.3	51.3 ± 23.0	40.2 ± 15.0 ***	51.5 ± 25.2

* P=0.01 EH vs PH and P= 0.04 EH vs CO, ** P=0.01 PHEO vs CO and P< 0.01 PH vs PHEO, *** P=0.05 PH vs PHEO

levels of the von Willebrand factor in patients with essential hypertension in comparison with the control group and with primary hyperaldosteronism. Patients with endocrine hypertension had increased levels of vWF compared to the control group, but these differences did not reach statistical significance. Levels of t-PA were increased in patients with pheochomocytoma in comparison with the control group and with primary hyperaldosteronism. Surprisingly, in case of E-selectin we found lower levels in patients with pheochromocytoma, particularly in comparison to patients with primary hyperaldosteronism.

Changes in levels of other biochemical markers of ED did not reach statistical significance.

We did not find any correlation between markers of endothelial dysfunction and hormonal levels (plasma aldosterone and catecholamines) (data not shown).

Discussion

Clinical as well as experimental studies showed endothelial dysfunction to be frequently associated with essential hypertension (Contreras *et al.* 2000, Haller *et al.* 2002). Patients with mild essential hypertension but without other conventional risk factors of atherosclerosis showed increased levels of endothelin-1, ICAM-1 and vWF (Hlubocká *et al.* 2002). However, severe essential hypertension with clinical manifestation of atherosclerosis was documented to be associated with higher levels of E-selectin and C-reactive protein (Huo and Levy 2001, De Caterina *et al.* 2001, Ballantyne and Entman 2002, Hillis 2003).

Von Willebrand factor is a glycoprotein produced by endothelial cells and megakaryocytes. Increased levels of circulating vWF may also have a prognostic value in cardiovascular mortality and morbidity (Lipp and Blann 1997). As expected from previous studies, in our study plasma vWF levels were higher in patients with moderate essential hypertension compared to the controls. In patients with endocrine hypertension increased levels of vWF were observed, but these differences did not reach statistical significance.

E-selectin is an adhesion molecule expressed by activated endothelial cells, and is thus considered to be a marker of endothelial activation. Patients with diabetes were reported to have significantly higher levels of this marker (Guerci *et al.* 2001). Surprisingly, we found lowest levels of E-selectin in patients with pheochromocytoma. This observation might be partly explained by the mild character of diabetes mellitus and relatively lower levels of blood pressure in pheochromocytoma, compared to other hypertensive patients. Bluher *et al.* (2002) suggested that E-selectin concentrations in type 2 diabetes might be related to insulin resistance and hyperinsulinemia. The lower levels of E-selectin might be due to the protective anti-inflammatory effect of HDL cholesterol (Navab *et al.* 2005) in endothelial dysfunction. Our examined patients with pheochromocytoma had significantly higher levels of HDL in comparison with other hypertensive groups.

Tissue plasminogen activator is produced in the endothelium. In case of t-PA we found highest levels in patients with pheochromocytoma, significantly higher in comparison with primary hyperaldosteronism and the control group. This might be explained by the stimulative effects of catecholamines (Makris *et al.* 1997) and by higher blood pressure variability in pheochromocytoma as we previously described (Zelinka *et al.* 2004, 2005).

Rizzoni *et al.* (1998) studied endothelial dysfunction in patients with primary and secondary hypertension such as primary hyperaldosteronism, pheochromocytoma and renovascular hypertension. They did not observe significant differences between groups in acetylcholine-induced vasodilatation and media/lumen ratio in small subcutaneus resistance arteries. Our study points to a similar conclusion that endothelial dysfunction

seems to be independent of the etiology of hypertension.

In conclusion we did not find any convincing differences in the levels of biochemical markers of endothelial dysfunction between patients with endocrine and essential hypertension except for the tendency towards elevated levels of vWF in patients with essential hypertension and higher levels of t-PA concentrations in patients with pheochromocytoma. Prolonged activation of catecholamines and mineralocorticoids in the circulation of patients with endocrine hypertension did not appear to lead to any marked signs of potent biochemical markers of endothelial dysfunction. This notion is further supported by the lack of correlation between above-mentioned vasopressor factors and markers of endothelial dysfunction.

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