Elevated plasma endothelin-1 levels as an additional risk factor in non-obese essential hypertensive patients with metabolic abnormalities

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Summary Circulating endothelin-1 concentration was evaluated in 93 lean patients with essential hypertension, of whom 16 had impaired glucose tolerance and hyperlipidaemia, 25 had impaired glucose tolerance, 28 had hyperlipidaemia and 24 had no metabolic abnormalities; we also studied 22 control subjects. All groups were age- and sex-matched. Plasma endothelin-1 levels were higher ($p < 0.05$) in hypertensive patients with impaired glucose tolerance and hyperlipidaemia than in the remaining groups, and

were directly correlated with fasting insulin levels ($r = 0.506$, $p = 0.045$). Therefore, circulating endothelin-1 concentrations are elevated in hypertensive patients with a high-risk profile due to the presence of metabolic abnormalities, and might favour the development of vascular damage. [Diabetologia (1997) 40: 100–102]

Keywords Essential hypertension, endothelium, endothelins, cardiovascular risk.

Insulin resistance and hyperinsulinaemia have been suggested to promote the onset of hypertension and metabolic abnormalities, i.e. reduced HDL-cholesterol levels, increased LDL-cholesterol and VLDL-cholesterol levels, and impaired glucose tolerance [1].

In this regard, insulin stimulates endothelin-1 (ET-1) secretion from human vascular endothelial cells [2], thus suggesting that circulating peptide levels might be elevated in hyperinsulinaemic humans. Accordingly, we described raised concentrations of plasma ET-1 in plasma from hyperinsulinaemic non-insulin-dependent diabetic [2] and obese patients [3].

Following these results, we studied whether an elevated concentration of circulating ET-1 was present in lean, non-diabetic patients manifesting the clustering of metabolic abnormalities associated with

hypertension [1], excluding any influential factor which is known to elevate per se the rate of ET-1 release into the bloodstream, such as diabetes mellitus [2], obesity [3], smoking [4], and atherosclerosis [5].

Subjects, materials and methods

Four subgroups were selected from consecutive essential hypertensive patients attending the out-patient clinics of our institutions. The first (Group I) consisted of hypertensive patients with impaired glucose tolerance [6] and elevated levels of serum total cholesterol (> 5.20 mmol/l) and triglycerides (> 1.8 mmol/l) ($n = 16$, 10 males and 6 females); the second (Group II) consisted of hypertensive patients with impaired glucose tolerance alone ($n = 25$, 15 males and 10 females); the third (Group III) consisted of hypertensive patients with hypercholesterolaemia and hypertriglyceridaemia but normal glucose tolerance ($n = 28$, 15 males and 13 females); the final (Group IV) consisted of hypertensive patients without metabolic abnormalities ($n = 24$, 13 males and 11 females). Clinical and laboratory tests excluded pregnant women, diabetic patients, and patients who suffered from secondary hypertension or concomitant diseases. Obese patients (body mass index > 26 kg/m²), smokers, and patients with a diastolic blood pressure less than 95 mm Hg or more than 114 mm Hg (on 8 different visits performed twice a week for 4 weeks, in a sitting

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Abbreviations: HDL, High density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; ET-1, endothelin-1.

Table 1. Characteristics of the study population

Variable	Groups				
	I	II	III	IV	V
Number	16	25	28	24	22
Age (years)	50.3 ± 4.2	50.1 ± 2.8	51.6 ± 3.9	51.2 ± 3.3	49.7 ± 3.1
Sex (male/female)	10/6	15/10	15/13	13/11	12/10
Body mass index (kg/m ²)	24.41 ± 1.01	23.87 ± 1.19	24.36 ± 0.85	24.25 ± 1.23	23.82 ± 0.95
Systolic blood pressure (mm Hg)	162.9 ± 8.4	159.8 ± 9.6	162.7 ± 9.5	161.4 ± 8.2	127.8 ± 4.6 ^a
Diastolic blood pressure (mm Hg)	103.4 ± 6.2	102.8 ± 4.9	102.5 ± 5.5	101.4 ± 5.6	86.4 ± 3.1 ^a
Heart rate (beats/min)	77.8 ± 3.2	74.1 ± 8.2	75.2 ± 4.6	75.1 ± 6.4	72.8 ± 2.1
Blood urea nitrogen (mmol/l)	5.79 ± 0.69	5.23 ± 0.97	5.27 ± 0.45	5.45 ± 0.36	5.21 ± 0.81
Serum creatinine (μmol/l)	71.7 ± 9.2	70.1 ± 10.4	72.5 ± 11.5	74.2 ± 10.7	72.3 ± 9.3
Serum total cholesterol (mmol/l)	6.52 ± 0.25 ^c	5.03 ± 0.08 ^d	6.67 ± 0.31 ^c	5.01 ± 0.09 ^d	4.71 ± 0.08
HDL-cholesterol (mmol/l)	0.53 ± 0.09	1.60 ± 0.18	0.74 ± 0.12	1.84 ± 0.16 ^b	1.98 ± 0.16 ^b
LDL-cholesterol (mmol/l)	5.57 ± 0.51 ^c	3.19 ± 0.38 ^d	5.51 ± 0.43 ^c	2.91 ± 0.37 ^d	2.51 ± 0.22
VLDL-cholesterol (mmol/l)	0.41 ± 0.08 ^c	0.27 ± 0.07 ^d	0.39 ± 0.04 ^c	0.25 ± 0.05	0.21 ± 0.05
Serum triglycerides (mmol/l)	2.01 ± 0.24	1.26 ± 0.12 ^{d,e}	2.12 ± 0.19 ^c	1.12 ± 0.16	1.02 ± 0.09

Group I, hypertensive patients with impaired glucose tolerance, serum total cholesterol > 5.20 mmol/l and triglycerides > 1.8 mmol/l.

Group II, hypertensive patients with impaired glucose tolerance alone

Group III, hypertensive patients with hypercholesterolaemia and hypertriglyceridaemia but normal glucose tolerance

Group IV, hypertensive patients with no metabolic abnormalities

Group V, control subjects.

^a $p < 0.05$ vs other groups; ^b $p < 0.04$ vs other groups; ^c $p < 0.05$ vs groups II, IV, V; ^d $p < 0.05$ vs group V; ^e $p < 0.05$ vs group IV

position) were also excluded. No patient was under antihypertensive treatment. Atherosclerotic lesions of the leg and limb vessels were excluded by clinical and ultrasound studies. A group of 22 healthy volunteers served as a control group (Group V).

After informed consent, patients and control subjects were asked to consume a weight maintaining diet for 1 week. Then, blood samples for ET-1 and other laboratory assays were drawn from an antecubital vein, 1 h after an intravenous catheter was inserted [2, 3].

Plasma ET-1 was measured by HPLC followed by radioimmunoassay (Peninsula Laboratories, Belmont, Calif., USA) [2, 3]. For this purpose, 2.5 ml of plasma was passed through C18 columns (Amersham, Amersham, Buckinghamshire, UK), in duplicate, dried under vacuum and then reconstituted in 500 μl starting HPLC solution. The two eluates were then pooled and eluted by reverse-phase HPLC. Fractions corresponding to the elution position of human ET-1 (Peninsula) were collected at 1-min intervals and evaporated before reconstitution in 500 μl assay buffer (50 mmol/l phosphate buffer, pH 7.4, containing 0.9 % NaCl, 0.05 % NaN₃, and 0.4 % bovine albumin). ET-1 immunoreactivity was then assayed, in duplicate, by RIA. The limit of detection of our method was 0.2 pg ET-1. Plasma ET-1 concentrations were calculated by semilog mathematical model. Inter- and intra-assay variations were less than 10 %. Cross-reactivity of the ET-1 antibody with ET-2 and ET-3 was less than 7 %, according to the supplier. Plasma insulin was assayed by radioimmunoassay (Ares Serono, Milan, Italy). Total serum cholesterol, triglyceride and HDL-cholesterol levels were measured by enzymatic methods, while VLDL- and LDL-cholesterol concentrations were calculated by the Friedewald formula [7].

Statistical analysis

Statistical tests were analysis of variance followed by the Bonferroni test, analysis of co-variance, Pearson's coefficient and, for descriptive parameters, chi-square analysis. Statistical significance was considered as a p value < 0.05. Unless otherwise stated, data are presented as mean ± SD.

Results

All subgroups were matched for age, sex, and body mass index (Table 1). Renal function and blood pressure levels were also similar among the four subgroups.

Plasma ET-1 was at the highest concentration in Group I, e.g. in patients manifesting both carbohydrate and lipid abnormalities, with a statistically significant difference vs the other groups ($p < 0.05$) (Fig. 1, A). Circulating ET-1 levels were also higher ($p < 0.05$) in Groups II and III than in Group IV, e.g. in patients with essential hypertension without metabolic abnormalities, and control subjects.

Interestingly, the behaviour of ET-1 was paralleled by that of fasting insulin, although there was no significant difference in plasma insulin concentrations between Groups I and II (Fig. 1, B). Plasma ET-1 levels correlated with fasting insulin concentrations in Group I ($r = 0.506$, $p = 0.045$). A similar correlation was found in Group II ($r = 0.496$, $p = 0.039$), but not in Groups III and IV. No other significant correlations were found. In particular, analysis of

Fig 1 A, B. Individual plasma endothelin-1 levels (ET-1, **A**) in hypertensive patients without metabolic abnormalities ($n = 24$, ▨), with hyperlipidaemia ($n = 28$, ▩), with impaired glucose tolerance ($n = 25$, ▤), with metabolic abnormalities as in the last two cases ($n = 16$, ■), and normal subjects ($n = 22$, □). Horizontal bars indicate the mean, vertical bars indicate SD. (**B**) Fasting insulin levels (mean $\pm$ SD) in the same subgroups.

* $p < 0.05$ vs the other hypertensive groups and control subjects; § $p < 0.05$ vs control subjects; ◇ $p < 0.05$ vs hypertensive patients, hypertensive patients with hyperlipidaemia, and control subjects

co-variance showed no significant correlations among plasma ET-1 levels and other continuous variables such as total cholesterol, cholesterol subfractions, etc.

Discussion

Our data indicate that hypertensive patients manifesting the cluster of different risk factors also display increased levels of circulating ET-1. In this regard, insulin is a well-known promoter of ET-1 release from human vascular endothelial cells in vitro [2], and a direct correlation between insulin and ET-1 levels has been already demonstrated in hyperinsulinaemic obese patients [3], regardless of the presence or the

absence of hypertension. Thus, the most likely explanation for our findings is related to the presence of marked hyperinsulinaemia in patients of both Groups I and II.

With regard to the clinical implications of our findings, the direct correlation between insulin and ET-1 levels in patients of Groups I and II suggests that an insulin-related ET-1 secretion could contribute to the atherogenic actions of insulin [1]. On the other hand, the increased levels of ET-1 in plasma might further increase insulin resistance by reducing flow-dependent insulin sensitivity [8], thereby contributing to continuous overstimulation of insulin secretion.

With regard to possible study limitations, confounding factors such as diabetes [2], obesity [3], smoking [4], atherosclerosis [5], antihypertensive treatment, and concomitant diseases were carefully excluded during patient selection.

In conclusion, circulating ET-1 levels are increased in hypertensive patients with impaired glucose tolerance, particularly when hyperlipidaemia is also present. The role of ET-1 as a possible independent risk factor for the development of vascular lesions and contributor to insulin resistance in hypertensives with metabolic abnormalities remains to be elucidated.

References

- DeFronzo RA, Ferrannini E (1991) Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerotic heart disease. *Diabetes Care* 14: 173-194
- Ferri C, Pittoni V, Piccoli A, et al. (1995) Insulin stimulates endothelin-1 secretion from human cultured endothelial cells and modulates its circulating levels in vivo. *J Clin Endocrinol Metab* 80: 829-835
- Ferri C, Bellini C, Desideri G, Baldoncini R, Santucci A, De Mattia G (1995) Plasma endothelin-1 levels in obese and non-obese hypertensive patients. *Diabetes* 44: 431-436
- Haak T, Jungmann E, Raab C, Usadel KH (1994) Elevated endothelin-1 levels after cigarette smoking. *Metabolism* 43: 267-269
- Lehrman A, Edwards BS, Hallet J, Heublein DM, Burnett JC Jr. (1991) Circulating endothelin immunoreactivity in advanced atherosclerosis. *N Eng J Med* 325: 997-1001
- ADA position statement (1989) Screening for diabetes. *Diabetes Care* 12: 588-589
- Friedewald WT, Levy R, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 18: 499-502
- Anderson EA, Mark AL (1993) The vasodilator action of insulin. Implications for the insulin hypothesis of hypertension. *Hypertension* 21: 136-141