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Endothelin-1 in hypertensive patients with ischemic heart disease

Carlo Moroni[1] · Stefano Tolone[1] · Francesco Bondanini[5] · Orazio Schillaci[4] · Cesare Affricano[2] · Rosario Cassone[1] · Achille Gaspardone[3] · Carlo Gaudio[1]

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Abstract
This study was aimed at evaluating whether transient dipyridamole-induced myocardial ischemia in hypertensive patients reflects on endothelin-1 plasma levels by comparing normotensives and hypertensives with or without stable angina. Endothelin-1 plasma levels were assessed in baseline conditions and after provocative stress test by dipyridamole. Four groups of ten age- and sex-matched subjects were retrospectively considered among patients referred for chest pain evaluation and submitted to high-dose Dipyridamole Echocardiographic-Scintigraphic combined test (DES). On the basis of DES results we considered: (1) control normotensives subjects; (2) essential hypertensives (for both groups negative result of DES); (3) essential hypertensives with stable angina; and (4) normotensives with stable angina (for both groups concordant DES detection of myocardial ischemia). Our data showed a marked post-DES increase of endothelin-1 plasma levels in hypertensives with stable angina (mean levels = 16.50 ± 4.19 pg/ml $p < 0.001$ vs. baseline = 9.05 ± 1.37 pg/ml) and a minor increase in stable angina pts (mean levels = 8.3 ± 1.75 pg/ml $p < 0.01$ vs. baseline = 6.74 ± 0.61 pg/ml) whereas non significant increase was observed both in control (mean levels = 5.09 ± 0.83 pg/ml p = n.s. vs. baseline = 4.91 ± 1.04 pg/ml) and hypertensives groups (mean levels = 6.34 ± 1.72 pg/ml p = n.s. vs. baseline = 5.95 ± 1.04 pg/ml). ET-1 involvement in hypertension-related ischemic heart disease patho-physiology appears to be considered.

Keywords Endothelin-1 · Myocardial ischemia · Hypertension · Ischemic heart disease

Introduction

Functional abnormalities in coronary microcirculation may contribute to myocardial ischemia both in the presence and in absence of epicardial obstructive coronary artery disease (CAD) [1]. Indeed, microvascular dysfunction distal to epicardial stenosis can significantly affect the severity and extent of myocardial ischemia [1–3]; on the other hand, a reduced coronary flow reserve has been demonstrated in patients with essential hypertension, despite the presence of angiographically normal coronary arteries and even in the absence of ventricular hypertrophy [4–6]. In this case, an impaired coronary endothelial function might be involved producing an increased microvascular vasoconstrictor activity or causing a reduced endothelium-dependent vasodilation in response to stress stimuli [6–9]. On this basis, endothelin-1 (ET-1), a widely studied potent vasoconstrictor peptide derived mainly from endothelial cells, might be involved in this abnormal response [10] as well as in other similar clinical conditions with impaired perfusion (cardiac transplantation [11], renal ischemia [12] and brain experimental ischemia [13]). However, discordant results have been reported when assessing ET-1 plasma levels in essential hypertensives and in subjects with ischemic heart disease, in baseline conditions [14] as well as during transient acute myocardial ischemia (provoked by physical or pharmacological stress-tests) [7, 15–18]. A previous work from our group suggested a potential involvement of ET-1 in the pathogenesis of myocardial ischemia in hypertensives with CAD since increased ET-1 plasma concentrations have been showed in hypertensives with chronic stable angina but

✉ Carlo Moroni
carlo.moroni@uniroma1.it

1 Cardiovascular Sciences Department, Sapienza University, Rome, Italy

2 Statistics, Sapienza University, Rome, Italy

3 U.O.C. di Cardiologia, S. Eugenio Hospital, Rome, Italy

4 Department of Biomedicine and Prevention, Tor Vergata University, Rome, Italy

5 Laboratory Medicine, S. Eugenio Hospital, Rome, Italy

not in hypertensives without CAD [10]. This finding is in keeping with the demonstration that ET-1 is detectable in the tunica media, and more precisely on VSMCs, of human arteries obtained ex vivo from patients with coronary artery disease and/or hypertension [19]. Moreover an increased tissue endothelin-1-like immunoreactivity has been found in atherosclerotic human coronary arteries [20], atherosclerotic plaques [21], as well as in active coronary atherosclerotic lesions in acute ischemic syndromes [22].

As both coronary endothelium and microvascular functions can be considered common targets of hypertension and coronary atherosclerosis, we undertook a study aimed at evaluating ET-1 changes during dipyridamole-induced myocardial ischemia in hypertensive subjects with ischemic heart disease.

Methods

Study populations

The study population included 40 sex and age-matched stable patients referred to our Institution for typical chest pain evaluation (Table 1). All subjects were submitted, in pharmacological wash-out, to dipyridamole echocardiography combined to myocardial perfusion scintigraphy (DES), a combined perfusion/function imaging method, with high sensitivity and high specificity, as previously described [23]. All subjects underwent, after 7 days, pharmacological wash-out, dipyridamole echocardiographic test (dipyridamole i.v. infusion: up to 0.84 mg/kg in 10 min) combined with myocardial scintigraphy (Tc^{99m}-sestamibi: 20 mCi i.v.) [23].

Four groups of patients were retrospectively defined on the basis of DES results (flowchart of the study: Fig. 1):

1. Essential hypertensives with chest pain without instrumental evidence of myocardial ischemia (HYP: 5 males, mean age 49 ± 7 years);
2. Essential hypertensives with chronic stable angina, evidence of angiographically significant (> 50%) epicardial CAD and instrumental evidence of myocardial ischemia (HYP-CAD: 5 males, mean age 50 ± 6 years, CCS Class II–III);
3. Normotensive patients with chronic stable angina evidence of angiographically significant (> 50%) epicardial CAD and instrumental evidence of myocardial ischemia (CAD: 5 males, mean age 50 ± 6 years, CCS Class II–III);
4. Normotensive subjects with chest pain, but without instrumental evidence of myocardial ischemia, constituted the control group (CONTR: 5 Males, mean age 49 ± 7 years).

All DES-positive patients underwent, for clinical purposes, to coronary angiography. Only patients with concordant coronary angiography and DES results were considered as CAD/HYP-CAD patients.

Hypertension was considered for BP values > 140/90 mmHg in three different measurements in

Table 1 Clinical features of the study populations

	Controls	HYP	CAD	HYP-CAD	
Men	5/10	5/10	5/10	5/10	p = n.s
Age ± SD	49 ± 7	49 ± 7	50 ± 6	50 ± 6	p = n.s
Weight ± SD (kg)	67 ± 7	68 ± 7	66 ± 9	67 ± 8	p = n.s
Height ± SD (mt)	1.70 ± 0.07	1.71 ± 0.07	1.69 ± 0.05	1.71 ± 0.06	p = n.s
Mean systolic/diastolic blood pressure (mmHg)	133/80	162/95	136/84	165/94	p = n.s
Mean LV ejection fraction %	54	53	51	53	p = n.s
Mean LDL cholesterol (mg/dl)	107	111	111	109	p = n.s
Mean triglyceridemia (mg/dl)	120 ± 21	121 ± 18	125 ± 24	123 ± 24	p = n.s
Creatinine clearance (ml/min)	82	80	76	73	p = n.s
24 h-microalbuminuria (mg/die)	22	25	24	26	p = n.s
ARBs %	–	30	20	30	p = n.s
Beta-blockers %	–	20	30	40	p = n.s
ACE inhibitors %	–	40	20	45	p = n.s
Calcium channel block %	–	20	30	50	p = n.s
Diuretics %	–	20	10	15	p = n.s

No significant differences were observed among groups

CONTROLS control subjects, *HYP* hypertensive subjects, *CAD* stable angina patients, *HYP-CAD* hypertensives with stable angina

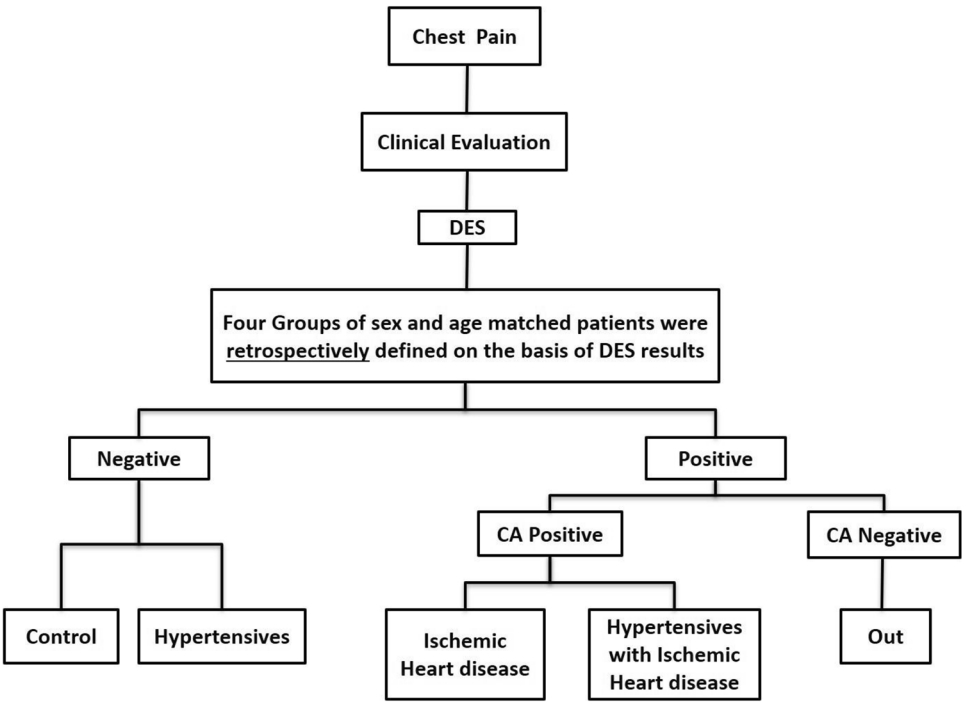


Fig. 1 Flowchart of the study population enrollment. *DES* dipyridamole echo test, *CA* coronary angiography

the supine position or ongoing antihypertensive treatment at the time of enrolment.

To exclude the presence of extravascular organ damage, or factors affecting ET-1 levels, all subjects included in the study had normal 24 h-microalbuminuria and creatinine clearance, echo-Doppler study of major arteries and echocardiographic left ventricular mass index. Patients with previous myocardial infarction and or coronary revascularization, type I and II diabetes, documented hypercholesterolemia (LDL cholesterol > 130 mg/dl), hypertriglyceridemia (> 150 mg/dl), and smoking habit were excluded from the study.

To assess possible variation of ET-1 plasma concentration, venous blood samples were collected before (baseline values: five samples within 1 h after at least 15 min of semi-supine resting) and immediately at the end (two blood samples within 5 min) of dipyridamole infusion, in ethylenediamine tetraacetic acid (EDTA)-containing tubes and centrifuged at 1800*g* for 20 min at 4 °C. Under these conditions, no decrease in measured ET-1 plasma concentration was observed. ET-1 was assayed using a radioimmunoassay system (Bachem AG, Switzerland).

Mean values were obtained for each subject and then for each group. Variance analysis, Student's *t* test and Aspen–Welch test were used to calculate differences vs. baseline values and between groups. Tests for paired data were used when appropriate. A p value < 0.01 was considered significant.

The present study was approved by the local Institutional Board and each participant gave written informed consent to the study.

Results

All patients with ischemic heart disease showed positive high-dose concordant DES results (transient left ventricular segmental dissinergy combined with a decrease in uptake in the same vascular territory) whereas those without ischemic heart disease showed concordant negative high-dose DES results. Individual ET-1 values before and at the end of dipyridamole infusion for the four groups are shown in Table 2 and Fig. 2; mean values for each group are reported in Fig. 3.

The variance analysis showed significant results when considering ET-1 baseline values of the four groups ($f = 8.55$, $p = 0.0013$), furtherly emphasized by DES ($f = 9.891$, $p = 0.0006$).

HYP-CAD patients presented the highest baseline concentration of ET-1 as compared to CAD, HYP and CONTR patients.

Furthermore, our data showed during dipyridamole-induced myocardial ischemia increase of endothelin-1 plasma levels in hypertensives with stable angina (mean levels = 16.50 ± 4.19 pg/ml $p < 0.001$ vs. baseline = 9.05 ± 1.37 pg/ml) and a minor increase in stable angina pts (mean levels = 8.3 ± 1.75 pg/ml

Table 2 Endothelin-1 plasma levels (pg/ml) for the four groups: baseline and after dipyridamole test values

Controls		HYP		CAD		HYP-CAD	
Baseline	Post-DES	Baseline	Post-DES	Baseline	Post-DES	Baseline	Post-DES
3.9	3.9	6.4	5.8	6.3	6.8	8.8	18.6
5.6	5.7	6.9	10	6.7	10	10.5	16.1
5	4.9	5.8	6	7.1	7.8	7.8	20.7
5.4	5.5	7.3	7.5	7.2	7.5	10.1	25
4	3.9	4.7	4.3	5.9	6.7	9.7	14
6.2	6.1	5.4	5.3	6.2	8	8.4	13
5.6	5.5	7	8	7.1	8	7.9	12.8
6	6.1	6.6	6.4	6.6	9.7	9.5	15.6
3	4.4	4.4	5.2	6.5	6.5	6.7	11.2
4.4	4.9	5	4.9	8	12	11.1	18

CONTROLS control subjects, *HYP* hypertensive subjects, *CAD* stable angina patients, *HYP-CAD* hypertensives with stable angina

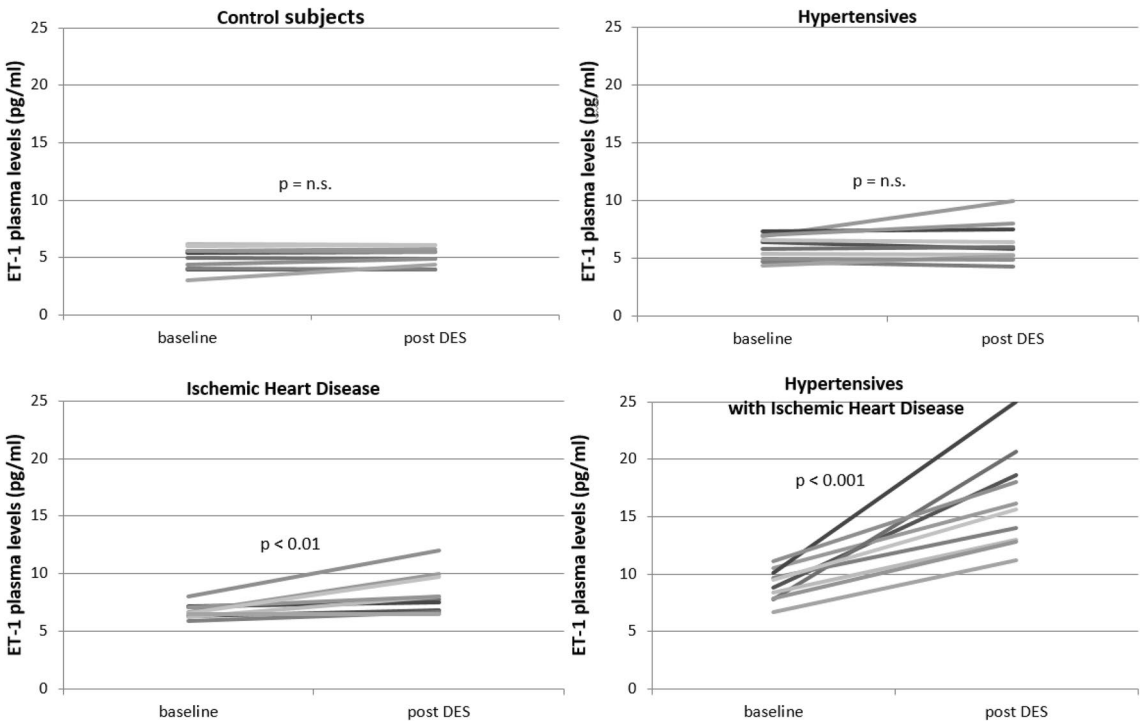


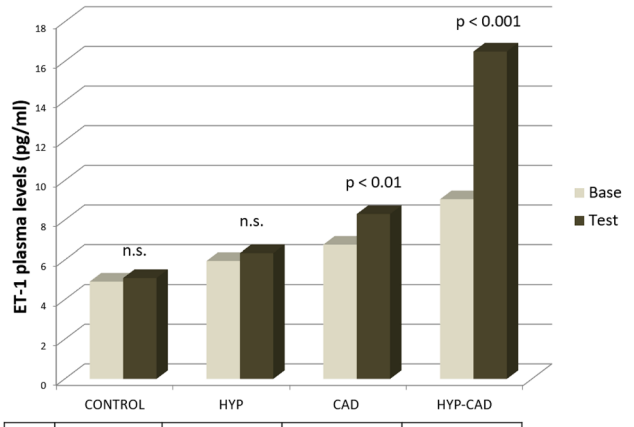
Fig. 2 Endothelin-1 plasma levels for each subject of the four groups: when compared to baseline values, no significant increases after dipyridamole echo-scintigraphic test (post-DEST) in control subjects and in hypertensives, whereas a moderate increase in stable angina patients and a marked increase in hypertensives with stable angina were showed

$p < 0.01$ vs. baseline $= 6.74 \pm 0.61$ pg/ml) whereas non significant increase was observed both in control (mean levels $= 5.09 \pm 0.83$ pg/ml $p =$ n.s. vs. baseline $= 4.91 \pm 1.04$ pg/ml) and hypertensives groups (mean levels $= 6.34 \pm 1.72$ pg/ml $p =$ n.s. vs. baseline $= 5.95 \pm 1.04$ pg/ml).

Of note, after dipyridamole, HYP-CAD presented the greatest increase of ET-1 ($p < 0.001$ vs. CAD; $p < 0.001$ vs. HYP and $p < 0.001$ vs. CONTR).

When comparing ET-1 values of patients with (CAD and HYP-CAD) vs. patients without ischemic heart disease (Controls and HYP), a significant difference was highlighted by variance analysis (baseline: $f = 17.556$, $p = 0.0002$; post-DES: $f = 16.83$, $p = 0.0002$).

Furthermore, when considering the effects of DES on ET-1 values, the variance analysis showed significant results only in patients with ischemic heart disease (CAD and HYP-CAD: $f = 9.968$, $p = 0.003$; Controls and HYP: $f = 0.466$,



Base	4,91 ± 1,04	5,95 ± 1,04	6,74 ± 0,61	9,05 ± 1,37
Test	5,09 ± 0,83	6,34 ± 1,72	8,3 ± 1,75	16,5 ± 4,19

Fig. 3 Baseline and after dipyridamole mean endothelin-1 plasma levels (± DS). *CONTROLS* control subjects, *HYP* hypertensive subjects, *CAD* stable angina patients, *HYP-CAD* hypertensives with stable angina

$p = 0.498$). Student's t test for paired data confirmed this observation (CAD and HYP-CAD: $p = 0.004$; Controls and HYP: $p = 0.133$).

Discussion

ET-1 is a 21-amino acid peptide with mitogenic, vasodilating and vasoconstricting properties. Increased levels of circulating ET-1 have been reported in patients with atherosclerosis and in the coronary circulation of patients with endothelial dysfunction and early atherosclerosis. ET-1 microcirculation response is mediated by two major receptors: ET-A and ET-B. ET-A receptors are located on vascular smooth muscle cells and mediate vasoconstriction; ET-B is located on both smooth muscle cells, where mediate vasoconstriction as well as ET-A, and on endothelial cells where they mediate vasodilation by releasing nitric oxide. Administration of atrasentan, an ET-A receptor antagonist, in a porcine hypercholesterolemic model has been shown to improve endothelial function and 6-month treatment with atrasentan of patients with multiple cardiovascular risk factors, nonobstructive coronary artery disease and coronary endothelial dysfunction, improved coronary endothelial function supporting the role of endogenous endothelin system in the regulation of endothelial function in early atherosclerosis. In patients with clinical coronary atherosclerosis, ET-1 evaluation during myocardial ischemia has yield conflicting results in literature [7, 15, 23]. Major reasons for discordant results may be represented by different methods of ET-1 assay, patient selection (age- and sex-matching), the presence of confounding factors (end-organ damage, hypercholesterolemia, smoking habits) and furthermore, different myocardial stressors.

The main finding of this study is that selected patients without confounding factors but with both hypertension and ischemic heart disease showed the highest increase of ET-1 plasma levels during transient myocardial ischemia. This finding is consistent with the hypothesis that myocardial ischemia in hypertensive patients might have peculiar features that are at least in part related to an abnormal increase of ET-1 plasma levels [10].

Indeed, ET-1 could represent a marker of a healing microvascular coronary endothelium, a target common to hypertension and ischemic heart disease. A role for ET-1 in microvascular function and structure control can be considered: a reduced vasodilator response of distal coronary vessels [4], an impaired endothelium-mediated vasodilatation [5] as well as a condition of ET-1 vascular overexpression or disreactivity to ET-1 (based on impaired NO availability) [9, 24, 25] can contribute to the patho-physiology of ischemic heart disease in hypertensive subjects.

Furthermore, in the presence of epicardial flow-limiting stenosis, ET-1 over-release, under induced myocardial ischemia, could represent an extreme compensatory mechanisms to maintain an adequate distal perfusion pressure in ischemic territories [4, 24]. However, in patients with hypertension and CAD, this feature, combined to reduced nitric oxide availability, might eventually lead to reduced distal perfusion thus worsening myocardial ischemia. In this contest, endogenous ET-1 might play an important role in the modulation of endothelial function and in the pathogenesis and progression of coronary endothelial dysfunction, a known predictor for cardiovascular disease. As coronary microcirculation plays a major role in modulating the severity and extension of myocardial ischemia, our results represent a rational basis for the use of ET-1 antagonist as a therapeutic option for hypertensive patients with nonobstructive coronary artery disease and endothelial dysfunction.

Limitations of the study

The small sample size due to the rigid exclusion criteria is an obvious limitation of the present study.

Furthermore, the use of a mature ET-1 assay instead of the C peptide, as well as the measure of ET-1 levels in peripheral venous blood instead in coronary veins have to be recognized as potential limitations of the study.

Conclusion

In conclusion, in the patho-physiological setting of hypertension-related ischemic heart disease, the highest baseline level of ET-1 and the maximal ET-1 rise during myocardial

ischemia, could represent a marker of healing coronary microvascular and endothelial functions and then, a role for ET-1 in mediating or potentiating myocardial ischemia should be considered.

According to Schiffrin [26], blocking the endothelin system could represent "a new therapeutic approach contributing to arrest of vascular damage, and, accordingly, improve prognosis".

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statements on human and animal rights The study has been approved by our Institutional Board, since respecting human rights.

Informed consent All patients gave informed consent to be enrolled in the study.

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