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Concentration of circulating plasma endothelin in patients with angina and normal coronary angiograms

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Abstract

***Background*—Some patients with angina pectoris and normal coronary arteriograms have reduced coronary flow reserve and abnormal endothelium dependent vasodilator responses. Endothelin-1 (ET-1), a potent vasoconstrictor, is an important modulator of microvascular function and may also have algogenic properties.**

***Method*—Plasma ET-1 was measured in peripheral venous blood in 40 patients (30 women) (mean (SD) age 56 (8) years) with angina and normal coronary arteriograms and 21 normal controls (17 women) (mean (SD) age 53 (7) years). Patients with systemic hypertension, left ventricular hypertrophy, or coronary spasm were excluded. Plasma ET-1 was measured using radioimmunoassay.**

***Results*—Thirty five patients had $\geqslant$ 1 mm ST segment depression during exercise. Left bundle branch block was present in four patients at rest and in one during exercise. Mean (SD) (range) concentration of ET-1 (pg/ml) was higher in patients than in controls (3·84 (1·25) (1.97–7·42) *v* 2·88 (0·71) (1·57–4·48) P < 0·0001). In patients with "high" (> control mean (one SD)) ET-1 concentrations (n = 23), the time to onset of chest pain during exercise was significantly shorter (6·21 (3·9) *v* 9·03 (3·9) min; p = 0·01) than in patients with "low" ET-1 concentrations. Of the five patients with left bundle branch block, four had plasma ET-1 concentration > 4·0 pg/ml.**

***Conclusion*—Plasma endothelin is raised in patients with angina and normal coronary arteriograms and is consistent with the demonstration of endothelial dysfunction in such patients. The association between "high" plasma ET-1 and an earlier onset of chest pain during exercise suggests that endothelin may also have a role in the genesis of chest pain in patients with normal coronary arteries.**

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Keywords: angina; endothelin; normal coronary arteriograms

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Up to 30% of patients undergoing coronary angiography for the investigation of angina pectoris have angiographically normal coronary arteries.[1] Several reports have suggested that some patients with chest pain and normal coronary arteriograms have reduced coronary flow reserve[2–5] and abnormal endothelium-dependent coronary vasodilatation.[6] Nevertheless, the interpretation of such findings remains controversial because of a lack of definitive evidence for myocardial ischaemia in most patients. Patients with syndrome X and microvascular angina have abnormal cardiac and somatic pain perception[7–9] and current hypotheses suggest that the paradox of typical angina in the absence of clinically detectable ischaemia may be explained by the release of algogenic substances within the myocardium that also disturb some aspects of normal cardiac function (microvascular blood flow, electrical activity etc.).[10 11]

Endothelin is a powerful vasoconstrictor and neuromodulator peptide, first isolated by Yanagisawa *et al*[12] in 1988 from porcine aortic endothelial cells. It is released from the endothelium in response to several stimuli, including shear stress, thrombin, and hypercholesterolaemia[13–15] and, although secretion is thought to be predominantly abluminal,[16] it can be detected in peripheral blood. The presence of increased plasma levels in acute myocardial infarction,[17] advanced atherosclerosis,[18] hypertension[19] and renal failure[20] suggests that raised plasma endothelin-1 (ET-1) may be a marker of endothelial abnormality and microvascular dysfunction. This together with the recent demonstration in an animal model[21–22] that endothelin may also stimulate nociceptors suggests that it could have a role in the genesis of symptoms and microcirculatory abnormalities in patients with chest pain and normal coronary arteriograms. The aim of this study was to compare the plasma concentration of ET-1 in patients with angina and normal coronary arteriograms with that in normal controls, and to determine its relationship to clinical and electrocardiographic variables.

Patients and methods

PATIENTS

Forty consecutive patients (30 women and l0 men of mean (SD) (range) age 56 (8) (41–72) years) referred between 1991 and 1993 for the assessment of chest pain associated with normal coronary arteriograms were prospectively studied. All had a history of typical angina pectoris and completely normal coronary

arteriography in the absence of valvular heart disease, diabetes mellitus, or systemic hypertension (defined as a blood pressure of $\geqslant$ 150/90 mm Hg on at least three occasions over a period of 3 months). Only patients with completely normal angiographic studies were included. No patient had evidence of left ventricular hypertrophy on the baseline electrocardiogram (ECG)[23] or by conventional cross sectional echocardiographic assessment[24 25] (septal thickness 0·93 (0·18) cm, posterior wall thickness 0·86 (0·10) cm, left ventricular mass 171·23 (46·47) g). No patient had a history of Prinzmetal's variant angina and coronary spasm was excluded in all patients using hyperventilation or ergometrine provocation tests, or both. None of the patients had a history of prior myocardial infarction. Six (three men and three women) were current smokers.

It was not a primary aim of this study to investigate the relation between female hormones and the concentration of plasma endothelin. However, oestrogen status was assessed in all female patients during routine clinical evaluation (mean (SD) oestradiol 168·3 (141·8) pmol/l).

Four patients had T wave flattening or inversion and three had ST segment depression (< 0·1 mV) on the baseline resting ECG. Oesophageal abnormalities were excluded in all patients using manometry, acid provocation tests, and ambulatory pH studies.

All patients underwent symptom limited treadmill exercise testing (supervised by the same observers both blinded to the plasma endothelin concentration) using the modified Bruce protocol. Calcium antagonists, β-blockers, and oral nitrates were discontinued for at least 5 half-lives before evaluation. Patients were allowed to continue with sublingual glyceryl trinitrate as required but no patient received nitrates in the 3 h before exercise testing. All patients had chest pain during exercise. The time of onset of anginal chest pain was noted in all patients and tests were discontinued in the presence of progressive symptoms (chest pain, dyspnoea or fatigue).

A positive electrocardiographic response was defined as horizontal or down sloping ST segment depression of $\geqslant$ 0·1 mV from baseline at 60 ms after the J point. Thirty five patients had positive tests. Four patients had left bundle branch block on the resting ECG, precluding interpretation of the exercise ECG and one developed left bundle branch block during exercise.

Single photon computerised thallium-201 emission tomography was performed in all patients using intravenous dipyridamole stress (0·56 mg/kg over 4 min) according to a previously published protocol.[26]

Controls

Twenty one normal controls (17 women and four men of mean (SD) (range) age 53 (7) (43–66) years) were studied. All had similar levels of physical activity and cardiovascular risk profiles to those of the patient group. None had a history of cardiovascular, renal or metabolic disease. Three were current smokers.

Endothelin assay

Venous blood was sampled from the antecubital vein after a period of 20 min supine rest. Blood was drawn into chilled citrate tubes on ice. Plasma was separated by centrifugation at 2500 g for 10 min at 4°C and stored at -20^{0}C until analysis. All samples were analysed within 2 months of venesection.

Plasma ET-1 was estimated using a radioimmunoassay (Nichols Institute, Diagnostics, Wychen, The Netherlands). Two ml plasma were acidified with 3 ml 4% acetic acid in a polystyrene tube mixed by vortex. Extraction was performed by gravity, decanting the acidified sample through a Seppak C-18 cartridge, pretreated with 5 ml 100% methanol, 5 ml distilled water, and 5 ml 4% acetic acid. After application of the plasma the cartridge was washed with 3 ml 25% ethanol in distilled water. Endothelin was eluted from the cartridge with 2 ml 4% acetic v/v in 86% ethanol into l6 100 mm borosilicate glass tubes. The eluates were dried and reconstituted in 500 μl radioimmunoassay buffer.

The sensitivity of the assay was 2 pg/ml, with 100% cross reactivity with ET-1, 52% with endothelin-2, 96% for endothelin-3 and 7% for big endothelin. Cross reactivity with atrial natriuretic peptide, angiotensin II, adrenocorticostrophic hormone, and vasopressin was < 0·1%.

Statistical Analysis

All results are reported as mean (one SD). Statistical analysis was performed using the unpaired Student's t test. A P value of < 0·05 was considered to be significant.

Results

The mean (SD) (range) concentration of plasma ET-1 in patients with angina and normal coronary arteriograms was 3·84 (1·25) (1·97–7·42) pg/ml compared with 2·88 (0·71) (1·57–4.48) pg/ml in controls (P < 0·0001). Of the five patients with left bundle branch block, four had a plasma endothelin concentration > 4·0 pg/ml (mean (SD) 4·43 (1·22). When patients with left bundle branch block were excluded the mean (SD) plasma endothelin concentration in the patient group was 3·75 (1·25) pg/ml (P < 0·001) (fig 1).

There was no was no correlation between ET-1 concentration and age in either patients or controls. The mean (SD) (range) concentration of plasma cholesterol was 6·2 (1.0) (4·4–8·6) mmol/l in patients and 6·8 (1·1) (4·5–8·9) mmol/l in controls. There was no correlation between the concentrations of total plasma cholesterol and ET-1 in either the patient or control group, nor between those of oestrogen and plasma endothelin.

Exercise electrocardiography

The table gives the results of exercise testing. Using the mean (SD) plasma endothelin concentration in the control group as an arbitrary cut off, patients with a "high" (> 3·59 pg/ml) plasma endothelin concentration developed chest pain earlier during exercise than those

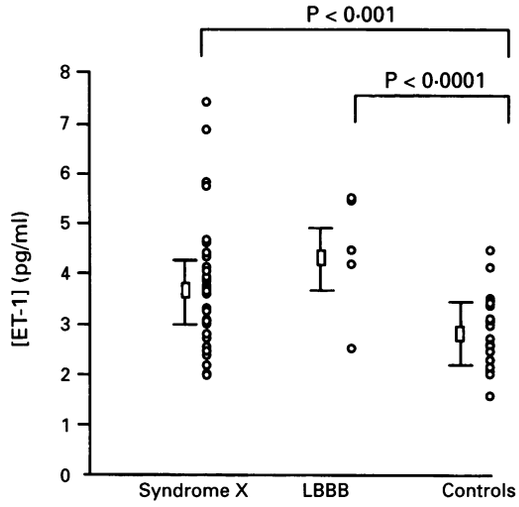

Figure 1 Plasma endothelin-1 (ET-1) concentration in patients with angina pectoris and normal coronary arteriograms (syndrome X) and controls. Values are mean one SD). LBBB, left bundle branch block.

Results of symptom limited exercise testing in patients with "high" (> 3·59 pg/ml) and "low" (⩽ 3·59 pg/ml) concentrations of plasma endothelin-1 (ET-1)

	ET-1 > 3·59 (n = 23)	*ET-1 ⩽ 3·59 (n = 17)*
Baseline		
Heart rate (bpm)	88 (12)	83 (13)
RPP	11 164 (2206)	10 580 (2070)
1 mm ST depression*		
Heart rate	138 (19)	130 (20)
RPP	19 878 (7124)	20 435 (3986)
Time (min)	6·7 (5)	7·4 (3·7)
Onset of chest pain		
Heart rate (bpm)	133 (19)	144 (17)
RPP	20 531 (5447)	24 005 (4285)
Time (min)	6·2 (3·9)	9·0 (3·9)†
Peak exercise		
Heart rate (bpm)	152 (18)	153 (17)
RPP	24 903 (5119)	26 254 (4999)
Time (min)	10·7 (5)	11·3 (3·6)
METS	7·5 (3·3)	7·3 (2·4)

*Excluding five patients with baseline left bundle branch block.
†P = 0·01 (Student's *t* test).
RPP, heart rate blood pressure product; METS, metabolic equivalent of the task.

with "low" endothelin (6·2 (3·9) *v* 9·0 (3·9) min; P = 0·01). When patients with left bundle branch block were excluded, the time to chest pain in patients with high and low endothelin levels was 6·2 (4·0) and 8·7 (3·9) min respectively, P = 0·04). Total exercise duration was similar in both groups.

Thallium-201 scintigraphy

Fourteen patients had one or more fixed defects and 16 had one or more reversible defects. Of these, seven had a combination of fixed and reversible ^{201}TI perfusion abnormalities. Plasma endothelin was higher in patients with reversible defects but the difference did not reach significance (4·06 (1·10) *v* 3·49 (1·13), P = 0·13) (fig 2). There was no significant difference in the concentration of plasma endothelin in patients with or without fixed defects (3·83 (1·05) and 3·75 (1·21) respectively).

Using the same cut off (3·59 pg/ml) to define "high" and "low" endothelin groups there was no correlation between the proportion of patients with fixed defects and those with reversible defects.

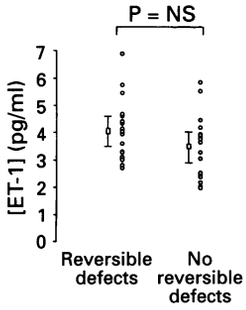

Figure 2 Plasma endothelin-1 (ET-1) concentration in patients with and without reversible thallium-201 perfusion defects. (Values are mean (one SD). NS, not significant.

Discussion

This prospective study demonstrated that the concentration of plasma ET-1 was significantly higher in a well characterised population of patients with angina and normal coronary arteriograms than in normal controls.

The endothelins are a group of peptides released from intact endothelial cells that have a paracrine effect on adjacent smooth muscle cells.[27] Although the relation of plasma ET-1 and vascular resistance in humans remains speculative, Haynes and Webb[28] have recently shown that endothelin exerts a continuous effect on vascular smooth muscle tone in the forearm. Lerman *et al*[29] have demonstrated that changes in blood concentrations within the physiological range have a biological effect in animals. Relatively low concentrations of endothelin may also indirectly influence vascular smooth muscle tone by modulating the interaction of other vasoactive substances with the endothelium.[30] This may be relevant in patients with angina and normal angiograms, as several studies have suggested a role for excessive sympathetic activity in some patients.[31 32]

Endothelin in patients with angina and normal coronaries

The diagnostic category "angina and normal coronary arteries" encompasses a heterogeneous patient population, and this more than any other factor, complicates the study of its pathophysiology. Despite this studies using various techniques have consistently demonstrated reduced coronary flow reserve in a proportion of patients and have suggested that microvessels in symptomatic patients may be more "sensitive" to vasoconstrictor stimuli.[2–5] More recently, studies using positron emission tomography[33] have shown that patients with angina, normal coronary arteriograms, and ST segment depression during exercise have lower coronary flow reserve than those patients without ST segment changes. As no organic lesion has yet been identified to explain this phenomenon in most of patients with angina and normal coronary arteriograms, it has been suggested that "functional" variations in prearteriolar tone ("microvascular angina") are responsible.[5] The underlying mechanism of this microvascular abnormality is unknown but the demonstration of abnormal endothelium dependent vasodilatation in some patients with angina and normal coronary arteries[34] suggests that endothelial dysfunction may have a key role. Recently, Egashira *et al*[6] investigated nine well characterised patients with syndrome X (without hypertension, hypercholesterolaemia, or diabetes) and demonstrated that acetylcholine induced increases in coronary blood flow were less than in controls. Responses to nitrates and papaverine were, however, similar in both groups indi-

cating the presence of a selective difference in endothelial responsiveness.

The mechanism by which endothelin might contribute to a microvascular abnormality remains speculative but its known effects on the coronary circulation suggest intriguing possibilities. Systemic infusion of endothelin can produce intense vasoconstriction in animals and humans; the effect being mediated by a direct action on vascular smooth muscle.[35 36] The coronary vessels seem particularly sensitive to endothelin[37] and high circulating levels in animals have been shown to cause not only coronary artery spasm but also myocardial ischaemia.[38] Similarly, administration of antibodies to endothelin seems to protect animals from experimental myocardial infarction.[39]

No significant differences in exercise variables (except the time to chest pain) were demonstrated in patients with "high" or "low" ET-1 concentrations. This might suggest that endothelin is not important in the generation of ST segment shifts observed in patients with angina and normal coronary arteriograms but the trend towards higher endothelin concentrations in patients with reversible ^{201}TI perfusion defects supports the hypothesis that this peptide may have a role in reducing coronary flow reserve in such patients. However, the small patient cohort and the difficulties of interpretation of ^{201}TI perfusion abnormalities in the absence of large vessel disease makes this attractive idea speculative.

The good long-term prognosis,[40] and a lack of clinically detectable myocardial ischaemia in most patients with angina and normal coronary arteriograms, makes interpretation of coronary flow abnormalities in this group controversial. Some workers have suggested that the paradox of angina without ischaemia may be explained by the release, within the myocardium, of algogenic substances (for example potassium[41] or adenosine[11]) or both that also influence other aspects of myocardial function. This hypothesis is supported by several studies that have demonstrated abnormal cardiac and somatic pain perception in a significant proportion of patients with syndrome X.[7-9] Raffa *et al*[21-22] have recently demonstrated that abdominal constriction in mice, produced by intraperitoneal injection of ET-1, can be antagonised by both subcutaneous and intracerebroventricular injection of morphine, indicating the presence of a centrally mediated nociceptor response. The demonstration of an earlier onset of chest pain (albeit by *post hoc* analysis) in patients with the highest plasma endothelin levels in the present study provides at least circumstantial evidence for a putative algogenic action of endothelin in humans.

The development of progressive ventricular dilatation and congestive cardiac failure[42] in some patients with angina, normal coronary angiograms, and left bundle branch block suggests that they represent a separate disease cohort. The five patients with left bundle branch block in the present study had normal left ventricular dimensions, but four had high concentrations of ET-1. Endothelin may, therefore, be a marker of risk in such patients although the size of this cohort is too small to draw any firm conclusions.

Plasma endothelin is probably derived from the "spill over" of endothelin produced locally at the vascular endothelium and is, therefore, likely to be a poor quantitative measure of its activity at a cellular level. However, the demonstration of increased blood concentrations in the present study is consistent with the presence of abnormal endothelial function in patients with angina and normal coronary arteriograms. The introduction of endothelin antagonists in the future will facilitate clinical evaluation of the importance of ET-1 in coronary blood flow and pain perception in this complex clinical entity.

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