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Circulating endothelin-1 levels in type 2 diabetic patients with ischaemic heart disease

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Abstract To investigate whether circulating endothelin-1 (Et-1) may be related to the increased incidence and severity of ischaemic heart disease in type 2 diabetes mellitus, we compared the concentrations in type 2 diabetic patients and in non-diabetic patients with coronary artery disease (CAD) angiographically documented. Plasma levels of Et-1 were determined in 34 type 2 diabetic patients with CAD (16 with stable angina, 6 with unstable angina, 12 with previous myocardial infarction) and in 19 non-diabetic patients with CAD (4 with stable angina, 5 with unstable angina, 10 with previous myocardial infarction). Fifteen diabetic patients without CAD and 9 healthy volunteers served as control subjects. In the type 2 diabetic patients, the mean Et-1 levels were 3.19±1.61 pmol/l in those with stable angina, 3.58±1.92 pmol/l in those with unstable angina, 4.24±2.53 pmol/l in those with myocardial infarction. These values were not significantly different one another, nor from the values obtained from type 2 diabetic controls (3.64±2.13 pmol/l). In the non-diabetic patients, the mean Et-1 levels were 3.92±0.73 pmol/l in those with stable angina, 4.35±1.67 pmol/l in those with unstable angina, 4.33±1.66 pmol/l in those with myocardial infarction. These values were not significantly different one another, but significantly higher than those obtained from healthy controls (2.07±0.67 pmol/l; $P<0.001$). No significant differences were found in Et-1 levels between diabetic and non-diabetic patients with stable, unstable angina and previous myocardial infarction. In contrast, a statistically significant difference was found in Et-1 levels between diabetic and non-diabetic control subjects ($P<0.05$). In conclusion, similar raised concentrations of Et-1 in diabetic and non-diabetic patients with stable, unstable angina and previous myocardial infarction do not support the hypothesis that higher levels of Et-1 in diabetic patients are responsible for the increased incidence of CAD in diabetes mellitus. However, the raised Et-1 levels found in diabetic patients in the absence of CAD strongly suggest that a generalised endothelial dysfunction, documented in our study by increased levels of Et-1, most probably precedes subsequent cardiovascular diseases.

Key words Endothelin-1 (Et-1) · Type 2 diabetes mellitus · Coronary artery disease (CAD)

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Introduction

Patients with type 2 diabetes mellitus have an increased risk of atherosclerotic vascular disease manifesting as coronary artery disease (CAD), cerebrovascular disease, and peripheral vascular disease [1–3]. In particular, the incidence of CAD is increased two- to fourfold and is responsible for 50%–60% of adult deaths [4], and the age-adjusted incidence of acute myocardial infarction is six times greater in diabetic men and four times greater in diabetic women than in non-diabetic patients [1]. Endothelin-1 (Et-1), the most potent vasoconstrictor substance present in plasma, perhaps as a result of spillover from the interface of endothelial and vascular smooth muscle cells [5] has been postulated to contribute to the atherosclerotic process through its known effect as a mitogen for fibroblasts [6] and rat aortic smooth muscle cells [7]; thus, Et-1 is a likely candidate in the mediation of several forms of vascular diseases, among which is CAD. This study was designed to determine whether type 2 diabetic patients with coronary artery disease had higher circulating levels of Et-1, compared with non-diabetic patients with CAD. If this were so, it might help to explain, at least in part, the increased incidence and severity of CAD in type 2 diabetes mellitus.

Subjects and methods

Thirty-four type 2 diabetic patients (12 men, 22 women) and 19 non-diabetic patients (18 men, 1 woman) with clinical syndromes of CAD, angiographically documented, were selected. Patients with abnormal liver or kidney function tests, recent myocardial infarction (<3 months) or congestive heart failure were excluded from the study. The control diabetic subjects (4 men, 11 women; mean age 60.0±10.4 years) and the control non-diabetic subjects (2 men, 7 women; mean age 51.4±11.4 years) were selected on the basis of a negative history for CAD and negative treadmill exercise test. Written informed consent was obtained from each subject before her/his participation to the study. The investigation was performed according to the principles outlined in the Declaration of Helsinki and approved by the local Ethical Scientific Board.

Among 34 diabetic patients with CAD, 16 patients (2 men, 14 women; mean age 65.4±7.0 years) had chronic stable angina, 6 patients (4 men, 2 women; mean age 60.0±12.1 years) had unstable angina, 12 patients (6 men, 6 women; mean age 64.3±7.5 years) had had a previous myocardial infarction. Among 19 non-diabetic patients with CAD, 4 patients (4 men; mean age 58.0±8.2 years) had chronic stable angina, 5 patients (4 men, 1 woman; mean age 64.0±10.5 years) had unstable angina, 10 patients (10 men; mean age 57.9±5.3 years) had had a previous myocardial infarction. Blood samples were taken after an overnight fast for preparation of plasma which was assayed for Et-1.

Test procedures

Plasma Et-1 was determined by a radioimmunoassay (RIA) system after extraction from plasma by absorption onto prewashed Sep Pak silica gel C_{18} cartridges (Waters, Millipore, UK). Eluates were evaporated to complete dryness under a controlled optimal stream of nitrogen in a waterbath at 37°C, then reconstituted in a RIA buffer (borate buffer, pH 8.4) and assayed by RIA. The interassay coefficient of variation (CV) was 4.3% at 5.33 pmol/l; intra-assay CV was 2.01% at 5.33 pmol/l; the sensitivity was 0.40 pmol/l (Nichols Institute, [^{125}I]endothelin-1, The Netherlands).

Statistical analysis

All results are given as mean ± SD. Differences in measurements among the groups were assessed by one-way analysis of variance (ANOVA) or by means of unpaired Student's *t*-test, as applicable. All statistical analyses were performed using the MINITAB, release 9.2 (Minitab, State College, Pa., USA) statistical package. *P* values <0.05 were considered statistically significant.

Results

The clinical features of the patients are shown in Table 1. All groups were comparable with regard to mean age, duration of CAD, systolic and diastolic blood pressure. Figure 1 shows that mean concentrations of Et-1 in diabetic patients with stable angina (3.19±1.61 pmol/l) or with unstable angina (3.58±1.92 pmol/l) or with previous myocardial infarction (4.24±2.53 pmol/l) did not differ from one another nor from diabetic control subjects (3.64±2.13 pmol/l). Mean concentrations of Et-1 in non-diabetic patients with stable angina (3.92±0.73 pmol/l), with unstable angina (4.35±1.67 pmol/l) or with previous myocardial infarction (4.33±1.66 pmol/l) did not differ from one another, but were significantly higher than those from healthy control subjects (2.07±0.67 pmol/l; $P<0.001$). In the pairwise comparison, no significant differences were found in Et-1 concentrations between diabetic and non-diabetic patients with stable, unstable angina and myocardial infarction, whereas a statistically significant difference was found between diabetic (3.64±2.13 pmol/l) and non-diabetic (2.07±0.67 pmol/l) control subjects ($P<0.05$).

Discussion

In this study we clearly demonstrated that plasma Et-1 levels are similarly raised both in diabetic and non-diabetic patients with stable, unstable angina and previous myocardial infarction. Thus, Et-1 does not seem to be associated with the increased incidence and severity of CAD in type 2 diabetes mellitus. Most interestingly, confirming our previous data [8], plasma Et-1 levels are increased even in diabetic patients without CAD. Several reports indicate that a generalised endothelial dysfunction is an early phenomenon in diabetes mellitus before vascular complications occur. For example, endothelium-dependent vasodilatation in response to acetylcholine is reduced in both the periph-

Table 1 Clinical characteristics of control subjects and patients

	n (M/F)	Age (year)	Duration CAD (year)	Systolic (mmHg)	Diastolic (mmHg)
Control					
Diabetic	15 (4/11)	60.0±10.4	–	139.00±14.17	79.00±7.61
Non-diabetic	9 (2/7)	51.4±11.4	–	129.44±19.11	78.89±6.01
Stable angina					
Diabetic	16 (2/14)	65.4±7.0	4.0±1.4	142.19±18.79	80.94±8.98
Non-diabetic	4 (4/0)	58.0±8.2	5.2±4.3	127.50± 6.45	80.00±8.16
Unstable angina					
Diabetic	6 (4/2)	60.0±12.1	10.6±6.3	130.00±20.98	84.17±8.01
Non-diabetic	5 (4/1)	64.0±10.5	8.6±3.7	155.00±13.23	83.33±5.77
Myocardial infarction					
Diabetic	12 (6/6)	64.3±7.5	10.5±6.9	144.58± 9.40	82.50±9.89
Non-diabetic	10 (10/0)	57.9±5.3	7.5±6.8	127.00±14.94	80.50±7.62

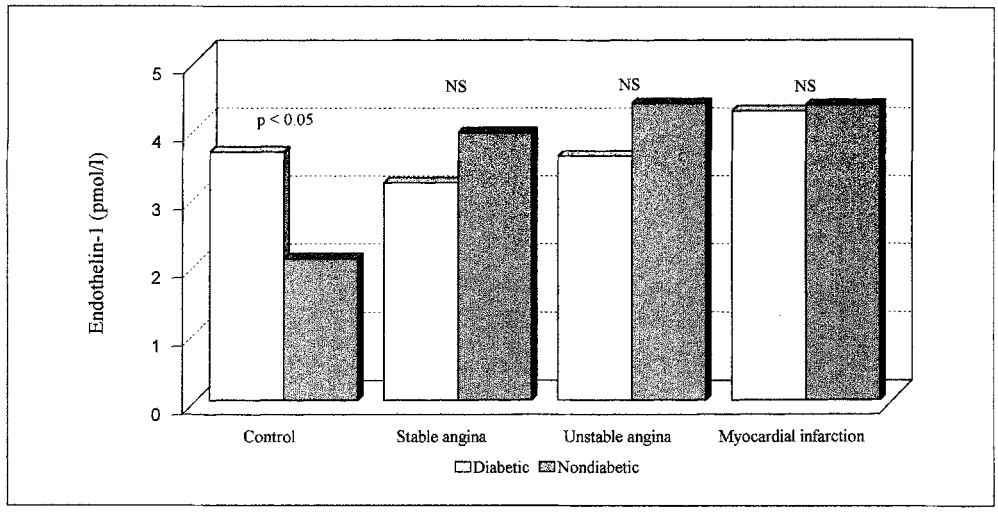

Fig. 1 Plasma endothelin-1 levels in control subjects and in patients with stable, unstable angina and previous myocardial infarction

eral and coronary circulation long before structural vascular changes or clinical symptoms arise [9]. An indicator of endothelial dysfunction, such as von Willebrand factor (vWF), has been reported to be increased in diabetes mellitus and predictive for the development of new vascular events [10]; also, thrombomodulin, which is considered a more specific indicator than vWF for endothelial dysfunction because it is produced only by endothelium (vWF is also synthesised by megakaryocytes), has been found to be increased in diabetes mellitus, independently of the age of the patient [11]. Similarly, an elevation of plasma Et-1 in diabetic patients without CAD may have functional importance as a participant in the atherogenic process through its known effects as a mitogen for vascular smooth muscle cells, interacting with specific cell-surface receptors in a dose-dependent manner [12]. More, although it is clear that raised plasma levels of Et-1 do not always correlate with receptor activity, Et-1 could also act by amplifying the contractions induced by other vasoconstrictors, such as norepinephrine [13] or serotonin [13, 14]. In conclusion, this preliminary work represents data in a small group of patients with no follow-up; future longitudinal studies are needed to corroborate that in diabetes mellitus raised plasma Et-1 should be considered as another sensitive marker of endothelial damage, most probably related to the occurrence of subsequent diabetic sequelae.

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