Elevated plasma endothelin-1 levels in coronary sinus during rapid right atrial pacing in patients with slow coronary flow

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Received 26 March 2003; received in revised form 24 June 2003; accepted 24 June 2003

Abstract

The aim of the study was to evaluate whether there was an imbalance between endothelin-1 (ET-1) and nitric oxide (NOx) release and diffuse atherosclerotic changes existed in patients with slow coronary flow (SCF). Baseline and post-atrial pacing coronary sinus ET-1 and NOx levels were measured in 19 patients with SCF (11 female, 56 ± 9 years) and in 14 control subjects (nine female, 54 ± 7 years). All patients underwent subsequent intravascular ultrasound (IVUS) investigation at the same setting with right atrial pacing. Baseline arterial (12.4 ± 9.9 vs. 6.3 ± 5.1 pg/ml, \( P < 0.005 \)) and coronary sinus (12.2 ± 11.1 vs. 6.4 ± 6.9 pg/ml, \( P < 0.005 \)) ET-1 plasma levels were higher in patients than in controls. After atrial pacing, concentration of ET-1 level from coronary sinus (24.7 ± 14.6) significantly increased as compared to baseline (12.4 ± 9.9, \( P < 0.0001 \)) and control levels (5.3 ± 6.3, \( P < 0.0001 \)). Additionally, coronary sinus ET-1 level increased significantly with atrial pacing compared to femoral artery ET-1 level (16.3 ± 8.5, \( P < 0.005 \)) in patients with SCF. After atrial pacing, the femoral artery ET-1 level also increased in patients compared to control level (\( P < 0.0001 \)). No significant differences in arterial and coronary sinus NOx plasma levels were found between the two groups, both at baseline and after pacing. Upon IVUS investigation, the common finding was longitudinally extended massive calcification throughout the epicardial arteries in patients with SCF. Mean intimal thickness was 0.59 ± 0.18 mm. The data of this study suggest that increased ET-1 levels and insufficient NOx response, as well as the pathological data of IVUS may be associated with coronary microvascular dysfunction and may be the manifestation of early diffuse epicardial atherosclerosis in these patients with SCF.

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Keywords: Slow coronary flow; Diffuse atherosclerosis; Endothelin-1; Nitric oxide

1. Introduction

In general, typical chest pain with angiographically normal coronary arteries is well known as syndrome X [1]. However, slow coronary flow (SCF) differs in a distinct manner characterized by delayed opacification of epicardial coronary arteries in the absence of epicardial occlusive disease as was introduced by Tambe et al. [2] in 1972. The exact etiology and pathogenesis of SCF is still unknown. Microvascular dysfunction and occlusive disease of small coronary arteries were suggested as its etiology [2–4]. Generally, it is believed to represent coronary microvascular dysfunction, because angiographic phenomenon is selectively ameliorated by microvascular vasodilators such as dipyridamole [4,5]. However, the pathogenesis of microvascular dysfunction remains poorly understood and previous studies [6,7] have demonstrated evidence of coronary endothelial dysfunction.

Endothelin (ET) is a potent vasoconstrictor peptide produced by vascular endothelium, which appears to play an important role in cardiovascular regulation and pathophysiology. Endothelin-1 (ET-1) is produced with ET converting enzyme and effective via ET-A and ET-B receptors on cardiovascular system. ET-A receptors play a role on vasoconstriction and smooth muscle cell proliferation [8]. ET-1 increases coronary vascular resistance and has positive inotropic effects on cardiomyocytes and is mitogenic for smooth muscle cells [9]. Because of these properties, ET contributes to the progression of atherosclerosis and increased ET-1 may substantially contribute to cell growth and the regulation of vascular tone in the very
early stages of plaque evolution, when a plaque is clinically still imperceptible [10,11]. All these data support the hypothesis that elevated ET levels may have pathophysiologic significance in angiographically normal coronary arteries. Accordingly, many intravascular ultrasound (IVUS) studies have shown the evidence of diffuse atherosclerotic changes in patients with SCF. We investigated whether coronary sinus ET-1 and NOx plasma levels were increased by rapid atrial pacing [15,16] which is an endothelium activating stimulus and may result in an abnormal response of ET-1 and NOx in patients with SCF, as compared to healthy subjects. Additionally, we investigated diffuse atherosclerotic changes in these patients, using intravascular ultrasonography (IVUS).

2. Methods

The study population consisted of 19 patients with SCF [11 female; 56 ± 9 years], who underwent coronary angiography to determine whether or not obstructive coronary artery disease existed because of typical and quasi-typical symptoms of angina and ECG changes between January 2001 and June 2002 at Cardiology Clinic of Mersin University. The patients with SCF had angiographically normal coronary arteries without luminal irregularities. The patients who suffered from one of the following diseases or associated disorders were excluded from this study: myocardial and/or valvular heart disease, tortuous coronary vessels, myocardial bridge, coronary ectasia, a proximal lumen diameter less than 3 mm, diabetes mellitus, hypertension and left ventricular hypertrophy. The patients who complied with study design were called back within the following month and were comprehensively informed about the procedure. Only 19 out of 45 patients were suitable and accepted such a procedure. After signed informed consent was obtained, all concomitant medication was stopped 48 h prior to the procedure. The following investigations were performed in patients and control group. As a control group we studied 14 patients [nine female, age 54 ± 7 years], who underwent a control electrophysiologic test after successful radiofrequency catheter ablation of an accessory pathway, performed because of paroxysmal supraventricular tachycardia. None of the control subjects had any history of chest pain or evidence of structural cardiac or systemic disease, and all had normal echocardiographic study and maximal exercise testing.

The study was carried out according to the principles of the Declaration of Helsinki and approved by Mersin University, School of Medicine, investigational review board.

2.1. Coronary angiography and the TIMI frame count

Coronary angiography was applied by femoral approach using standard Judkins technique. Coronary arteries in left and right oblique planes, cranial and caudal angles were demonstrated. Left ventricular and aortic pressures were obtained. During the coronary angiography, lopromide (Ultravist-370, Schering AG) was used as contrast agent and was manually injected (6–8 ml contrast agent at each position). Proximal coronary lumen diameter was measured by quantitative computer-assisted (QCA) facility and those with a caliber of 3 mm or more were enrolled for further SCF measurements. For the quantitative measurement of coronary blood flow, the time elapsed from the appearance till the contrast agent reached the distal end of coronary arteries in terms of cineframe count was considered to be the TIMI frame count [5,17]. Thereafter, the final count was subtracted from the initial and the exact TIMI frame was calculated for the given artery. The TIMI frame counts cut-off values are taken from study of Gibson et al. [17] in which 78 normal coronary arteries were evaluated. The cut-off values due to the length for normal visualization of coronary arteries were 36.2 ± 2.6 frames for left anterior descending coronary artery (LAD), 22.2 ± 4.1 frames for left circumflex coronary artery (LCX), 20.4 ± 3 frames for right coronary artery (RCA). Gibson et al. [17] proposed that TIMI frame count for LAD was 1.7 times higher than those of LCX and RCA. In this study, the reason of this difference was explained as LAD is 1.5 times longer than RCA and 1.6 times longer than LCX anatomically. Therefore, the TIMI frame count was divided by 1.7 when LAD coronary artery was the case, for adjusted correction [17]. The corrected cut-off value for LAD coronary artery was 21.1 ± 1.5 frames [17]. Any values obtained above these thresholds were considered to be SCF. The TIMI frame counting was undertaken by two separate cardiologists. In case of conflict, the frames were referred to a third one. All TIMI frame counts were measured in matched projections with use of Medcon Telemedicine Technology (version 1.900, Israel).

2.2. Study protocol

All patients were studied in the morning and in a fasting state. 6Fr pacing catheter was positioned in the right atrium through the right femoral vein. Then a 6Fr USCI multipurpose sample catheter was placed in the coronary sinus through the right subclavian or right femoral vein under radioscopic control. Ten minutes after heart rate and blood pressure stabilization, baseline blood samples were drawn from the coronary sinus. Then, incremental atrial pacing was performed, starting at a rate of 100 beats per min and increasing the rate of stimulation by 20 beats every 3 min until reaching a rate of 160 beats per min. Maximal paced heart rate was maintained for 3 min. Blood samples were drawn from the coronary sinus immediately after the end of pacing. Blood pressure and 12-lead electrocardiogram were
monitored and symptoms recorded throughout the entire procedure.

2.3. Intravascular ultrasound

All patients with SCF enrolled in the study underwent subsequent IVUS investigation at the same setting with right atrial pacing. By using standard Judkins technique, 7Fr guiding catheter was positioned in the given coronary artery. Fifteen minutes after atrial pacing (before IVUS), 2 µg of nitroglycerin was administered via intracoronary injection. “Endosonics In Visions Imaging System” was utilized during IVUS. The imaging catheter (The Endosonic Visions Five-64 F/X catheter) had a 30 frames per s maximum frame rate and 20-MHz single-piezoelectric crystal transducer mechanically rotating at 1800 rpm within a 3.5-F monorail catheter, was then advanced over the 0.014-in. guide wire into the coronary artery as distally as possible and was then carefully pulled back to continuously image the wall morphology. The size of Judkins catheter was used to calibrate the length of the coronary segment. Images were analyzed frame by frame having the external elastic lamina border manually traced, maximal and minimal intimal thicknesses were measured within the same segment. The following criteria [14] were chosen for lesion characteristics and severity; atherosclerotic lesion; in any segment intimal thickness ≥ 5 mm, eccentric lesion; if maximal thickening exceeded 2-fold minimal thickening the lesion was considere eccentric, calcified lesion; focal or diffuse calcification leading to acoustic shadowing. All images were recorded on recordable compact disk for subsequent data analysis. Each IVUS image was analyzed off-line by two independent experienced IVUS analysts.

2.4. Analysis of endothelin-1

Plasma samples were drawn into chilled EDTA tubes (1 mg/ml blood) containing aprotinin (500 kIU/ml of blood). The whole blood samples were centrifuged at 1600 × g for 15 min at 0 °C. The plasma fractions were transferred to a plastic tube and stored at −70 °C for long-term storage. After a short incubation period, the excess sample was washed out and a polyclonal antibody to ET-1 was labeled and the enzyme horseradish peroxidase was added. This labeled antibody was bound to the ET-1 and was captured on the plate. After a short incubation period, the excess labeled antibody was washed out and substrate was added. The substrate, which reacted with the labeled antibody was bound to the ET-1 and was captured on the plate. The color generated with the substrate was read at 450 nm, and was directly proportional to the concentration of ET-1 in the sample (Human Endothelin-1, catalog no EIA-3111, DRG International, USA).

2.5. Analysis of serum nitrite and nitrate

The levels of nitrite and nitrate were determined using a procedure based on the Griess reaction. Blood samples were centrifuged at 4000 rpm for 10 min. Serum samples were then separated and stored at −70 °C until used for assay. Equal volumes of serum and potassium phosphate buffer were placed in an ultrafilter and centrifuged at 4000 rpm for

<table>
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<tr>
<th>Clinical characteristics of the patients with SCF and control subjects</th>
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<tr>
<td>SCF (n = 19)</td>
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<tr>
<td>Smoking, n (%)</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
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<tr>
<td>Heredity, n (%)</td>
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<td>Systolic blood pressure (mmHg)</td>
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<td>Diastolic blood pressure (mmHg)</td>
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<td>Heart rate (beats per min)</td>
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<td>NS: nonsignificant.</td>
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Fig. 1. Baseline and post-pacing ET-1 plasma levels in the coronary sinus (A) and femoral artery (B) circulations of patients with SCF and control subjects.
45 min. The ultrafiltrate was collected and used in the test. Nitrates were quantitatively converted to nitrites for analysis. Enzymatic reduction of nitrate to nitrite was carried out using coenzymes (NADPH, FAD) in the presence of nitrate reductase in step of incubation assay. N-1-(Naphthyl) ethylenediamine dihydrochloride, sulfanilamide and incubation solutions were mixed at a ratio of 1:1:2 (v/v). These mixtures were incubated for 5 min at room temperature and measured at 540 nm. Sodium nitrite of 1.00 mM was used as standard for determination of nitrite and potassium nitrate of 80 mM was used as standard for determination.

2.6. Statistical analysis

Statistical analysis was performed using SPSS software package (Version 10.0, SPSS, Chicago, IL, USA). Differences between groups in baseline continuous clinical variables were compared by Student’s t-test, whereas categoric variables were compared by Pearson χ²-test or Fisher’s exact test. Continuous variables were expressed as mean ± S.D. Three-way analysis of variance (ANOVA), with a two-factor repeated measurement design, was used to compare baseline and post-pacing values of NOx and ET-1 plasma levels from the femoral artery and coronary sinus in patients with SCF and controls. In case of statistically significant differences, paired between-group and within-group comparisons were performed by planned contrast analysis. Pearson correlation test was used to figure out any relation among ET-1, NOx, TIMI frame count and intimal thickness. All hypothesis testing was two-tailed. A P value of < 0.05 was considered significant.

3. Results

All clinical characteristics of the patients with SCF are given in Table 1. Two groups did not differ by means of age, sex, blood cholesterol, baseline heart rate and blood pressure. During contrast injection at angiography, five patients had a ST segment depression of 1–2 mm and another three had typical anginal pain. Maximal pacing rate was similar in both groups. Pacing was stopped in four patients because of chest pain with the heart rate of 120 beats per min and, in three patients because of ST segment depression (>3 mm) during pacing with the heart rate of 120 beats per min (one patient) and 140 beats per min (two patients). Control
subjects completed the whole protocol. None of the subjects in the control group had ST-segment changes, tachyarrhythmias or chest pain throughout the procedure.

The group–time–vessel interaction of ET-1 in three-way repeated measurement ANOVA with a two-factor within two groups was shown statistically significant ($P = 0.006$, Observed Power = 0.824) (Fig. 1). Baseline and post-pacing coronary sinus ET-1 and NOx levels of the patients with SCF and the control group are given in Table 2. There were significant differences in baseline ET-1 plasma levels between two groups with both arterial and coronary sinus levels being higher in patients with SCF. After atrial pacing, concentration of ET-1 level from coronary sinus significantly increased compared to basal and control levels. Additionally, coronary sinus ET-1 level increased significantly with atrial pacing compared to femoral artery ET-1 level in patients with SCF. After atrial pacing, the femoral artery ET-1 level also increased in patients compared to control level. Although, the femoral artery ET-1 level increased in patients compared to basal level, it was not statistically significant. There was no statistically significant difference between basal and after pacing femoral artery and coronary sinus ET-1 levels in control subjects.

The group–time–vessel interaction of NOx in three-way repeated measurement ANOVA with a two-factor within two groups was shown statistically insignificant ($P = 0.871$, Observed Power = 0.053). The mean coronary sinus NOx level was lower in patients with SCF compared to control subjects; but this was not statistically significant. Although, coronary sinus NOx level increased after pacing both in patients with SCF and control group, there was no significant difference according to increase in NOx levels with atrial pacing between them. There was no difference between groups regarding gender, baseline and post-pacing ET-1 and NOx levels. No positive correlation was determined between age and either ET-1 or NOx.

Upon IVUS investigation in patients with SCF, the common finding was longitudinally extended massive calcification throughout the epicardial arteries in 13 patients and regional calcification in six patients. Mean intimal thickness was $0.59 \pm 0.18$ mm and in 13 patients, eccentric lesions were observed (Fig. 2). Mean vessel diameter was $3.6 \pm 0.4$ mm and there was determined positive correlation between ET-1 and intimal thickness in patients with SCF (Fig. 3A). The TIMI frame counts were $97.8 \pm 21.28.3$ frames for LAD ($n = 13$), $44.0 \pm 2.8$ frames for LCX ($n = 2$), and $41.3 \pm 5.4$ frames for RCA ($n = 4$) in patients with SCF. The corrected TIMI frame count was $57.3 \pm 12.5$ frames for LAD. There was determined positive correlation between ET-1 and the TIMI frame count in patients with SCF (Fig. 3B). Also, a strong positive correlation was seen between the TIMI frame count and intimal thickness in these patients (Fig. 4).

Due to the relatively small number of patients involved in the study, other coronary artery disease risk factors such as smoking, heredity and lipid parameters etc. were not applied to any kind of statistical methods to figure out any possible correlation.

Fig. 3. (A) Correlation between ET-1 and intimal thickness. (B) Correlation between ET-1 and the TIMI frame count in patients with SCF.

Fig. 4. Correlation between the TIMI frame count and intimal thickness in patients with SCF.
4. Discussion

The results of this study show that baseline ET-1 plasma levels were higher in patients with SCF than in a matched group of control subjects. The differences regarding coronary sinus ET-1 levels between the two groups became higher after rapid right atrial pacing, as a result of a significant increase in ET-1 in these patients with SCF. Furthermore, coronary sinus ET-1 levels increased significantly with atrial pacing compared to femoral artery ET-1 levels in these patients.

ET-1 and NOx are key molecules in normal autoregulatory mechanisms e.g. modulating vasodilator response to tachycardia and exercise [18,19]. Endothelial injury causes increase in levels of plasma ET [20]. Epicardial coronary artery dilatation induced by pacing is found as depending on the release of endogenous NOx [21]. With right atrial pacing, coronary sinus NOx levels increased in a similar fashion in both SCF and control groups; however, they were statistically insignificant. Tousoulis et al. [22] reported that endothelium in the region of atheromatous stenosis can produce NOx even in a normal amount. The increase in NOx levels in both groups in a similar manner may be explained with this data.

Some biopsy studies of patients with SCF [3,4] showed that SCF could be the result of increased resistance in arterioles. Mangieri et al. [4] and Kurtoglu et al. [5] have observed remarkable progress in restoring coronary flow when they studied dipyridamole in this group of patients. All these data support the theory that the pathophysiology underlying this disorder is closely related to the microvasculature and has a dynamic character. Accordingly, the present study demonstrated that levels of ET-1 from coronary sinus were correlated with TIMI frame count.

However, all these data do not exactly and clearly delineate the borders of this disorder neither does it imply any interaction between micro- and macrovasculature of the heart. Accordingly, some post-mortem studies [23,24] revealed a co-incidence of epicardial and small vessels disease, which supports our opinion. According to preliminary data, in the early phase of atherosclerosis or with intensive coronary artery disease risk factors, vasodilation capacity of coronary resistive arterioles by pharmacologic and physical stress was disturbed before development of angiographic atherosclerotic disease [25,26]. Besides, IVUS imaging can detect intimal thickening and is suitable for detection of early atherosclerosis, which cannot be detected by conventional angiography [12–14]. In the present study, we found diffuse calcification and intimal thickening in all segments of the vessels despite the absence of focal stenosis or plaques in coronary angiography of SCF patients. Also, levels of ET-1 were correlated with intimal thickness of coronary artery detected with IVUS in these patients. Thus we speculate that SCF may be a form or preliminary phase of diffuse atherosclerotic process that involve both small vessels and epicardial coronary arteries.

Endothelial dysfunction in patients with atherosclerotic coronary disease is also associated with an increased release of ET-1 after acetylcholine administration [20]. Accordingly, Lubov et al. found that ET-1 is indeed released during exercise and is related to the severity of the ischemia as reflected by perfusion defects on SPECT sestamibi [27]. Furthermore, myocardial coronary flow is altered by several complex factors including endothelial-dependent and -independent vasodilatory function, diffuse atherosclerosis due to arterial wall fibrosis, and/or atheromatous plaque and abnormal smooth muscle cell proliferation [28].

Overall, the results of this study revealed that there was an increased vasoconstrictor activity of the endothelium and it may play some pathophysiological role in SCF. It is possible that, abnormal slow flow pattern in coronary arteries of these patients may lead to diffuse atherosclerotic disease by damaging endothelium. Alternatively, the release of ET-1 in response to pacing might be secondary to the induction of myocardial ischemia [29]. These results strongly suggest that increased ET-1 level could be an early manifestation of coronary atherosclerosis before progression to coronary artery disease. Both baseline and post-pacing increased ET-1 levels in these patients may play a role in the abnormal coronary flow pattern.

4.1. Study limitations

Baseline and post-atrial pacing blood samples were obtained during electrophysiologic test in control subjects. So, none of the subjects underwent coronary angiography and no IVUS study could be performed. Because no IVUS criteria exist in patients with SCF, we compared our findings with the criteria, which were reported in a study performed in the subjects with normal coronary angiography [14].

5. Conclusion

The data presented in this study lend further support to the hypothesis that increased ET-1 levels and the pathological data of IVUS may be associated with the manifestation of early diffuse epicardial atherosclerosis in patients with SCF.

References


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