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BRIEF COMMUNICATION

# Endothelin-1 and Cold Provocation in Health, Primary Raynaud's Phenomenon, and Progressive Systemic Sclerosis

JANINE DÖRFFLER-MELLY,* THOMAS F. LÜSCHER,† MARTIN WENK,‡ SHENGHUA WEN,* ALFRED BOLLINGER,* AND U. K. FRANZECK*

**Department of Medicine, Angiology Division, University Hospital, Zürich; †Department of Medicine, Cardiology Division, University Hospital, Bern; and ‡Department of Medicine, Clinical Pharmacology Division, University Hospital, Basel, Switzerland*

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## INTRODUCTION

Endothelin-1 (ET-1) is known as a potent vasoconstrictive peptide present in the circulating blood and produced by endothelial cells (Yanagisawa *et al.,* 1988). Because of its vasoconstrictive activity, ET-1 concentrations in venous plasma have been repeatedly investigated in small series of patients with Raynaud's phenomenon as well as in healthy subjects after cold exposure; however, there have been conflicting results (Fyhrquist *et al.,* 1990; Hynynen *et al.,* 1991; Harker *et al.,* 1991; Zamora *et al.,* 1990; Biondi *et al.,* 1991; Smits *et al.,* 1991; Bierbrauer *et al.,* 1991; Kanno *et al.,* 1991). We studied whether patients with primary Raynaud's phenomenon have different ET-1 plasma levels before and after cold provocation (CP) compared to patients with secondary Raynaud's phenomenon due to progressive systemic sclerosis (PSS) and to healthy volunteers.

## PATIENTS AND METHODS

### *Patients*

The control group (CG) consisted of 12 healthy volunteers (8 women, 4 men; mean age 32.7 years, 24 to 52 years) without any clinical signs or symptoms of illness. None had any history of vasospastic attacks or migraine.

In the group with primary Raynaud's phenomenon (PRP) 12 patients (9 women, 3 men; mean age 37.2 years, 21 to 54 years) with a history of typical cold-induced vasospastic attacks for at least 5 years duration, with negative immunology tests and without any microangiopathy of the nailfold capillaries, were included.

Six patients (4 women, 2 men; mean age 40.9 years, 18 to 65 years) were suffering from secondary Raynaud's phenomenon (SRP) due to PSS. The diagnosis of PSS was based on the criteria of the American Rheumatism Association (Subcommittee for



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Scleroderma Criteria of the ARA Diagnostic and Therapeutic Criteria Committee, 1980), positive immunology tests (antinuclear antibody, anti-Scl-70), and a pronounced microangiopathy of the nailfold capillaries with a typical scleroderma-like pattern (Mariq, 1986). All of the patients with PSS had multiple digital artery occlusions as shown by noninvasive diagnostic means with electronic oscillography and digital artery pressure measurements. None of them had acute ulcerations of the digits. Any medication was stopped 2 weeks before the patients were investigated. All study participants were nonsmokers.

All study participants had given their informed consent. The study was approved by the Ethical Committee of the Department of Medicine, University Hospital, Zürich.

*Study Protocol and Methods*

All individuals were investigated on 2 different days. In the first session physical examination was performed (inspection of the extremities and digits to discover finger swelling, color changes, skin and nail dystrophy, ulcerations; palpation of the pulses of the upper extremity, Allen test). Blood pressure was measured in both arms according to the Riva-Rocci technique, and systolic pressure of the ulnar and radial arteries was measured with a cw-Doppler (Parks Electronics, Portland, OR). Systolic digital blood pressure was measured using the laser Doppler technique. Laser Doppler flux and cuff pressure were recorded on a chart recorder (Gould 2600 S, Gould Brush, Cleveland, OH). Electronic oscillography was performed on all fingers. Red blood cell velocity measurements of the nailfold capillaries were measured on a finger with known cold-induced vasospastic attacks or on the fourth right finger in the control group. Capillary blood flow was studied by video microscopy (Bollinger *et al.*, 1974) and recorded on videotape for off-line evaluation (CapiFlow, IM-CapiFlow, Stockholm, Sweden). A local cooling test was performed in every subject (Mahler *et al.*, 1986).

In a second session the CP test was performed with immersion of the right hand and forearm in ice-water for 60 sec. Blood samples (EDTA tubes) were drawn from indwelling catheters of an antecubital vein on both arms simultaneously by before and 2, 4, 6, and 10 min after cooling. The samples were centrifuged immediately at 3000 rpm for 10 min, and the plasma was stored at −60°. Plasma ET-1 levels were measured by radioimmunoassay after solid-phase extraction using SepPak C18 cartridges (Millipore-Waters, Milford, MA) similar to the method of Sørensen (1991). Extraction recoveries were 76%. For the radioimmunoassay an antiserum against ET-1 from Peninsula Laboratories (Belmonst, CA) was used. After preincubation of the samples with antiserum for 24 hr, $^{125}$I-ET-1 (Biomedica, Vienna, Austria) was added and the incubation was continued for another 24 hr. Bound and free ET-1 were separated using a second antibody system (Peninsula Laboratories). The test sensitivity was 0.7 pg/ml.

*Statistics*

From all ET-1 data median and mean values plus standard deviations were calculated. The Wilcoxon test for paired data was used to compare ET-1 concentrations before and after CP, whereas the unpaired Mann–Whitney U test was used to compare ET concentrations between the different patient and control groups (StatView 512$^+$, Abacus Concepts, Inc.). The statistics were performed on a personal computer (Macintosh Classic).

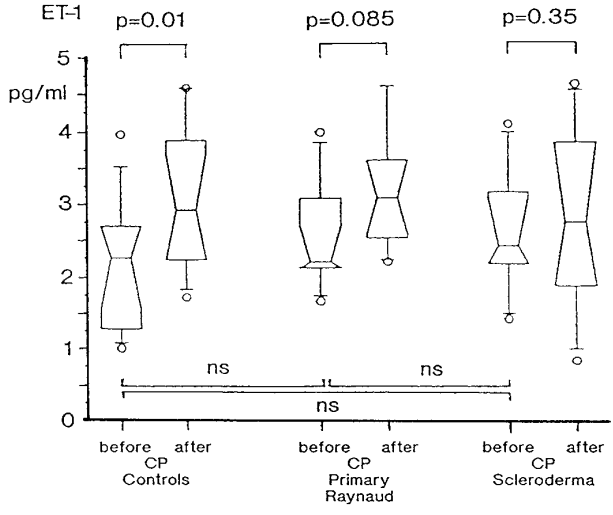


FIG. 1. Box plots of ET-1 plasma concentrations (pg/ml) before and after cold provocation in controls and patients with primary and secondary Raynaud's phenomenon.

## RESULTS

Baseline ET-1 plasma concentrations (see Fig. 1) were not different in the three groups ($P > 0.05$). In controls mean ET-1 level of the test arm increased significantly ($P = 0.01$) from 2.2 ± 1.0 pg/ml at rest to 2.9 ± 1.0 pg/ml after CP. On the contralateral arm ET-1 values were 2.9 ± 0.8 pg/ml before and 2.9 ± 0.8 pg/ml after CP ($P = 0.91$).

Patients with PRP had a baseline ET-1 plasma level of the test arm of 2.6 ± 0.8 pg/ml. Mean ET-1 concentration increased to 3.2 ± 1.0 pg/ml ($P = 0.085$) after CP. On the contralateral arm values of 3.4 ± 1.5 pg/ml before and 3.2 ± 1.1 pg/ml after CP ($P = 0.31$) were obtained.

In the SRP group mean plasma ET-1 at rest on the cooled arm was 2.6 ± 0.9 pg/ml before and 2.8 ± 1.5 pg/ml after CP ($P = 0.46$), whereas on the second arm ET-1 concentrations were 2.7 ± 1.3 pg/ml and 2.8 ± 1.0 pg/ml ($P = 0.35$), respectively.

## DISCUSSION

The results of our study confirm previous findings (Smits *et al.,* 1991; Bierbrauer *et al.,* 1991; Kanno *et al.,* 1991) that basal ET-1 concentrations in healthy subjects, patients with primary Raynaud's phenomen, and patients with secondary Raynaud's phenomenon due to progressive systemic sclerosis are not different and are in the normal range. This in contrast to results reported by others (Zamora *et al.,* 1990; Biondi *et al.,* 1991), where even threefold higher baseline ET-1 levels were measured in patients with primary Raynaud's phenomenon. In the series with a higher number of healthy subjects no significant differences in basal ET-1 could be found (Zamora *et al.,* 1990; Biondi *et al.,* 1991); therefore the low ET-1 concentrations in normals found by Zamora (1990) may be due to the small number of subjects studied. Normal basal ET-1 levels of patients with PRP are in agreement with clinical and noninvasive

test findings that these patients present no vasospastic condition in a regular environment without cold exposure.

Previous studies found a significant increase in ET-1 concentrations (Zamora *et al.*, 1990; Bierbrauer *et al.*, 1991; Kanno *et al.*, 1991) after cold provocation in patients with primary vasospastic Raynaud's phenomenon and suggested that the increased venous concentrations of ET-1 represent local release in the forearm. In our study there was a significant ET-1 increase after immersion of hand and forearm in ice water only in healthy volunteers; in patients with PRP there was a trend toward higher concentrations, but in patients with secondary Raynaud no change in ET-1 could be measured. The conflicting results show that there is obviously a rather wide range of possible individual reactions to cold exposure. An unspecific systemic effect by the cold provocation can be excluded, because the ET-1 levels from the contralateral arm not exposed to ice water were always in the normal range in all study participants and did not increase during cold exposure.

In our patients with secondary Raynaud's phenomenon due to progressive systemic sclerosis we were able to verify a typical microangiopathy of the nailfold capillaries of the scleroderma pattern by video microscopy (Maricq *et al.*, 1986; Franzeck *et al.*, 1983). In addition clinical examination and digital blood pressure measurements revealed digital artery occlusions in the hand where the cold provocation was performed. Our data suggest that in this particular group of patients obviously the vasospastic element is of minor importance. The organic pathologies with the pronounced microangiopathy and the digital artery occlusions seem to be more important. ET-1 obviously plays no major role in the cold induced vasospasm in these patients with progressive systemic sclerosis. It can be speculated that alterations in endothelial function or cell damage in these patients may even be responsible for the fact that ET-1 production in the hand and forearm is not stimulated by cold provocation.

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