A Pathogenic Role for Endothelin in Raynaud's Phenomenon?

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Plasma endothelin response to a standardized cold challenge in 7 patients with primary Raynaud's phenomenon and 7 controls using a sensitive radioimmunoassay was measured. There was no difference between resting levels of plasma endothelin in patients with primary Raynaud's phenomenon (2.6 fmol/ml) and controls (2.4 fmol/ml). A decrease in plasma endothelin levels in both groups of patients during the initial phase of the cold challenge was detected; this was more pronounced in the patients with Raynaud's phenomenon. These results suggest that there is no persistent stimulus to overproduction of endothelin. The fall in levels in patients with Raynaud's phenomenon during the initial phase of the cold challenge might suggest that a different vasoconstrictive factor is initiating the start of the vasospastic process, with the decreased endothelin levels being a reactive response to increased vasoconstriction produced by this alternative factor.

The recently discovered peptide endothelin, an extremely potent vasoconstrictor, has been implicated in the vascular changes occurring in Raynaud's phenomenon (RP). Endothelins were originally isolated from the supernatant of cultured endothelial cells and are potent constrictors of vascular smooth muscle, producing prolonged in vitro and in vivo vasoconstrictor activity (1). This group of peptides, and in particular endothelin-1 (ET-1), are therefore of natural interest in the pathogenesis of a condition where the principal abnormality is that of episodic vasospasm. Several groups (2, 3) have investigated alteration in plasma ET-1 in secondary RP; however, these findings cannot be used to draw any valid conclusions regarding the pathogenesis of primary RP. Only Zamora et al. (4) have investigated the changes in plasma ET-1 in patients with primary RP in response to a cold challenge. They showed that patients with primary RP had significantly higher resting plasma ET-1 levels than controls, with a further late rise in plasma ET-1 levels only when there had been a marked reduction in arterial pulsatility. Given the continuing debate regarding the role of ET-1, we have tried to validate this original finding, with particular emphasis on the very early dynamic changes occurring in ET-1 levels following a cold challenge.

MATERIALS AND METHODS

Patients

Seven female patients (aged 25–55) with RP, all of whom had either biphasic cold induced symmetrical colour changes or uniphasic cold induced changes that were associated with either numbness or paraesthesia, were selected. They were all non-smokers and had no other significant medical problems. General physical examination and the nailfold capillaries were normal. All were screened for antinuclear, anticentromere and scleroderma 70 antibodies profile to exclude any secondary disease; all were negative. All drug treatment for RP was stopped at least one week prior to the assays. Seven age and sex-matched controls were selected; all were non-smokers with no significant previous medical history. All were screened for the above antibodies and were negative.

Cold challenge test

The patients were rested in a constant temperature room (at 23°C) 20 min prior to the start of the investigation. A plastic cannula was inserted into a vein in the antecubital fossa at the start of this period, and only normal saline was used to maintain its patency. A tourniquet was not used, as this might have caused local release of ET-1. The cold challenge was produced by placing the hand in a sealed box and a constant stream of cool air was passed through crushed ice prior to passing over the fingers. This method has been validated as a satisfactory cold challenge for patients with RP by Goodfield et al. (5). The cold challenge lasted for 10 min; during this time blood was withdrawn from the cannula at 0, 1, 3, 5 and 10 min and 15 min after the finish of the challenge.

Serum ET measurements

Blood samples were collected in tubes containing ethylenediamine-tetra-acetate and stored in ice until the final sample was taken and then immediately centrifuged at 2,500 rpm for 15 min at 4°C. The plasma was then frozen and stored at −70°C until all samples were collected, when they were all transported in dry ice and then stored again at −70°C prior to analysis. When required, the samples were thawed at room temperature and the plasma was acidified by the addition of 2 mmol/l HCL. They were then centrifuged at 1,000 g for 5 min at room temperature and loaded onto ethyl silica mini-columns, each of which had been washed with 5 ml trichloroacetic acid in 80% methanol. The eluent was then dried under vacuum centrifugation. The radioimmunoassay was carried out using the endothelin assay system RPA 555 (Amersham International PLC, Aylesbury, Bucks, UK). Samples were incubated with 100 μl of the assay buffer, containing polyclonal antibody raised against ET-1. After vortexing, samples were incubated for up to 24 h at 4°C before buffer containing ET-1 was added. The vortexing and incubation were repeated with buffer containing donkey anti-rabbit antibody coated onto magnetizable polymer particles and incubated at room temperature for 10 min. Following magnetic separation, the level of radioactivity in the antibody-bound fraction was determined by gamma spectrophotometry. The sensitivity of the assay, defined as the amount of immunoactivity that could be distinguished from blank/zero value with a 95% confidence level, was 1 fmol/tube. All assays were performed in duplicate.

Statistical analysis

All values were expressed as means ± the standard error of the difference of the mean. For statistical evaluation the probability was related to multiples of standard deviations for a normal population (6).

RESULTS

Resting plasma endothelin levels (fmol/ml) were the same in subjects with RP (2.6 fmol/ml) and controls (2.4 fmol/ml). In both groups of patients there was an initial drop in ET-1 levels when the challenge was performed. This was more exaggerated in the patients with RP, reaching its maximum (1.2 fmol/ml) after 5 min. Thereafter, plasma ET-1 gradually increased (Table I and Fig. 1).

Table I. *Plasma endothelin-1 levels during and after cold challenge*

Values are mean levels fmol/ml plasma. SEM values are in parentheses.

Time	Control	Raynaud's
0 min	2.4 (0.45)	2.6 (0.6)
1 min	2.7 (0.55)	2.5 (0.55)
3 min	2.6 (0.6)	1.98 (0.5)
5 min	2.1 (0.6)	1.2 (0.2)
10 min	2.1 (0.5)	1.65 (0.3)
Post 15 min	2.2 (0.5)	2.8 (0.7)

DISCUSSION

RP was first described in 1862 by Maurice Raynaud (7), who believed that the abnormality lay in a defect of the sympathetic vascular innervation. However, others, the first of whom was Lewis in 1929, favoured the idea that the abnormality lay within the arteries themselves (8). To date this debate has not been satisfactorily resolved. Several different endogenous factors have been postulated to be involved in the excessive peripheral vasoconstrictive response seen in RP. These include calcitonin gene related peptide (9), platelet aggregation (10) and serotonin (11). As the most powerful vasoconstrictor yet discovered, ET-1 (2) is a likely candidate in the involvement in the pathogenesis of RP. However, the overall pressor effect of ET-1 varies; and at low concentrations it is limited by the release of endothelium-derived vasodilators, including endothelium-derived relaxing factor and prostacyclin. Unfortunately there are also inherent difficulties in assessing the role of ET-1 in the vascular tone of the peripheral circulation in both health and disease, as ET-1 secretion is directed principally at the basal side of the endothelial cell, with more than 80% of the total amount secreted towards the underlying smooth muscle. Thus plasma ET-1 represents only the overspill of ET-1 directed towards the vascular bed and those molecules that have not become attached to endothelin receptors.
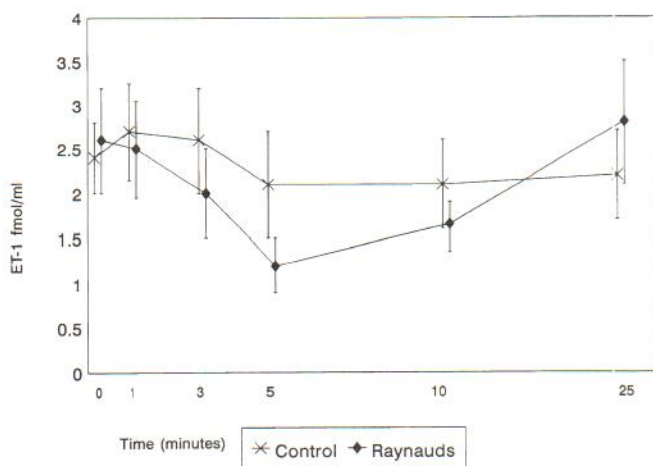

Fig. 1. ET levels during and after cold challenge.

This study has shown no difference in resting plasma ET-1 levels in patients with RD and controls. There is also an initial decrease in ET-1 levels in RD patients, which is maximal after 5 min of the cold challenge. These results suggest that there is no chronic persistent stimulus to overproduction of ET-1 and that the initial vascular response to the cold challenge in RD is not ET-1-mediated but possibly due to a reflex sympathetic hyperactivity producing a secondary temporary suppression of ET-1 levels. It is difficult to explain the difference between the normal resting level of ET-1 in RP found in this study and the raised levels found by Zamora et al. (4), even when account is taken of their use of heparin to ensure the patency of the cannula and possible use of a tourniquet, both of which might affect the measurements. The dynamic early ET-1 samples in this study are not directly comparable with those of Zamora, as no early samples were taken before symptom onset in their study.

In summary these results do not support the hypothesis of a role for ET-1 in the pathogenesis of RD and suggest that an alternative factor is operating in the early stages of an episode of RD. However, definite confirmation of the role of ET-1 must wait for the development of either specific ET-1 antagonists or the ability to accurately sample the peripheral microcirculation.

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