Haemorrhheological effects of prostaglandin E₁ infusion in Raynaud's syndrome

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SUMMARY Eighteen patients with severe Raynaud's syndrome had impaired deformability of erythrocytes, as measured by filtration through 5 μ m diameter pores, compared with 19 healthy controls. The patients were given prostaglandin E₁ (PGE₁) or placebo by intravenous infusion for 72 h to assess the haemorrhheological action of PGE₁. Contrary to a previous report, PGE₁ did not improve erythrocyte filterability. Infusion of PGE₁ did, however, evoke an acute phase response with hyperproteinaemia and a leucocytosis and is a potentially important mediator of this stress response in patients with vascular disease.

Raynaud's syndrome is characterised by episodic impairment of flow in the microcirculation of the extremities. Although the primary abnormality may lie in the peripheral vessels,¹ the blood of patients with Raynaud's syndrome shows several haemorrhheological abnormalities which may contribute to impaired flow in the microcirculation. These include plasma hyperfibrinogenaemia,^{2,3} an increase in whole blood viscosity,^{4,5} and a reduction in erythrocyte deformability.^{6,7}

Prostaglandin E₁ (PGE₁), a potent vasodilator,⁸ has been reported to improve erythrocyte deformability⁷ and also to be of clinical benefit^{9,10} in Raynaud's syndrome. We have therefore investigated the rheological effect of a 72 h infusion of PGE₁, in comparison with placebo, in 18 patients with Raynaud's syndrome.

Patients and methods

PATIENTS

Eighteen patients with severe chronic Raynaud's syndrome (Table 1), who had given informed consent, were admitted to hospital for insertion of a central venous catheter under local anaesthesia. After 24 h they were randomised to receive placebo or PGE₁ (at a dose of 10 ng/kg/min in ethanol in normal saline) by intravenous infusion for 72 h. Both the patients and the investigators were "blind" as to treatment. The patients were then discharged and assessed as outpatients after two and four weeks. Blood samples were taken, using minimal venous stasis, on admission, 24 h later (immediately

before the infusion), immediately after the infusion, and two and four weeks later.

METHODS

Erythrocyte deformability

Blood anticoagulated with 15 IU/ml dry lithium heparin (Sterilin Ltd, Teddington, Middlesex) was passed through Imugard IG 500 cotton wool (Terumo Corporation, Tokyo, Japan) and washed once with phosphate buffered saline (PBS), pH 7.4, to remove leucocytes, platelets, and plasma proteins.¹¹ The resulting erythrocytes were resuspended in PBS at a packed cell volume of about 0.07, and erythrocyte deformability (filterability) was measured at ambient temperature using a Hemorheometre SPO2 (Bell System (Telephones) Ltd, Barnet) and disposable polycarbonate membranes of 5 μ m pore diameter (Nuclepore Corporation, Pleasanton, California). The filtration time for erythrocytes was determined in relation to that of buffer alone, then adjusted for packed cell volume

Table 1 Clinical details of the 18 patients with Raynaud's syndrome

	Prostaglandin E ₁ infusion	Placebo infusion
No of patients (male)	9 (2)	9 (4)
Mean age (range) (yr)	51.2 (39-68)	42.4 (21-57)
Type of Raynaud's syndrome		
Idiopathic	6	5
Secondary	3*	4†

*Secondary to systemic sclerosis (3).

†Secondary to systemic sclerosis (2), mixed connective tissue disease (1), and calcinosis-Raynaud's syndrome-sclerodactyl-telangiectasia syndrome (1).

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Table 2 Erythrocyte deformability, measured as index of filtration, in the prostaglandin E₁ (PGE₁) treated and placebo groups

	Baseline	Immediately post-infusion	Week 2	Week 4
PGE ₁ group (n = 9)				
Mean index of filtration (SEM)	13.49 (0.36)	14.13 (0.47)	13.56 (0.34)	13.07 (0.38)
Placebo group (n = 9)				
Mean index of filtration (SEM)	13.13 (0.35)	13.73 (0.41)	14.04* (0.39)	13.68 (0.50)

*p < 0.05 compared with baseline measurement using paired Wilcoxon's ranking.

as measured by a Coulter S counter (Coulter Electronics, Luton), and expressed as an index of filtration according to Hanss.¹² An increase in index of filtration indicated loss of erythrocyte filterability.

Leucocyte and platelet counts were measured using K₂EDTA anticoagulated blood and Coulter S and Coulter Thrombocyte-Thrombocounter systems, respectively.

Plasma viscosity was measured at 25°C using K₂EDTA anticoagulated blood and a Coulter-Harkness capillary viscometer.

Plasma fibrinogen was measured by the Laurell rocket technique¹³ and serum C reactive protein by laser nephelometry¹⁴; antisera were supplied by Dako Immunoglobulins, Copenhagen, Denmark.

Statistical methods

Paired data were compared using Wilcoxon's rank sum test, and unpaired data by the Mann-Whitney U test. Yates' correction for small samples was used for χ^2 testing of frequency (Table 1).

Results

There was no significant difference in age, sex, or type of Raynaud's syndrome between the PGE₁ treated and placebo groups (Table 1). The two preinfusion test results (on admission and at 24 h) were not significantly different either between the two patient groups or within the groups, apart from a significantly raised (p < 0.05) plasma fibrinogen concentration in the placebo group at 24 h (mean 3.79 g/l, SEM 0.51) compared with the admission value (mean 3.19 g/l, SEM 0.39). The mean of the two preinfusion measurements for each group was

therefore used as the baseline for all statistical comparisons.

ERYTHROCYTE DEFORMABILITY

The mean baseline for index of filtration for all 18 patients (13.31, SEM 0.25) was significantly higher (p < 0.05) than that of a control group of 19 healthy individuals of age range 20–59 years (mean index of filtration 12.27, SEM 0.20). After the infusion of PGE₁ there was no significant change in erythrocyte filterability (Table 2) during the four week study; a significant increase (p < 0.05) in index of filtration occurred, however, in the placebo group two weeks after infusion.

ACUTE PHASE RESPONSE

An acute phase response was seen in the PGE₁ treated patients, as shown by significant increases in leucocyte count (immediately post-infusion), serum C reactive protein concentration (post-infusion), and plasma fibrinogen concentration (post-infusion, week 2, and week 4) (Table 3). Plasma hyperproteinaemia was the likely cause of the plasma hyperviscosity at weeks 2 and 4. The mean platelet count decreased significantly immediately after the PGE₁ infusion and rebounded to show a significant increase at week 2. No significant acute phase changes were seen in the placebo group (Table 4), except for an increase in plasma viscosity immediately post-infusion, thus excluding catheter insertion as the cause of the acute phase response in the treatment group.

In the PGE₁ treated group, three patients had Raynaud's syndrome secondary to systemic sclerosis and all three showed acute phase responses immedi-

Table 3 Mean (SEM) values for acute phase reactants in nine patients treated with prostaglandin E₁

	Baseline	Immediately post-infusion	Week 2	Week 4
Leucocyte count ($\times 10^9$ /l)	6.7 (0.48)	7.8 (0.64)†	6.0 (0.61)	6.1 (0.74)
C reactive protein (mg/l)	2.3 (1.7)	44.0 (11.8)*	6.6 (1.8)	2.3 (1.2)
Fibrinogen (g/l)	3.31 (0.22)	4.56 (0.31)†	4.52 (0.44)*	4.37 (0.23)*
Plasma viscosity (mPa s)	1.72 (0.03)	1.77 (0.04)	1.79 (0.03)*	1.80 (0.03)*
Platelets ($\times 10^9$ /l)	238 (21)	212 (16)*	301 (32)*	232 (21)

*p < 0.05 and † p < 0.01, compared with baseline measurement using paired Wilcoxon's ranking.

Table 4 Mean (SEM) values for acute phase reactants in nine patients treated with placebo

	Baseline	Immediately post-infusion	Week 2	Week 4
Leucocyte count ($\times 10^9/l$)	7.3 (0.66)	6.9 (0.51)	6.7 (0.70)	6.6 (0.63)
C reactive protein (mg/l)	6.8 (2.4)	14.0 (7.0)	6.9 (2.1)	8.8 (4.9)
Fibrinogen (g/l)	3.50 (0.44)	3.69 (0.39)	3.61 (0.32)	4.15 (0.37)
Plasma viscosity (mPa s)	1.69 (0.03)	1.77 (0.04)*	1.71 (0.03)	1.72 (0.03)
Platelets ($\times 10^9/l$)	252 (23)	210 (10)	268 (31)	257 (23)

*p < 0.05 compared with baseline measurement using paired Wilcoxon's ranking.

ately after the infusion—mean leucocyte count $8.7 \times 10^9/l$ (baseline 7.4), mean C reactive protein concentration 77.7 mg/l (baseline 5.1), and mean fibrinogen concentration 4.93 g/l (baseline 2.79). Although the subgroups were too small for statistical analysis, PGE₁ appears to have caused an acute phase response in systemic sclerosis as well as in the six patients with primary Raynaud's syndrome.

Discussion

Earlier reports of impaired erythrocyte deformability in patients with Raynaud's syndrome were based on filtration methods that are affected by plasma proteins⁶ and leucocytes.^{6,7} Both plasma fibrinogen¹⁵ and a small number of leucocytes^{11,16} can significantly impair filtration through 5 μ m diameter pores and give a spurious impression of loss of erythrocyte filterability (deformability). Since patients in the acute or chronic phase of vascular disease, including Raynaud's syndrome,³ may show a stress response with hyperproteinaemia and a leucocytosis,¹⁷ it is essential to remove these extrinsic contaminants before the measurement of erythrocyte filterability.¹⁸ In the present study this was achieved by prefiltration of whole blood through Imugard IG 500 cotton wool followed by washing in buffer to remove leucocytes, platelets, and plasma protein.¹¹ Using the resulting pure erythrocyte suspension we found a small loss of filterability, through 5 μ m diameter pores, of Raynaud's syndrome erythrocytes.

In vitro studies of the effect of PGE₁ on erythrocyte filterability have given conflicting results, with reports of improved filterability,^{19,20} no effect,^{21,22} and decreased filterability.²³ This variation may reflect either differences in the concentration of PGE₁ used, since improved erythrocyte filterability has been reported at 10^{-11} – 10^{-10} mol/l but not at higher concentrations, or the effects of PGE₁ on leucocytes and platelets, which may have contaminated the test erythrocyte suspensions to a variable extent. Infusion of PGE₁ in patients with Raynaud's syndrome and systemic sclerosis has been reported⁷ to increase erythrocyte filterability through 3 μ m

diameter pores; however, the method used to prepare the erythrocyte suspensions does not reliably remove sufficient leucocytes¹¹ and we have not been able to show any PGE₁ effect when testing a pure erythrocyte suspension filtered through 5 μ m diameter pores. Our findings suggest that any beneficial effect of PGE₁ in Raynaud's syndrome^{9,10} is unlikely to be mediated via an effect on erythrocytes.

The acute and chronic phase stress responses of patients with vascular disease may contribute to their haemorrhological abnormality and prothrombotic tendency.^{17,18} The nature of the presumed mediators which feed back from thrombus formation on damaged endothelium to induce hepatic protein synthesis and stimulate bone marrow release of leucocytes and platelets in these patients is, however, uncertain. One potential mechanism is the stimulation, by fibrinogen fragments D and E, of reticuloendothelial cells to release interleukin-1, which can cause hepatic synthesis of fibrinogen^{24,25} and bone marrow release of leucocytes.²⁶ Prostaglandins, however, may also be mediators of these stress responses since we have shown that PGE₁ administration to patients with Raynaud's syndrome can induce an acute phase response by the liver (hyperfibrinogenaemia and raised C reactive protein concentration) and also bone marrow (leucocytosis). In addition, previous workers have reported that PGE₁ infusion increases serum haptoglobin concentrations in animals²⁷ and also C reactive protein concentrations in patients with atherosclerosis.²⁸ Thus PGE₁, in contrast to PGE₂, which may suppress interleukin-1 production,²⁹ could evoke an acute phase response either by stimulating interleukin-1 production or by acting synergistically with it.

Patients with Raynaud's syndrome and systemic sclerosis have considerably raised plasma PGE₁ concentrations of about 5×10^{-9} mol/l³⁰ and could therefore be refractory to a PGE₁ infusion. Whicher *et al*²⁸ found no rise in C reactive protein concentration after a 72 h infusion in 11 patients with systemic sclerosis. In contrast, all three of our patients with Raynaud's syndrome and systemic sclerosis showed acute phase responses to PGE₁, and a prolonged

clinical response to PGE₁ infusion has also been reported.³¹ Thus patients with extensive vascular disease and persistently raised plasma PGE₁ concentrations do not seem to become refractory to PGE₁, which could therefore act as a mediator of the chronic phase, as well as the acute phase, stress response.

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