Effect of prostaglandin E₁ on deposition of autologous labelled platelets onto human atherosclerotic lesions *in vivo*

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Summary: The effects of infusions of prostaglandin E₁ (PGE₁) on platelet deposition at atherosclerotic sites *in vivo* in man have been examined. Thirteen patients with atherosclerotic vascular disease received intravenous PGE₁ 25 mg/kg/min for 6 hours daily for 5 days. Prostaglandin E₁ had no effect on platelet uptake at atherosclerotic sites but prolonged platelet half-life significantly ($P < 0.001$).

Introduction

Abnormalities of epoprostenol (prostacyclin, PGI₂) generation have been implicated¹ in the development of human atherosclerosis and both PGI₂² and prostaglandin E₁ (PGE₁)³ have been advocated for the treatment of atherosclerotic ischaemic vascular disease. The development of stable and orally active drugs which mimic the effects of these compounds in inhibiting platelet aggregation has been suggested as a long term goal in the treatment of atherosclerosis and the prevention of its thromboembolic complications.⁴ The results of clinical trials with both PGI₂⁵ and PGE₁⁶ have so far been conflicting. Recently it has been possible to monitor the effects of these drugs on platelet deposition at sites of atherosclerosis *in vivo* in man. This has been achieved using gamma-camera imaging of human atherosclerotic lesions after ¹¹¹In-oxine-sulphate labelling of autologous platelets.⁷ With this technique we have demonstrated that PGI₂ reduced platelet deposition at sites of active human atherosclerotic lesions as monitored by a decrease in the platelet uptake ratio.⁸ This effect was surprisingly long lasting after discontinuing infusion of PGI₂⁸ and similar results were obtained for implanted vascular grafts.⁹ The present study examines for the first time the effects of PGE₁ on platelet deposition at atherosclerotic sites *in vivo* in man.

Materials and methods

Platelet labelling was carried out using a kit developed by one of us.¹⁰ Essentially, 16 ml of blood withdrawn

from an antecubital vein was added to 4 ml acid-citrate-dextrose as anticoagulant. After 5 minutes sedimentation at room temperature platelet-rich plasma was obtained by centrifugation at 150 g for 5 minutes. The platelet-rich plasma was transferred to another cuvette and a platelet pellet obtained by further centrifugation at 500 g. This platelet pellet was dissolved in 1,000 µl of tyrode buffer (pH 6.2) and 100 µCi of ¹¹¹In-oxine-sulphate added followed by incubation at 37°C for 5 minutes. The incubation mixture containing the labelled platelets was resuspended in platelet-poor plasma and the solution reinjected into the patient. Platelet half-lives were calculated from counts of radioactivity in blood samples (2 ml) obtained from each patient three times daily.

Platelet uptake ratios were obtained by gamma-camera imaging using a 5 minute response time. The radioactivity over the lesion was quantified by counting radioactivity in an area over the lesion (region of interest) and comparing the counts with those obtained over a similar area on the contralateral side.

Regions of interest were selected on the basis of appearance on gamma-camera imaging over the site of the active lesion. For comparative purposes, a region of the same size was inserted (a computer manipulation) on the contralateral side and counts over this area compared with those on the side of the active lesion.

The platelet uptake ratio thus derived was calculated repeatedly to assess the effect, if any, of PGE₁. A fall in platelet uptake ratio implies that the number of radio-labelled platelets over the lesion has decreased and an increase in the ratio the converse. Radiolabelled platelets circulate in the blood for their whole life span and the radiolabel is not substantially

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released or reutilized. Platelet uptake ratios of greater than 1.30 and less than 1.20 have been taken to represent active and inactive atherosclerotic lesion, in terms of platelet deposition.^{8,9}

Thirteen patients with ischaemic atherosclerotic peripheral vascular disease (Fontaine stage II) were studied. Their age ranged from 47 to 61 years, 9 were male and 4 were female. The patients studied were selected from a larger group of similar patients on the basis of the results of autologous platelet labelling and gamma-camera imaging. Six of the 13 patients selected (4 male and 2 female) had 'active' atherosclerotic lesions characterized by a platelet uptake ratio of greater than 1.30 and 7 patients (5 male and 2 female) had 'inactive' lesions with a platelet uptake ratio of less than 1.20. Written informed consent was obtained from all patients included in the study.

For all thirteen patients, platelets were labelled *ex vivo* and re-infused as described above. The platelet uptake ratio was then measured by gamma-camera visualization over the previously identified atherosclerotic lesion site. This measurement was carried out on the day of platelet labelling and again on the morning of the following day. An intravenous infusion of PGE₁ was then administered to each patient at a dose of PGE₁ 25 mg/kg/minute for 6 hours daily for 5 days. The platelet uptake ratio was measured again by gamma-camera imaging, before starting infusion of PGE₁, just before each infusion ended and also 2 hours later.

Statistical significance of differences was assessed using Student's *t*-test.

Results

The platelet half-life was similar in patients having lesions identified as active or inactive (Table I). These platelet half-lives are shorter than the normal range for

this laboratory (100–115 hours). The platelet uptake ratio for the 'active' atherosclerotic lesions showed no significant reduction during PGE₁ infusion (Figure 1). There was no change in platelet uptake ratio over 'inactive' lesions during PGE₁ infusion. However, a small but significant ($P < 0.01$) prolongation of platelet half-life occurred in both groups of patients in response to infusion of PGE₁ (Table I).

Discussion

It is more than 10 years since the first claims¹¹ that PGE₁ was of benefit in the treatment of ischaemic peripheral vascular disease. Prostaglandin E₁ at doses between 1 and 10 ng/kg/min administered intra-arterially increases calf blood flow.¹² Despite the extensive first pass metabolism of PGE₁ in the lung, intravenous infusion of PGE₁ 20 ng/kg/min has been shown to be equivalent to intra-arterial administration of 0.1 to 0.3 ng/kg/min in terms of increase in blood flow.

The therapeutic benefit from PGE₁ administered intravenously^{11–14} or intra-arterially^{3,15,16} has been inconsistent. Prostaglandin E₁ administered to 14 patients at a dose of 20 ng/kg/min intravenously has been reported to produce clinical benefit in patients with ischaemic vascular disease.¹⁷ However, a large double-blind controlled trial⁶ of intravenous PGE₁ showed no beneficial effect of PGE₁ in patients suffering from ulcers secondary to ischaemic vascular disease. In this study the spontaneous healing rate of around 50% in the placebo group was not exceeded in the PGE₁ treated group.

We have not yet directly compared the effects of PGE₁ and of PGI₂ on *in vivo* platelet deposition. However, the results of the present study suggest that the effects of PGE₁ are much smaller than those reported for PGI₂ under very similar conditions.⁸ We chose an intermittent infusion scheme for the present

Figure 1 Effect of PGE₁ on platelet uptake ratio (PUR). PGE₁ 20 ng/kg/min was administered on days 2–6 of the second labelling occasion. PUR was measured just before start, just before end and 2 hours after end of each daily infusion period. Values are mean $\pm$ standard deviation.

Table 1 Effect of PGE₁ on platelet uptake ratio (PUR) and platelet half-life (*t*₁) in patients with active and with inactive atherosclerotic lesions. Values shown as mean $\pm$ standard deviation.

	Active lesions	Inactive lesions
n	6	7
PUR	1.33 $\pm$ 0.05	1.14 $\pm$ 0.04
<i>t</i> ₁ before PGE ₁	76 $\pm$ 8	78 $\pm$ 7
<i>t</i> ₁ after PGE ₁	*82 $\pm$ 6	*83 $\pm$ 5

*Indicates significant difference between values before and after PGE₁ ($P < 0.01$).

study because significant platelet rebound after PGE₁ has been reported during continuous infusion¹⁸ and also in order to duplicate the infusion schedules used previously for PGI₂. In addition, the prolongation of platelet half-life seen in the present study is less than we have observed in similar studies with PGI₂ (unpublished data). The findings with PGE₁ given intravenously at 20 ng/kg/min indicate that there is a modest

beneficial effect on *in vivo* platelet consumption. Whether this is sufficient to produce therapeutic improvement in patients with atherosclerotic peripheral vascular disease is uncertain. It remains to be established whether other infusion regimes perhaps using different doses and even different routes of administration might improve on the results found in the present study.

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