Development of optimal infusion regimens for epoprostenol using radio labelled platelet uptake over atherosclerotic lesions in man

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1 Epoprostenol (prostacyclin, PGI₂) has been evaluated in clinical trials in peripheral vascular disease and other conditions chiefly on the basis of its platelet inhibitory properties. These therapeutic evaluations have proceeded in the absence of evidence as to the optimum infusion regimen for epoprostenol and the choice of schedules of administration has been arbitrary. We have tried to establish an optimum infusion regimen in patients with peripheral vascular disease in terms of maximal inhibition of platelet deposition on atherosclerotic lesions *in vivo* together with maximal inhibition of platelet aggregation *ex vivo*.

2 One hundred and twenty three patients with atherosclerotic peripheral vascular disease and increased platelet uptake at atherosclerotic sites were selected. Epoprostenol was administered at a fixed dose of 5 mg kg⁻¹ min⁻¹ for 0.5–24 h daily for 3–7 days.

3 Infusion of epoprostenol for 6 h daily for up to 5 days caused maximum decrease in platelet uptake without tachyphylaxis and without loss of the inhibitory effect of epoprostenol on platelet aggregation responses. Longer daily infusion periods were associated with progressive loss of the anti-aggregatory effect of epoprostenol without any greater decrease in platelet uptake. Shorter daily infusion periods produced smaller decreases in platelet uptake.

Keywords epoprostenol platelet atherosclerosis

Introduction

Epoprostenol (prostacyclin, PGI₂), a potent inhibitor of platelet aggregation and a vasodilator, is predominantly synthesized by the endothelial lining of blood vessels (Moncada & Vane, 1978). Generation of epoprostenol has been suggested as an important mechanism for protecting against inappropriate intra-vascular thrombosis (Moncada & Vane, 1978). Decreased production of epoprostenol by atherosclerotic vascular tissue from experimental animals and man together with early claims of low blood levels of 6 oxo

PGF_{1α}, an epoprostenol metabolite, led to the hypothesis that the thromboembolic complications of atherosclerotic vascular disease might be due to a deficiency of epoprostenol production (Moncada & Vane, 1981). On this basis many clinical trials of epoprostenol in patients with ischaemic vascular disease were carried out. The conflicting results (Lewis & Dollery, 1983) obtained may have resulted in part from inadequacies of design and of patient numbers. In addition clinical trials in this condition employed

a variety of infusion regimens for epoprostenol ranging from infusion 12 h daily for 3 days (Olsson & Nilsson, 1981) to continuous infusion for 7 days (Hossmann *et al.*, 1984). These schedules were empirical and based on no obvious scientific rationale. However, the infusion schedule may be a critical determinant of the effect of epoprostenol since rebound platelet activation (Sinzinger *et al.*, 1981) occurs after prolonged continuous infusion.

The extent of platelet deposition on human atherosclerotic lesions can be quantified *in vivo* by gamma camera imaging from autologous platelet labelling (Sinzinger & Fitscha, 1984). Whereas the majority of atheromatous lesions do not show enhanced platelet deposition, some lesions do, positive imaging developing within a few hours of labelling and persisting for at least 7 days. We have employed this measure of platelet deposition *in vivo*, together with conventional measurement of platelet aggregation responses *ex vivo*, to measure the effects of different schedules of epoprostenol administration.

Methods

Data were obtained from a total of 123 patients aged 45–70 years with symptomatic ischaemic peripheral vascular disease (Fontaine stages IIa or IIb). None had diabetes mellitus or hyperlipidaemia. Patients received epoprostenol at a fixed dose of $5 \text{ ng kg}^{-1} \text{ min}^{-1}$ according to various schedules as shown in Table 1. The results of several studies carried out under similar conditions have been collated.

Immediately before the start and before the end of each daily infusion measurements were

made of peripheral platelet count, platelet aggregation responses to adenosine diphosphate (ADP), platelet sensitivity to the antiaggregatory effect of epoprostenol *ex vivo* and platelet uptake of autologous labelled platelets over atherosclerotic sites. Platelet half-lives were calculated for each patient during each separate infusion schedule.

Autologous platelet labelling was performed using the Thrombo Kit (Sinzinger *et al.*, 1984a) with $100 \text{ } \mu\text{Ci } ^{111}\text{In}$ -oxine sulphate. Platelet uptake was expressed as platelet uptake ratio (PUR), the ratio of radioactive counts in a region of interest inserted over the lesion as compared with the counts in a similar region of interest over the contralateral artery. The higher the PUR then the more active the platelet deposition on the lesion. Gamma camera images were obtained for 4–5 min exposure. Platelet half-life was calculated from the radioactive counts in 2 ml blood samples withdrawn 2–3 times daily for 7 days.

Platelet aggregation responses were measured using a Payton aggregometer. Aggregation was induced by $1 \text{ } \mu\text{M}$ ADP in $100 \text{ } \mu\text{l}$ added to $600 \text{ } \mu\text{l}$ platelet rich plasma (PRP). Platelet counts were adjusted to $250 \times 10^3/\mu\text{l}$ by addition of autologous platelet poor plasma. Platelet sensitivity to the antiaggregatory effects of epoprostenol was measured as described previously (Sinzinger *et al.*, 1984b) using at least three concentrations of synthetic epoprostenol. Values were expressed as the concentration of epoprostenol in ng ml^{-1} (ID_{50}) needed to inhibit aggregation responses in PRP by 50%.

Results were analysed using analysis of variance and significance of differences assessed by

Table 1 Epoprostenol infusion schedules and patient characteristics (n = total number of patients in each treatment group)

Duration of infusion (h day ⁻¹)	Number of days	n	M	F	Age (years)
24	3	10	6	4	51–67
24	5	10	6	4	55–66
24	7	11	9	2	51–69
12	7	8	5	3	49–67
10	7	9	4	5	52–65
10	5	10	7	3	49–66
8	5	11	5	6	54–70
6	5	14	10	4	50–68
4	5	6	5	1	54–67
2	5	9	7	2	53–68
1	5	7	5	2	54–66
0.5	5	9	4	5	53–69
0.5 × 2	5	9	6	3	55–64

M male, F female

Student's *t*-test. Values are shown throughout as mean $\pm$ s.d.

Epoprostenol for infusion was synthesized by Upjohn and formulated and supplied by the Wellcome Foundation Limited. Epoprostenol for *in vitro* use was synthesised and supplied by Upjohn.

Results

Uninterrupted infusion of epoprostenol for more than 72 h causes platelet activation. Thus the initial inhibition of platelet aggregation (Figure 1) observed on day 1 disappears between the second and third day of infusion and continuing infusion thereafter results in a progressively increased aggregation response to ADP. In contrast intermittent infusion of epoprostenol for 6 h daily (Figure 1), though allowing complete recovery of aggregation responses between infusion periods, is associated with identical inhibition of aggregation in response to each daily infusion of epoprostenol. Similar responses are seen in terms of platelet sensitivity to epoprostenol *ex vivo* (Figure 2). Continuous infusion results in a progressive decrease in sensitivity of the platelet to epoprostenol, this decrease beginning between days 2 and 3 of infusion. Intermittent infusion for 6 h daily however, produces no change in platelet sensitivity to epoprostenol. All daily infusion periods exceeding 6 h were associated with similar degrees of

platelet activation while decreasing the infusion period below 6 h conferred no measurable benefit in terms of these platelet responses.

The deposition of labelled platelets at atherosclerotic sites as visualized by gamma camera imaging is shown in Figure 3 together with the effects of epoprostenol infusion. The platelet uptake ratios (Figure 4) show that daily epoprostenol infusion periods of 1 and 2 h are much less effective in decreasing platelet uptake than infusion periods of longer duration. However, the results from 6, 8 and 10 h daily infusion periods were similar.

Platelet half-life (Figure 5) was not significantly altered by daily infusion periods of 1 and 2 h but was increased by an infusion period of 4 h and further increased by infusion periods of 6, 8 and 10 h, the half-lives for the last three infusion periods being similar.

Discussion

As judged by the variables measured in this study, in patients with peripheral vascular disease, the shortest infusion schedule for epoprostenol at a dose of $5 \text{ ng kg}^{-1} \text{ min}^{-1}$ which produces a maximal response is a 6 h daily infusion period. Such a regimen results in a maximal decrease in platelet deposition at atherosclerotic sites *in vivo* without loss of the sensitivity of the platelets to the anti-aggregatory effects of epoprostenol and without development of hyper-aggregability.

Figure 1 Platelet aggregation responses to ADP ($1 \mu\text{M}$) during continuous (—●—) and 6 h daily (—○—) infusion of epoprostenol $5 \text{ ng kg}^{-1} \text{ min}^{-1}$. Results shown as mean ($\pm$ s.d.) percentage change in maximal amplitude of aggregation responses from pre-infusion values. $n = 10$, * indicates significant difference between groups ($P < 0.01$).

Figure 2 Platelet sensitivity to epoprostenol *ex vivo* during continuous (—●—) and 6 h daily (—○—) infusion of epoprostenol (PGI₂) 5 ng kg⁻¹ min⁻¹. Results shown as mean (± s.d.) concentration of epoprostenol required to produce 50% inhibition of aggregation response to ADP. *n* = 10, * indicates significant difference between groups (*P* < 0.01).

Longer or shorter daily infusion periods resulted in one or both of these disadvantages. It is fortuitous that although infusion periods of 8 and 10 h were equally effective, a 6 h infusion regimen in the context of clinical trials of epoprostenol in patients with ischaemic peripheral vascular disease would have practical advantages and would interfere less with mobilization of the patients than more prolonged infusion periods.

Though inhibition of platelet uptake has been shown to correlate with anti-aggregatory potency in experimental models (Steele *et al.*, 1983) its immediate clinical relevance is unknown. Neither is it clear whether such a change in platelet activity is attributable to decreased platelet aggregate formation, decreased rate of accumulation of platelets or change in the residence time of platelets at the site of local vascular pathology. Changes with time in the thrombogenicity and platelet attractant properties of vascular surfaces have been demonstrated in experimental animals on morphological examination (Kinlough-Rathbone *et al.*, 1983) and in man using platelet labelling (Sinzinger *et al.*, 1984c). Hence treatment of atherosclerotic vascular disease might more usefully be directed to patients with early rather than later disease and toward patients who have active but perhaps clinically symptomatic vascular lesions such that the cycle of enhanced platelet deposition can be interrupted. At the moment it is speculative whether such intervention would delay the progress of atherosclerotic lesions and the incidence of thromboembolic events in the long term.

While some investigators (Angelo *et al.*, 1978) have found the capacity of atherosclerotic vessels to produce epoprostenol *in vitro* is less than that of healthy vessels, others have not (Weksler *et al.*, 1985). Indeed, epoprostenol biosynthesis *in vivo* is increased (Fitzgerald *et al.*, 1984) in patients with severe atherosclerotic disease, questioning the rationale for the use of epoprostenol as a 'substitution therapy'.

The finding of platelet hyper-aggregability associated with more prolonged daily infusion periods is consistent with reports from others for epoprostenol (Blattler *et al.*, 1981) and for a chemically stable analogue (T. Kraus, personal communication). The pathophysiological features of such a desensitized platelet population *in vivo* have not been established and the clinical relevance is uncertain. Others have questioned whether myocardial infarction occurring in a child receiving epoprostenol infusion for pulmonary hypertension has resulted from these phenomena (Drummond *et al.*, 1982). However, despite the finding of such desensitization with prolonged daily infusion periods in the present study such infusion regimens were still associated with a prolongation in the platelet half-life. It would seem from this then that desensitization of the platelets and hyper-aggregability in *ex vivo* tests does not necessarily correlate with an increased rate of platelet consumption *in vivo*. Further investigation of these aspects is in progress. In the meantime, as inhibition of platelet aggregation has been one of the goals of epoprostenol administration, it seems reasonable

Figure 4 Platelet uptake ratio (PUR), as mean in response to 5 days infusion of epoprostenol $5 \text{ ng kg}^{-1} \text{ min}^{-1}$ administered for different daily infusion times: ■ 1 h, ▲ 2 h, □ 4 h, ● 8 h, ○ 6 h, △ 10 h.

to avoid infusion regimens which achieve the opposite effect.

For the purposes of the present study a single dose of epoprostenol $5 \text{ ng kg}^{-1} \text{ min}^{-1}$ was chosen as the highest dose consistently well tolerated by patients and as the dose used in some recent major clinical trials. Further studies should examine the feasibility of using lower dose rates. These might minimize occurrence of side effects and facilitate the conduct of double-blind trials with epoprostenol.

It may also be appropriate to question whether therapeutic benefits claimed from epoprostenol administration, if borne out in properly controlled trials, are the result of its actions on the platelet or rather a consequence of its vasodilator or of its so-called 'cytoprotective' properties. As an extreme example the reported beneficial effects of epoprostenol in pulmonary hypertension (Rubin *et al.*, 1982) are clearly related to its

Figure 5 Platelet half-life and the effect of epoprostenol (PGI_2) administered for different daily infusion periods: △ 1 h, ● 2 h, ■ 4 h, ▲ 6 h, □ 8 h, ○ 10 h. Same patients as in Figure 4. Daily epoprostenol infusion periods of 4 h and greater significantly ($P < 0.01$) prolong platelet half-life, values for 6, 8 and 10 h infusion periods were similar.

vasodilator effects while its platelet inhibitory properties are responsible for epoprostenol's application in facilitating haemodialysis in the absence of heparin (Zusman *et al.*, 1982). In clinical trials in some indications as in acute myocardial infarction, stable angina and acute non-haemorrhagic stroke, it has been unclear which pharmacological property of the drug it has been intended to exhibit and often no attempt has been made to select an appropriate infusion schedule. This makes it difficult to assess the results of such trials, particularly when negative results might have occurred because of the use of inappropriate dosing schedules.

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