The effect of a prostaglandin DP-receptor partial agonist (192C86) on platelet aggregation and the cardiovascular system in healthy volunteers

S. J. GRAY*, H. GILES¹ & J. POSNER

Departments of Clinical Pharmacology and ¹Biochemical Sciences, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS

- 1 PGD₂ (DP)-receptors mediate inhibition of platelet aggregation and vasodilatation. If receptor reserve were greater on platelets it might be possible to separate these effects. To determine whether such a difference in receptor reserve exists, we have examined the effects of a highly selective DP-receptor partial agonist 192C86 on platelet aggregation and the cardiovascular system in healthy volunteers.
- 2 Using an open, dose-escalating study design, four male volunteers received constant rate intravenous infusions of 192C86 for up to 60 min. *Ex vivo* platelet aggregation to ADP and collagen in platelet-rich plasma (PRP) and whole blood (WB) was studied at baseline, after 15, 30 and 60 min of each infusion and at 180 min post-infusion. Heart rate (HR), systolic and diastolic (DBP) blood pressure were measured at frequent intervals. Adverse experiences were monitored by checklist. Facial flushing was assessed by the volunteer using a visual analogue scale, by an observer using a numerical scale and by full-face colour photographs. Blood was taken for assay of plasma 192C86 concentrations by radio-immunoassay (r.i.a.).
- 3 192C86 (0.007–0.058 µg kg⁻¹ min⁻¹) inhibited platelet aggregation to ADP and collagen both in PRP and WB in a dose-dependent manner. However, this was always accompanied by a decrease in DBP, increase in HR and facial flushing. Plasma concentrations of 192C86 were at or below the limits of sensitivity of the r.i.a. (0.5 ng ml⁻¹).
- 4 The highest infusion rate was stopped after 20 min due to symptomatic hypotension on standing. Principal reported adverse experiences were related to symptoms of vasodilatation (facial flushing, nasal stuffiness and headache) and their severity was dose-related.
- 5 The dose-response relationships for inhibition of platelet aggregation and vasodilatation by 192C86 were not separable and there was no evidence of a difference in receptor reserve which could be exploited to obtain selective inhibition of platelet aggregation by DP-receptor partial agonists.

Keywords 192C86 DP-receptors platelet aggregation cardiovascular healthy volunteers

Introduction

Blood platelets play an important role in the pathophysiology of arterial thrombosis and aspirin, which inhibits platelet behaviour, has been shown to reduce the incidence of thromboembolic disorders such as myocardial infarction and stroke (Antiplatelet Trialists'

Collaboration, 1988; Patrono, 1989; Steering Committee of the Physicians Health Study Research Group, 1989). Pharmacological agents which increase the levels of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in platelets also inhibit platelet behaviour (Haslam, 1975).

Correspondence: Dr H. Giles, Biochemical Sciences, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS

*Present address: Amgen Ltd, 321 Cambridge Science Park, Milton Road, Cambridge CB4 4WG

Prostaglandin (PG) I_2 and PGD_2 act on separate and specific receptors to increase cellular cyclic AMP levels (Miller & Gorman, 1979). PGI_2 (epoprostenol sodium; Flolan) has been developed and marketed for use as an anti-thrombotic agent but vasodilatation usually accompanies its inhibitory effect on platelet aggregation (Data *et al.*, 1981; Fitzgerald *et al.*, 1979; Hassan *et al.*, 1982; O'Grady *et al.*, 1979; Reece *et al.*, 1983). In an attempt to obtain a more selective agent, research has been directed towards examining and comparing the receptors for prostanoids (in particular PGD_2) on platelets and blood vessels.

The PGD_2 (DP)-receptors on platelets and vascular smooth muscle have been shown to be of the same type (Giles *et al.*, 1989; Leff & Giles, 1992). Therefore, selective inhibition of platelet aggregation could only be achieved if the receptor reserve were greater on platelets than on blood vessels. This hypothesis can only be tested in man, since DP-receptor density patterns are not consistent between species (Town *et al.*, 1983; Whittle *et al.*, 1978) and also because animal models are of limited value for assessing vasodilatation which manifests as headache, facial flushing and nasal stuffiness in man.

192C86 [(±)-5-(3-carboxypropylthio)-1-(2-cyclohexyl-2-hydroxyethylideneamino)-3-ethylhexahydrocyclopentadimidazol-2(1H)-one] is a highly selective DP-receptor agonist. The compound is of low pharmacological efficacy and behaves as a partial agonist in tissues with high DP-receptor reserve, but in tissues with a lower receptor reserve it is without agonist effects (Leff & Giles, 1992). For example, in human platelets a supra-maximal concentration of 192C86 (10 μM) causes 50% inhibition of ADP-induced aggregation, but elicits minimal relaxation of histamine-contracted rings of rabbit jugular vein. Animal toxicology of 192C86 was entirely satisfactory and this compound was therefore chosen to test the hypothesis that if there were a greater DP-receptor reserve on platelets than on the vasculature in man, 192C86 could inhibit platelet aggregation at concentrations that are without effects on the cardiovascular system.

Methods

Four healthy male volunteers of mean age 31.3 years (range 26–34) and mean weight 67.3 kg (range 56.7–82.7) were recruited from the Volunteer Panel of Wellcome Foundation employees. All subjects were non-smokers in good general health with no abnormal findings on physical examination, electrocardiogram, lung function tests, full blood count, plasma biochemistry, urinalysis and tests of platelet aggregation and coagulation. The volunteers abstained from ingestion of any other drugs (in particular those known to inhibit PG synthesis) for at least 14 days prior to and subsequently during the study. The subjects gave written informed consent before the start of the study and received an honorarium on its completion. The study protocol was approved by an independent ethics committee.

Study design

Using an open, dose-escalating study design, volunteers received constant rate intravenous infusions of 192C86

for up to 60 min. The infusion rates were 0.007, 0.014, 0.028 and 0.058 $\mu g\ kg^{-1}\ min^{-1}$, administered with an interval of at least 7 days between occasions. *Ex vivo* platelet aggregation to adenosine diphosphate (ADP) and collagen in platelet-rich plasma (PRP) and whole blood (WB) was studied at baseline, after 15, 30 and 60 min of each infusion and at 180 min post-infusion. Heart rate, systolic and diastolic blood pressure were measured at frequent intervals. Facial flushing was assessed by the volunteers using a visual analogue scale, by an observer using a numerical scale and by full-face colour photographs. Adverse experiences were recorded using a checklist and blood was taken for assay of plasma 192C86 concentrations by radio-immunoassay (r.i.a.). A full 12 lead ECG and measurement of lung function were carried out before and after the infusion. Full blood count and plasma biochemistry were monitored before and 24 h after each study occasion.

Cannulation and infusions

At the beginning of each study day, a teflon cannula was inserted into an antecubital vein of both the left and right arms under local anaesthesia. One cannula was used to infuse 192C86 and the other for blood sampling. This second cannula was kept patent with an occluding stylet, and at each sampling point the first 2 ml of blood was discarded. Immediately before commencing infusions, the 192C86 stock solution (1 mg ml^{-1} in a vehicle containing 0.25 M sodium hydroxide, Tris USP and Glycine BP; pH 9.5) was diluted with saline to give the required concentration for infusion in a volume of 40 ml. A Braun 'Perfusor' pump was used to administer the 192C86 solutions at a rate of 30 $ml\ h^{-1}$.

Platelet aggregation

For platelet aggregation studies two samples of blood (4.5 ml and 9 ml) were placed in polystyrene tubes containing 3.18% (w/v) trisodium citrate dihydrate (ratio 9:1 v/v) as anticoagulant. PRP was prepared by centrifuging the 10 ml sample for 3 min at slow speed setting in a Petalfuge (Ortho Diagnostics Systems) and platelet-poor plasma (PPP) by recentrifuging the sample for 4 min at high speed setting after the PRP had been removed. Platelet aggregation in PRP was measured using the Payton dual-channel aggregometer (Model 300 BD-5), calibrated with both PRP and PPP at each time-point. Concentration-effect curves to collagen (range 0.1–10 $\mu g\ ml^{-1}$) and at least three concentrations of ADP (range 0.5–20 mM) were constructed. EC_{50} values (concentration of collagen causing 50% of maximum response) were estimated graphically and used to calculate the rightward shift of the collagen curve during 192C86 infusion. Platelet aggregation in WB was examined in the 5 ml sample with one concentration of collagen (0.5 $\mu g\ ml^{-1}$) and ADP (2 μM) using the Ultra-Flo 100 Whole Blood Platelet Counter (Fox *et al.*, 1982; Lumley & Humphrey, 1981). The number of single platelets in WB was measured at baseline, after 2, 4, 6 and 8 min of adding collagen and after 1, 2, 4, 6 and 8 min of adding ADP. The percent of single platelets present at each time point (compared with baseline) was then plotted

against time and the area under the aggregation-time curve measured using the linear trapezoidal rule.

Heart rate, systolic and diastolic blood pressure

Heart rate, systolic and diastolic blood pressure were measured supine and after 2 min standing using a Hewlett Packard cardiovascular monitor (Model HP78352A), which employs an oscillometric method for determination of blood pressure.

Facial flushing

Facial flushing was assessed at baseline, at 15, 30 and 60 min of each infusion and at 30, 60, 90, 120 and 180 min post-infusion. Volunteers were presented with a 100 mm visual analogue scale for the sensation of skin flushing which was annotated 'None' and 'Maximum'. A single observer also performed subjective assessments of facial flushing in volunteers using a three point numerical scale (0 = none, 1 = mild, 2 = moderate, 3 = severe). A 2.25" square colour transparency was also taken at these times using a Hasselblad single lens reflex camera with a lens of 150 mm focal length. Photographs were taken at a fixed distance from the volunteer and lighting conditions were standardised using two Bowen's mono-Lite 400 Joule flash heads positioned at 45° to the subject.

Adverse experiences

Volunteers were presented with an adverse experience checklist immediately after each assessment of facial flushing. They were requested to score the severity of any symptoms on a three point numerical scale (0 = none, 1 = mild, 2 = moderate, 3 = severe).

Assay of 192C86

Blood samples (4 ml) were taken at baseline, after 15, 30 and 60 min of each infusion and at 30, 60, 90, 120 and 180 min post-infusion and placed in tubes containing lithium-heparin as anti-coagulant for assay of 192C86 plasma concentrations using a double-antibody r.i.a.

Safety procedures

The 12 lead ECG was recorded using a Marquette cardiographic computer (MAC PC). Lung function, forced expiratory volume after 1 s (FEV₁), was measured using either a Vitalograph dry-wedge spirometer (S-model) or a portable turbine spirometer (Micro-Medical Ltd). For each study day only one type of spirometer was used. The mean of three consecutive values of FEV₁, considered to have been performed in a technically satisfactory manner, were recorded. Blood samples (5 ml) were taken in tubes containing EDTA and lithium-heparin as anti-coagulant for measurement of full blood counts and plasma biochemistry respectively. Blood counts were performed using a Coulter Counter (Model-S) and plasma biochemistry was determined using a Roche Diagnostics Analyser.

Data analysis

As there was no control group and only four subjects were studied, no formal statistical analysis was performed on the pharmacodynamic or pharmacokinetic data.

Results

All four subjects completed the first two study occasions, on which 192C86 was infused at 0.007 and 0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$ and one subject completed a study occasion on which the infusion rate was 0.028 $\mu\text{g kg}^{-1} \text{min}^{-1}$. However, when the rate was increased to 0.058 $\mu\text{g kg}^{-1} \text{min}^{-1}$ in one subject, 192C86 was poorly tolerated and the infusion was stopped after 20 min due to symptomatic hypotension on standing.

Platelet aggregation

The effects of 192C86 on collagen-induced aggregation in PRP are shown in Figures 1 and 2 and Table 1. 192C86 inhibited platelet aggregation to a concentration of collagen causing > 70% maximal aggregation and the maximum extent of this inhibition increased in a dose-dependent manner as the infusion rate increased from 0.007 to 0.058 $\mu\text{g kg}^{-1} \text{min}^{-1}$ (Figure 1). At the lower infusion rates (0.007 and 0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$) inhibition of aggregation increased during the infusion period, with maximum inhibition at 60 min and only partial return to baseline by 3 h post-infusion. At the two higher

Figure 1 Effect of 192C86 on collagen-induced aggregation in PRP. Results are expressed as a percent of the response to a concentration of collagen causing maximal aggregation and are mean $\pm$ s.e. mean at the 0.007 and 0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$ infusion rates.

Figure 2 Collagen dose-response curves in PRP prior to (●) and following (○) 60 min infusions of $0.014 \mu\text{g kg}^{-1} \text{min}^{-1}$ 192C86.

Table 1 Mean and range for the maximum rightward shift of the collagen dose-response curve for platelet aggregation in PRP during 192C86 infusions compared to values pre-infusion

Infusion rate ($\mu\text{g kg}^{-1} \text{min}^{-1}$)	log shift	fold shift
0.007 ($n = 4$)	0.17 (0.025–0.275)	1.5 (1.1–1.9)
0.014 ($n = 4$)	0.40 (0.11–0.6)	2.5 (1.3–4.0)
0.028 ($n = 1$)	0.71	5.0
0.058 ($n = 1$)*	0.50	3.0

*Infusion stopped after 20 min.

infusion rates (0.028 and $0.058 \mu\text{g kg}^{-1} \text{min}^{-1}$) platelet aggregation to the selected concentration of collagen was completely inhibited after 15 min of the infusion and aggregation remained inhibited up to 3 h post-infusion for the $0.028 \mu\text{g kg}^{-1} \text{min}^{-1}$ dose, although some reversal of the effect was apparent from a reduction in the rightward-shift of the collagen dose-response curve. The infusion of $0.058 \mu\text{g kg}^{-1} \text{min}^{-1}$ was terminated after 20 min and platelet aggregation had returned to baseline by 60 min. The mean maximum rightward-shifts of the collagen dose-response curve during infusions of 192C86 increased in a dose-dependent manner as the infusion rate increased (Table 1), with the greatest shift occurring after 60 min infusion of $0.028 \mu\text{g kg}^{-1} \text{min}^{-1}$ (0.71 log units or 5 fold). 192C86 was less effective at inhibiting ADP-induced aggregation in PRP as it had no effect on platelet aggregation induced by $5 \mu\text{M}$ ADP, a concentration which caused $> 70\%$ maximal aggregation. However, when the concentration of ADP was lower and elicited just $> 40\%$ maximal aggregation, 192C86 had a similar effect to that observed on collagen-induced aggregation.

The effects of 192C86 on collagen-induced aggregation in WB are shown in Figure 3. 192C86 inhibited the maximum extent of platelet aggregation to $0.5 \mu\text{g ml}^{-1}$ collagen in a dose-dependent manner as the infusion rate increased from 0.007 to $0.028 \mu\text{g kg}^{-1} \text{min}^{-1}$. At these infusion rates inhibition of aggregation also in-

Figure 3 Effect of 192C86 on collagen-induced aggregation in WB. Results are expressed as the area under the aggregation-time curve and are mean $\pm$ s.e. mean at the 0.007 and $0.014 \mu\text{g kg}^{-1} \text{min}^{-1}$ infusion rates.

creased during the infusion period, with maximum inhibition at 60 min and only partial return to baseline by 3 h post-infusion. At the highest infusion rate ($0.058 \mu\text{g kg}^{-1} \text{min}^{-1}$) inhibition of aggregation was maximum at 15 min, the infusion was terminated after 20 min and aggregation had returned to baseline by 3 h post-infusion. Similar effects of 192C86 were observed on platelet aggregation to $2 \mu\text{M}$ ADP in WB.

Heart rate, systolic and diastolic blood pressure

The mean maximum changes in heart rate and blood pressure during each infusion of 192C86 compared to the pre-infusion baseline are shown in Table 2. The mean maximum rise in heart rate increased in a dose-dependent manner as the infusion rate increased from 0.007 to $0.058 \mu\text{g kg}^{-1} \text{min}^{-1}$. The mean maximum fall in diastolic blood pressure was almost identical at the three lowest infusion rates, but was greater at the highest infusion rate. The mean maximum changes in systolic blood pressure showed a progressive rise over the three

Table 2 Mean ($\pm$ s.e. mean) for the maximum changes in heart rate (HR) (beats min^{-1}) and blood pressure (BP) (mmHg) during 192C86 infusions compared with values immediately pre-infusion

Infusion rate ($\mu\text{g kg}^{-1} \text{min}^{-1}$)	HR	Diastolic BP	Systolic BP
0.007 ($n = 4$)	+8 (± 4)	-12 (± 3)	-5 (± 4)
0.014 ($n = 4$)	+14 (± 5)	-11 (± 2)	+11 (± 4)
0.028 ($n = 1$)	+28	-11	+17
0.058 ($n = 1$)*	+28	-16	-17

*Infusion stopped after 20 min.

lower infusion rates and then a marked fall at the highest infusion rate.

Facial flushing

The effects of 192C86 on volunteers' sensation of facial flushing assessed by a visual analogue scale are shown in Figure 4. The mean scores for facial flushing increased during infusions of 0.007 and 0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$, reaching a maximum at 60 min and returning to baseline by 2 h post-infusion. There was a greater maximum score during the 0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$ infusion. The effects of 192C86 on the assessment of facial flushing made by an observer are shown in Figure 5 and results are qualitatively similar to those of the volunteers. Full-face colour photographs confirmed the results of the subjective assessment of facial flushing made by volunteers and the observer.

Adverse experiences

The principal adverse experiences reported by volunteers using the checklist were headache, facial flushing and nasal stuffiness. The severity of these adverse experiences was dose-related and all had resolved within 60 min of stopping the infusions.

Two other adverse experiences occurred during the course of the study, both related to the cardiovascular actions of 192C86. Postural hypotension accompanied by tachycardia was recorded 8 min after completing the 60 min infusion at 0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$ in one subject. No intervention was required apart from lying the subject down and at no time did he lose consciousness or feel

Figure 4 Effect of 192C86 on facial flushing as assessed by the volunteers using a 100 mm visual analogue scale. Results are expressed as mean $\pm$ s.e. mean at the 0.007 and 0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$ infusion rates.

Figure 5 Effect of 192C86 on facial flushing as assessed by an observer using a 3-point numerical scale (0 = none; 1 = mild; 2 = moderate; 3 = severe). Results are expressed as mean $\pm$ s.e. mean at the 0.007 and 0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$ infusion rates.

faint. A second subject experienced marked postural hypotension accompanied by bradycardia after 17 min of the 0.058 $\mu\text{g kg}^{-1} \text{min}^{-1}$ infusion. The volunteer was standing prior to measurement of heart rate and blood pressure, when he reported feeling faint. The subject lay down and after measurement of blood pressure confirmed hypotension the infusion was turned off. No intervention was required apart from maintaining a supine position and all signs and symptoms of postural hypotension had fully resolved 100 min after stopping the infusion.

Assay of 192C86

At the lowest infusion rate (0.007 $\mu\text{g kg}^{-1} \text{min}^{-1}$) plasma concentrations of 192C86 were below the limits of sensitivity of the assay (0.5 ng ml⁻¹). At the higher infusion rates, the mean $\pm$ s.d. maximum plasma concentrations of 192C86 were 0.78 $\pm$ 0.11 ng ml⁻¹ (0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$ for 60 min), 2.57 ng ml⁻¹ (0.028 $\mu\text{g kg}^{-1} \text{min}^{-1}$ for 60 min) and 2.33 ng ml⁻¹ (0.058 $\mu\text{g kg}^{-1} \text{min}^{-1}$ for 15 min). At most of the other time points, the plasma concentrations of 192C86 were at or below the limits of sensitivity of the assay.

Safety data

There were no clinically significant abnormalities in the 12 lead ECG performed pre- and post-infusion. Minor repolarisation changes were noted post-infusion in one subject at 0.007 $\mu\text{g kg}^{-1} \text{min}^{-1}$ and in a second subject at 0.014 and 0.028 $\mu\text{g kg}^{-1} \text{min}^{-1}$. A slight reduction in T-wave amplitude was noted in two subjects after infusion of 0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$ 192C86. There were no changes

in lung function that were considered to be related to 192C86 infusion. There were no clinically significant abnormalities in blood counts or plasma biochemistry taken before or 24 h after each study occasion.

Discussion

The principal aim of this study was to investigate dose-response relationships for the effects of 192C86 on *ex vivo* platelet aggregation to ADP and collagen in PRP and WB and on the cardiovascular effects associated with vasodilatation. The hypothesis was that if DP-receptor reserve were higher on human platelets than blood vessels, then a DP-receptor partial agonist such as 192C86 might inhibit platelet aggregation at concentrations that are without effects on the cardiovascular system. However, it is very clear from the results of this study that no separation could be made between the platelet and cardiovascular effects of 192C86 in man. The dose-response relationships for inhibition of platelet aggregation *ex vivo*, increase in heart rate, decrease in diastolic blood pressure and facial flushing were indistinguishable. Even a minimal degree of platelet aggregation inhibition was accompanied by changes in heart rate and blood pressure and by facial flushing. Furthermore, there was no evidence that the cardiovascular effects had reached a maximum. The fact that the platelet and cardiovascular effects of 192C86 were inseparable over the entire dose-response range suggests that there is no difference in receptor reserve which could be exploited to achieve selective inhibition of platelet aggregation by DP-receptor partial agonists. Consequently, studies on 192C86 analogues with greater or lesser efficacy are unlikely to produce qualitatively different results to those obtained with 192C86.

Platelet aggregation to ADP and collagen in PRP and WB was measured before, during and after each infusion of 192C86. At the three dose levels where the infusion was given for the full 60 min (0.007, 0.014 and 0.028 $\mu\text{g kg}^{-1} \text{min}^{-1}$) inhibition of aggregation increased during the course of each infusion and a clear relationship existed between the infusion rate of 192C86 and the maximum extent of inhibition of aggregation. At the highest dose level (0.058 $\mu\text{g kg}^{-1} \text{min}^{-1}$) only one subject received the infusion of 192C86 because it was poorly tolerated and the infusion had to be stopped after 20 min. Consequently, an accurate dose-response comparison cannot be made between this and the three previous dose levels. In general, inhibition of platelet aggregation by 192C86 had reversed, either fully or partially, by 3 h post-infusion.

The extent of inhibition of aggregation by 192C86 *ex vivo* can be compared directly with that *in vivo* for this group of volunteers. In platelet aggregation studies performed during volunteer screening, the effect of a single concentration of 192C86 (3 μM) was examined *in vitro* on the collagen dose-response curve in PRP. This concentration of 192C86 has previously been shown to produce the greatest rightward shift of the collagen dose-response curve (Giles, unpublished observation). In the four volunteers taking part in this study, the mean maximum rightward shift was 1.63 log units (range 0.09

to 2.18) or 43 fold (range 10 to 151) *in vitro*. At the highest infusion rate that all four volunteers were studied (0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$), the mean maximum rightward shift of the collagen dose-response curve *ex vivo* was only 0.4 log units (range 0.11 to 0.6) or 2.5 fold (1.3 to 4) and the greatest shift of the curve (0.71 log units or 5 fold) was observed in the volunteer receiving 0.028 $\mu\text{g kg}^{-1} \text{min}^{-1}$. It is clear from this comparison that the effects of 192C86 *ex vivo* are probably at the lower end of the dose-response curve for inhibition of platelet aggregation by this compound.

The principal reported adverse experiences following infusion of 192C86 were all related to vasodilatation e.g. headache, facial flushing and nasal stuffiness and their severity was dose-related. These symptoms have also been reported in man following infusion of PGD₂ (Heavey *et al.*, 1984) and BW 245C (a stable PGD₂ mimetic) (Orchard *et al.*, 1983) and, excluding nasal stuffiness, following infusion of other vasodilator prostanoids e.g. PGI₂ (Data *et al.*, 1981; Fitzgerald *et al.*, 1979; Hassan *et al.*, 1982; O'Grady *et al.*, 1979; Reece *et al.*, 1983) and PGE₁ (Bergstrom *et al.*, 1959). There was no evidence of the abdominal discomfort, gastrointestinal upset or general restlessness and agitation which were frequently reported during infusions of PGI₂ (Data *et al.*, 1981; O'Grady *et al.*, 1979; Reece *et al.*, 1983). Two other adverse experiences occurred during the course of the study, both related to vasodilatation. Postural hypotension accompanied by tachycardia occurred in one subject after completing the 0.014 $\mu\text{g kg}^{-1} \text{min}^{-1}$ infusion, and a second subject experienced marked postural hypotension accompanied by bradycardia during the 0.058 $\mu\text{g kg}^{-1} \text{min}^{-1}$ infusion. Tachycardia is a predictable cardiac response to vasodilator drugs which occurs as a result of activation of baroreceptor reflexes following a fall in blood pressure. The bradycardia which accompanied postural hypotension during the highest dose infusion is likely to be vagally mediated. Although there were no clinically significant abnormalities in the 12 lead ECG performed pre- and post-infusion of 192C86, minor repolarisation changes (mainly reduction in T-wave amplitude) were noted on four study occasions. Similar non-specific and clinically insignificant reductions in T-wave amplitude have been reported following PGI₂ infusions (Data *et al.*, 1981; Hassan *et al.*, 1982).

Direct comparison of the *ex vivo* inhibition of platelet aggregation by 192C86 with other prostanoids is difficult, principally because most studies have used different infusion schedules, different methods to assess platelet aggregation and different concentrations of aggregating agents. However, in general the platelet inhibitory effects of 192C86 in man (1.5–5 fold rightward-shifts of the collagen dose-response curve) were similar to or greater than those reported for PGD₂, and BW245C, two agonists which also activate DP-receptors on platelets. For example, intravenous infusion of PGD₂ at concentrations which caused vasodilatation was without effect on ADP-induced aggregation and caused a small but non-significant rightward-shift of the collagen dose-response curve (Heavey *et al.*, 1984). BW245C reduced *ex vivo* platelet aggregation to a sub-maximal concentration of ADP (2–3 μM), but like 192C86 these changes were accompanied by flushing, nasal congestion and increases in heart rate (Orchard *et al.*, 1983). Infusion of IP-receptor agonists

(e.g. prostacyclin, carbacyclin and iloprost) caused 2–5 fold rightward-shifts of the ADP aggregation curve (Data *et al.*, 1981; Fitzgerald *et al.*, 1979; Hutton *et al.*, 1983; O'Grady *et al.*, 1984), but like the DP-receptor agonists these were generally accompanied by vasodilatation. It was hoped that 192C86 would cause fewer vascular side-effects than PGD₂ and BW245C not only because it is a partial agonist, but also because it is highly selective for the DP-receptor, unlike PGD₂ and BW245C which can elicit vasodilatation through both EP₂- and DP-receptors (Cambridge *et al.*, 1988; Giles *et al.*, 1989). However, as their profile of action is so similar to 192C86 in these studies, one must conclude that in man vasodilatation to all three agonists is mediated predominantly via the DP-receptor.

There were no changes in lung function (assessed by FEV₁) that were considered to be related to 192C86 infusions. This is not an unexpected finding, since although inhaled PGD₂ is a potent bronchoconstrictor agent in man (Hardy *et al.*, 1984), this action appears to be mediated via interaction with thromboxane (TP)-receptors in the airways (Coleman & Sheldrick, 1989; Hamid-Bloomfield *et al.*, 1990). 192C86 is a highly selective DP-receptor partial agonist which does not bind to TP-receptors at concentrations up to 10⁴ times the DP-receptor K_B (Leff & Giles, 1992) and so would not be expected to induce bronchoconstriction.

We had hoped to examine the pharmacokinetic profile

of 192C86 in man but, unfortunately, despite using a very sensitive r.i.a. with a lower detection limit of 0.5 ng ml⁻¹, the plasma concentrations of 192C86 were mostly at or below this limit in the present study. The maximum plasma concentrations achieved were in the region of 2–3 ng ml⁻¹ and at these levels platelet aggregation to ADP and collagen was markedly inhibited.

In conclusion, 192C86 inhibited *ex vivo* platelet aggregation to ADP and collagen in a dose-dependent manner. However, the dose-response relationships for inhibition of platelet aggregation and vasodilatation by 192C86 were not separable and there was no evidence of a difference in receptor reserve which could be exploited to obtain selective inhibition of platelet aggregation by DP-receptor partial agonists.

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