A chemically stable analogue, 9 β -methyl carbacyclin, with similar effects to epoprostenol (prostacyclin, PGI₂) in man

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1 The effects of 9 β -methyl carbacyclin, a chemically stable analogue of epoprostenol (prostacyclin, PGI₂) were studied, in comparison with epoprostenol, both *in vitro* and *in vivo* in man.

2 *In vitro* 9 β -methyl carbacyclin and epoprostenol inhibited platelet aggregation induced by ADP, collagen, the endoperoxide analogue U46619 and arachidonic acid. The potency of 9 β -methyl carbacyclin relative to epoprostenol was comparable in ADP and collagen-aggregated platelet rich plasma (PRP), 9 β -methyl carbacyclin being 0.01 times as active as epoprostenol. The anti-aggregatory potencies of the two compounds were comparable in PRP and whole blood.

3 The phosphodiesterase inhibitor isobutyl methyl xanthine enhanced the anti-aggregatory activity of both compounds *in vitro*. 9 β -methyl carbacyclin and epoprostenol elevated platelet cyclic AMP, 9 β -methyl carbacyclin being 0.04 times as active as epoprostenol.

4 In a placebo controlled trial both drugs produces significant headache and facial flushing when compared with placebo. Nasal stuffiness, abdominal discomfort and nausea were reported on all three treatments.

5 Both drugs caused significant and comparable increase in heart rate and decrease in pre-ejection (PEP) and PEP/left ventricular ejection time (LVET) ratio compared with placebo. Systolic and diastolic blood pressure, LVET and QS₂ index were unchanged.

6 Platelet aggregation responses to ADP were significantly inhibited by all three doses of both drugs compared with placebo.

7 Bleeding time was significantly longer during epoprostenol infusion than either placebo or 9 β -methyl carbacyclin infusion. Neither drug had significant effect, compared with placebo, on kaolin activated clotting time in PPP, PRP or in PRP in the presence of heparin, prothrombin time, partial thromboplastin time, thrombin clotting time, fibrinogen, fibrinogen degradation products or euglobulin clot lysis time.

8 The pharmacodynamic effects and duration of action of 9 β -methyl carbacyclin and of epoprostenol are similar; 9 β -methyl carbacyclin is approximately 100 times less potent than epoprostenol in man.

Keywords 9 β -methyl carbacyclin epoprostenol (PGI₂) prostaglandins platelet aggregation cardiovascular effects

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Introduction

Epoprostenol (prostacyclin, PGI_2) is a naturally occurring prostaglandin (Moncada *et al.*, 1976) with potent platelet inhibitory (O'Grady *et al.*, 1980) and vasodilator (Warrington *et al.*, 1980) properties in man. It has been used to inhibit platelet activation during extracorporeal circulation procedures in man (Bunting *et al.*, 1981; Gimson *et al.*, 1980) and in renal dialysis it can be employed as the sole anticoagulant in place of heparin (Smith *et al.*, 1982). Clinical trials are in progress to assess the possible therapeutic benefits of epoprostenol in acute myocardial infarction, unstable angina, acute thrombotic stroke and ischaemic peripheral vascular disease. However, epoprostenol is an unstable compound, its formulation is complex and reconstituted solutions have limited stability. 9β -methyl carbacyclin [(5Z,9S)-9-methyl-6a-carba-prostaglandin I_2] (Figure 1) has been developed by the Wellcome Foundation Limited and the Upjohn Company as a chemically stable analogue to mimic as closely as possible the biological effects and duration of action of epoprostenol.

The present studies were designed to examine the effects of 9β -methyl carbacyclin *in vitro* and the effects of short term intravenous infusion of 9β -methyl carbacyclin in healthy volunteers and to compare its effects with those of epoprostenol and of placebo.

Methods

In vitro studies

Preparation of platelet rich plasma (PRP): approximately 200 ml of blood was collected

from healthy volunteers, who had not taken any non-steroidal anti-inflammatory drug for at least 7 days, into a plastic 150 cm^2 tissue culture flask (Corning) containing trisodium citrate (3.15% wt/vol. solution) to give a final citrate concentration of 0.315% wt/vol. Whole blood was centrifuged in plastic tubes at 1100 rev/min (200 g) in an MSE bench centrifuge for 15–20 min at room temperature. The PRP was then carefully removed using a plastic syringe, mixed and transferred to plastic universal 30 ml containers (Sterilin) and kept at room temperature.

Platelet aggregation was measured in a Payton dual channel aggregation module connected to a W + W recorder 1200 as described previously (Whittle *et al.*, 1978). Aliquots (0.5 ml) of PRP were incubated for 1 min at 37°C and stirred with teflon coated magnetic stirrers at 900 rev/min with or without the prostaglandin under investigation, prior to addition of submaximal doses of adenosine diphosphate (ADP) (6–10 μM) or collagen (2–4 $\mu\text{g/ml}$) to just cause a non-reversing control aggregation. Dose-inhibition curves were constructed for epoprostenol and for 9β -methyl carbacyclin and the ID_{50} (dose causing 50% inhibition) was calculated as the dose required to reduce the aggregation to 50% of its control amplitude.

For studies on whole blood aggregations, blood was collected into trisodium citrate as described above, aliquots (0.5 ml) placed into siliconized glass cuvettes of a Payton Dual Channel aggregation module and after 1 min stirring at 37°C , collagen (1.6–4 $\mu\text{g/ml}$), in a dose which produced near maximal aggregation, was added. Blood samples (10 μl) were withdrawn from the cuvette at 0, 1 and 2.5 min intervals and the platelet count recorded using a whole blood platelet counter (Ultra Flo 100,

Figure 1 Chemical structure of 9β -methyl carbacyclin

Clay Adams). The platelet count values were expressed as % inhibition using the following calculation:

$$\% \text{ inhibition} = 100 - 100 \times \frac{(\text{count at time 0} - \text{test count at 2.5 min})}{(\text{count at time 0} - \text{control count at 2.5 min})}$$

Cyclic adenosine monophosphate (cAMP) assay

To determine the effects on cAMP concentrations, PRP (500 μ l) was pre-incubated in an aggregometer at 37°C for 1 min prior to adding epoprostenol or 9 β -methyl carbacyclin. After 1 min the reaction was terminated and the cAMP extracted by the addition of ice cold absolute ethanol (1 ml). Following evaporation, the residue was dissolved in 125–150 μ l assay buffer (50 mM Tris buffer, pH 7.5, containing 4 mM EDTA) and centrifuged (1,400 g at 4°C for 30 min) to remove insoluble material. Aliquots (50 μ l) of the supernatant were removed for cAMP binding assay. The protein binding assay utilizes the competitive binding of ³H-cAMP with unlabelled cAMP to a specific high affinity protein, and measures cAMP in the range 0.5–16 pmol. Tritiated (8-³H) cAMP (50 nCi, Amersham International) and 100 μ l of a 1:15 dilution, in assay buffer, of a binding protein (BDH) was added to an aliquot (50 μ l) of the reconstituted sample, and incubated for 2 h at 4°C. Unbound cAMP was removed by centrifugation (1,400 g at 4°C) with a 100 μ l suspension of 2.5% charcoal in 0.5% BSA. Radioactivity (bound cAMP) in the supernatant was determined by liquid scintillation counting.

Drugs

Epoprostenol as the sodium salt was freshly dissolved in 1 M Tris buffer (pH 9.6 at 4°C) and stored on ice; subsequent dilutions were made with ice cold isotonic sodium bicarbonate solution (1.25% wt/vol.; pH 8.6) and used immediately. 9 β -methyl carbacyclin synthesized (Aristoff *et al.*, 1983) by The Upjohn Company was obtained as the calcium salt and a stock solution of 10 mg/ml in ethanol was kept at 4°C. On the day of use, aliquots were taken, dried under N₂ and dissolved in 50 mM Tris buffer (pH 7.4 at 37°C). The solution of ADP (Sigma Chemical Co.) was made up in distilled water and kept on ice, as was the suspension of collagen in isotonic glucose solution (suspended fibrils, Horman-Chemie, Munchen). Isobutyl methyl xanthine (IBMX, Sigma Chemical Co.) was dissolved in distilled water and incubated at 37°C.

Arachidonic acid (Grade I, Sigma Chemical Co.) was stored in *N*-hexane (10 mg/ml) at –20°C and used freshly as the sodium salt. Following evaporation of the *N*-hexane from

1 ml aliquots, sodium hydroxide (0.5 M in methanol, 0.5 ml) was added and subsequently evaporated to dryness. The residue was dissolved (10 mg/ml) in 1 M Tris buffer (pH 9.6 at 24°C), and stored in a light proof container on ice. The endoperoxide analogue 11 α , 9 α -epoxy methano PGH₂ (U-46619), kindly supplied by The Upjohn Company, was stored in ethanol at –20°C and dissolved in isotonic saline when required.

Healthy volunteer study

Open dose ranging trial Subjects attended the laboratory after an overnight fast and abstinence from smoking. They rested supine on a couch and indwelling cannulae (Angiocath 18G) were inserted into right and left forearm veins for infusion of drug and for blood sampling. A sphygmomanometer cuff was applied to the right arm and ECG leads, connected to an oscilloscope display, were placed on the chest. An i.v. infusion of saline (0.9% wt/vol.) was administered for 30 min followed by up to three incremental doses of 9 β -methyl carbacyclin, each for 30 min, followed by an infusion of saline (0.9% wt/vol.) for up to 60 min. The rate of infusion for saline was 0.25 ml/min and 9 β -methyl carbacyclin was infused at 0.25 ml/min for the first dose on each occasion, incremental doses being administered by increasing the infusion rate up to 1 ml/min.

Immediately prior to the start of saline infusion and during minutes 12 to 15 and 27 to 30 of each saline/drug infusion period, measurements were made of: (a) heart rate counted at the radial pulse over 1 min, (b) arterial blood pressure, using a Hawksley random zero sphygmomanometer, the diastolic blood pressure being taken as the point of disappearance of the Korotkoff sound (Phase 5), (c) platelet aggregation responses to adenosine diphosphate (ADP). Blood samples (9 ml) were collected in plastic tubes containing 1 ml of 3.15% trisodium citrate. Platelet rich plasma (PRP) was prepared as the supernatant resulting from centrifugation of the blood sample at 120 g for 3 min using a Petaluge centrifuge (F.T. Scientific Instruments). After the PRP had been removed with a plastic pipette, the remainder was respun at 600 g for 10 min to obtain platelet poor plasma (PPP). Platelet aggregation responses in 0.5 ml PRP were measured, using a Payton dual channel aggregometer, in response to ADP concentrations ranging between 0.5 and 64 μ M (final concentration) as described above. Measurements of platelet aggregation responses were completed within 20 min of withdrawal of blood samples from the subjects and (d) headache, facial

flushing, abdominal discomfort and nasal stuffiness were recorded using 100 mm visual analogue scales, where 0 represented the effect as absent and 100 represented the effect as severe as possible. These were completed by the subjects except for facial flushing which was assessed by the investigator.

Blood samples were obtained for haematological and biochemical screening tests during the saline infusion period prior to drug infusion, at the end of infusion of the highest dose administered on any one occasion and to 24 h after each drug administration. Urine samples for routine urinalysis were collected prior to and immediately after each experimental occasion and also 24 h later.

Placebo controlled comparison with epoprostenol Subjects attended the laboratory at the same time on each of three occasions, at least 2 days apart, having fasted and abstained from smoking overnight. They rested supine on a couch, indwelling cannulae (Angiocath 18G) were inserted into right and left forearm veins, ECG and phonocardiograph leads attached and a sphygmomanometer cuff applied to the arm. The experimental design is shown in Figure 2. An intravenous infusion of saline 0.9% wt/vol. at 0.25 ml/min was administered for 20 min before and after the following treatments, in random balanced order: (a) 9 β -methyl carbacyclin 150, 300 and 600 ng kg⁻¹ min⁻¹, (b) epoprostenol 1.5, 3 and 6 ng kg⁻¹ min⁻¹ and (c) saline 0.9% wt/vol. (placebo).

Each dose of drug/placebo was administered for 20 min, the infusion rate for the first dose of drug/saline was 0.25 ml/min and the second and third doses were administered by increasing the infusion rate to 0.5 and 1 ml/min, respectively.

The drug/placebo solutions were prepared, according to a code not known to the investigators, by a colleague otherwise unconnected with the study and handed to the investigator labelled only with the subject's number and treatment occasion indicated.

Measurements were made (Figure 2) immediately prior to start of saline infusion (baseline) and then in minutes 15–20 of each 20 min drug/saline infusion period, as follows: (1) heart rate and blood pressure, as described for the open dose ranging trial, (2) systolic time intervals and high speed ECG Lead II (Burgess *et al.*, 1978). Ten cardiac cycles were measured and averaged for each determination of systolic time intervals and for each ECG. The QS₂ interval, left ventricular ejection time (LVET) and the QT interval on the ECG were corrected for heart rate using regression equations derived previously (Burgess *et al.*, 1978; Bryson *et al.*, 1978): QS₂ index = QS₂ (in ms) + (1.2 $\times$ heart rate); LVET index = LVET + (1.1 $\times$ heart rate); pre-ejection period (PEP) requires no correction for heart rate; QT index = QT + (1.7 $\times$ heart rate) and corrected QT interval (QT_c) = $\frac{QT}{\sqrt{RR}}$, (3) headache, facial flushing, abdominal discomfort and nasal stuffiness as

Figure 2 Experimental protocol for comparison of 9 β -methyl carbacyclin with epoprostenol and placebo.

described for the open dose ranging trial and (4) platelet aggregation response to ADP as described for the open dose ranging trial.

During the initial saline infusion the following additional measurements were made and repeated during infusion of the highest dose of drug/placebo. (1) Cutaneous bleeding time in duplicate using the Mielke template technique (Mielke *et al.*, 1969) during application of a standard congesting pressure of 40 mm Hg over the upper arm, incisions being made over the surface of the forearm. To minimize scarring the measurement was performed during the initial saline infusion period on only one of the three experimental occasions for each subject. (2) Blood samples were obtained for prothrombin time (PT) (Quick one stage PT using 'Diagen' rabbit brain thromboplastin), partial thromboplastin time with kaolin (PTTK) (using 'Diagen' kaolin/platelet substitute) (Denson, 1976), thrombin clotting time (TT) (Austin & Rhymes, 1975), fibrinogen titre (FT) (serial dilution method (Hardisty & Ingram, 1965), fibrinogen degradation products (FDPs) (using Thrombo-Wellcotest, Wellcome Reagents Ltd) and for euglobulin clot lysis time (ECLT). PRP and PPP were prepared and 200 μ l aliquots of each incubated for 20 min with kaolin before the addition of calcium chloride and the clotting time measured (Hardisty & Hutton, 1965). The clotting time was also measured similarly in PRP and PPP in the presence of heparin (1 iu/ml), added to the PRP/PPP 2 min before kaolin. (3) Blood samples were taken for haematological and biological screening tests which were repeated 2 h after each experimental occasion.

Urinalysis was carried out on samples collected immediately before and after each experimental occasion and 24 h later.

Subjects

The studies were approved by the Ethics Committee of St Bartholomew's Hospital and all subjects gave written consent. Subjects for both the dose ranging and the placebo controlled trial were healthy male volunteers, with normal results of haematological and biochemical screening tests and urinalysis. Subjects took no medication for 14 days prior to study. The five subjects in the dose ranging study were aged 21–31 years and weighed 65–78 kg. The six subjects in the placebo controlled study were aged 21–30 years and weighed 52–79 kg.

Materials

9 β -methyl carbacyclin as the calcium salt was

synthesized by The Upjohn Company and formulated by the Wellcome Foundation Ltd. It was prepared as a sterile solution in vials containing 10 ml of a 1 mg/ml solution of 9 β -methyl carbacyclin formulated to remove the calcium and dissolved in tris buffer (50 mM, pH 8.0). The contents of the vials were further diluted with saline (0.9% wt/vol.) immediately prior to use. Epoprostenol was synthesized by The Upjohn Company and formulated by the Wellcome Foundation Ltd as a sterile freeze dried formulation in vials containing 0.5 mg epoprostenol as the sodium salt and stored at 5°C. Immediately before use the contents of the vials were reconstituted with sterile glycine buffer diluent (Wellcome Foundation Ltd) pH 10.5 and further dilutions made with a saline glycine buffer diluent mixture with a ratio of saline:glycine buffer diluent less than 6:1.

ADP was supplied by the Sigma Chemical Company and dissolved in distilled water to give final concentrations of 0.5, 1, 2, 4, 8, 16, 32 and 64 μ M and stored frozen until use.

Statistical analysis

For the *in vitro* studies the significance of differences between groups was assessed using the Student's *t*-test for paired or for unpaired data where appropriate.

Statistical tests were only appropriate to the placebo controlled phase of the volunteer studies. Variables were subject to a split plot analysis of variance with drug, occasion, subject and infusion as factors to give an estimate of variance. The three treatments were then compared using the Newman-Keuls multiple range test. The analysis of platelet aggregation responses was performed on the log ADP dose ratio to baseline values and was calculated by fitting a set of parallel logistic curves for each subject and occasion. The scores for headache and for facial flushing were subjected to a log transformation in order to stabilize the variances.

Results

In vitro studies

Pre-incubation of epoprostenol and 9 β -methyl carbacyclin with PRP for 1 min at 37°C caused a dose-dependent inhibition of platelet aggregation induced by ADP (Figure 3a) and collagen (Figure 3b) with the ID₅₀ values shown in Table 1. The potency of 9 β -methyl carbacyclin relative to epoprostenol was comparable in ADP and collagen aggregated PRP (potency ratio 0.01). 9 β -methyl carbacyclin and epoprostenol

likewise inhibited platelet aggregation, in PRP, induced by the stable endoperoxide analogue U-46619 and by arachidonic acid (Table 1). There was no loss of anti-aggregating activity of aqueous solutions of 9 β -methyl carbacyclin when stored at room temperature for 30 days.

The inhibition of collagen induced platelet aggregation by epoprostenol was very similar in PRP and in whole blood (Figure 3c). Likewise, in human PRP and in whole blood, 9 β -methyl

carbacyclin had a similar potency in inhibiting collagen-induced aggregation. Thus the ID₅₀ for the inhibition of collagen-induced aggregation in whole blood was 0.88 ± 0.15 ng/ml for epoprostenol and 73 ± 20 ng/ml for 9 β -methyl carbacyclin.

The phosphodiesterase inhibitor isobutyl methyl xanthine (IBMX 2–8 μ g/ml) enhanced the anti-aggregatory activity of 9 β -methyl carbacyclin in PRP aggregated with ADP and

Figure 3 Inhibition of (a) ADP-induced, (b) collagen-induced platelet aggregation in human PRP and (c) collagen-induced human platelet aggregation in whole blood with epoprostenol (○) and 9 β -methyl carbacyclin (■). Results, expressed as % inhibition of platelet aggregation compared to the initial controls, are shown as the mean $\pm$ s.e. mean of three to five experiments.

Table 1 Inhibition of human platelet aggregation *in vitro*

	Epoprostenol	ID ₅₀ (ng/ml) 9 β -methyl carbacyclin	Potency ratio
ADP	0.55 $\pm$ 0.1	86.6 $\pm$ 11	0.01
Collagen	0.91 $\pm$ 0.08	123 $\pm$ 32	0.01
U-46619	0.15 $\pm$ 0.03	15.4 $\pm$ 1	0.01
Arachidonic acid	1.8 $\pm$ 0.7	55.8 $\pm$ 17.6	0.03

Comparison of the ID₅₀ values of epoprostenol and 9 β -methyl carbacyclin in human PRP aggregated with different agents, ADP, collagen, U-46619 (epoxy-methano PGH₂) and arachidonic acid. Values shown are mean $\pm$ s.e. mean ($n = 4-13$).

shifted the dose-response curves to the left, in a dose related manner (Figure 4). Higher concentrations of IBMX (8 μ g/ml) alone markedly inhibited the ADP-induced aggregation and hence it was difficult to analyse its effect on 9 β -methyl carbacyclin.

The platelet levels of cAMP were elevated with increasing concentrations of epoprostenol and 9 β -methyl carbacyclin, epoprostenol being more potent than 9 β -methyl carbacyclin (Figure 5). For concentrations elevating cAMP by 50% of maximum, 9 β -methyl carbacyclin was 0.04 times as active as epoprostenol.

Open dose ranging trial

The results of the open phase of the study are described in detail here only where they provide

information additional to that from the placebo controlled trial.

9 β -methyl carbacyclin at 20, 40 and 80 ng kg⁻¹ min⁻¹ produced no measureable or subjective effects except for headache during infusion of 80 ng kg⁻¹ min⁻¹. Doses of 160, 320 and 640 and later 150, 300 and 600 ng kg⁻¹ min⁻¹ produced increases in heart rate, not exceeding 15 beats min⁻¹, headache and facial flushing without change in blood pressure. One subject receiving 800 ng kg⁻¹ min⁻¹ developed severe headache, restlessness and nausea in the final 5 min of the 30 min infusion period, heart rate had increased by 12 beats/min and blood

Figure 4 Effect of isobutyl methyl xanthine (IBMX; 2-4 μ g/ml) on the inhibition of ADP-induced aggregation in human PRP by 9 β -methyl carbacyclin. Results, expressed as % inhibition of platelet aggregation compared to the initial controls, are shown as the mean $\pm$ s.e. mean of three experiments.

Figure 5 Effects of epoprostenol (○) and 9 β -methyl carbacyclin (■) on cyclic AMP levels in human PRP, using a binding assay technique. Results, expressed as multiples of basal cAMP levels (in the range 0.5-16 pmol), are shown as the mean $\pm$ s.e. mean of four to six experiments.

pressure was unchanged. These effects subsided within 20 min of the end of the infusion period. One subject receiving $1,280 \text{ ng kg}^{-1} \text{ min}^{-1}$ developed, after 10 min infusion at this dose, pallor, sweating, nausea, yawning and epigastric pain. Heart rate increased by 20 beats/min but blood pressure was unchanged. These effects required that the infusion be discontinued whereupon these effects reversed within 20 min.

Platelet aggregation responses were unaffected by doses of 9β -methyl carbacyclin 20, 40 and $80 \text{ ng kg}^{-1} \text{ min}^{-1}$ but showed a dose-related inhibition at all doses of and greater than $150 \text{ ng kg}^{-1} \text{ min}^{-1}$.

There were no consistent differences between the cardiovascular, platelet inhibitory or subjective effects at 15 and 30 min after starting infusion of 9β -methyl carbacyclin at a given dose. The effects observed in response to infusions of the drug reversed within 20 min of discontinuing infusions.

Placebo controlled comparison with epoprostenol

There were no significant differences, for any of the variables measured, between treatments for baseline or pre-treatment saline values. Facial

Figure 6 Headache and facial flushing ($\log_n$ scores) in response to 9β -methyl carbacyclin ($\blacksquare$ 150, 300 and $600 \text{ ng kg}^{-1} \text{ min}^{-1}$), epoprostenol ($\circ$ 1.5, 3 and $6 \text{ ng kg}^{-1} \text{ min}^{-1}$) and placebo ($\bullet$). Values shown as means together with the standard error of the difference between treatments (s.e.d.). B represents baseline values, S the values during saline before and after drug/placebo, 1, 2 and 3 the values during each dose of drug/placebo.

**Significantly ($P < 0.01$) different from placebo value, † and †† significantly different from epoprostenol value, $P < 0.05$ and $P < 0.01$.

flushing (Figure 6) was significantly ($P < 0.01$) increased by all doses of epoprostenol but only by the two higher doses of 9β -methyl carbacyclin, compared with placebo, and the response to the lowest dose of epoprostenol was significantly different ($P < 0.01$) from the response to the lowest dose of 9β -methyl carbacyclin. Values during post-treatment saline infusion were similar for all treatments.

Headache scores (Figure 6) were significantly greater ($P < 0.01$) for the higher two doses of both drugs than for placebo and the value for 9β -methyl carbacyclin was significantly higher ($P < 0.05$) than for epoprostenol at the highest dose. Whereas the score for epoprostenol returned to a similar value to placebo during post-treatment saline, the score for 9β -methyl carbacyclin was still significantly higher ($P < 0.05$) than for placebo, and for epoprostenol, at this time.

Nasal stuffiness was reported by one subject on all three treatments and by two other subjects on 9β -methyl carbacyclin only. Abdominal discomfort was experienced by one subject on both placebo and 9β -methyl carbacyclin, by one subject on epoprostenol alone and by one subject on both 9β -methyl carbacyclin and epoprostenol. Nausea was experienced together with the abdominal discomfort on each occasion in this last subject. One subject complained of restlessness and one of sore throat during infusion of epoprostenol.

Figure 7 Heart rate in response to 9β -methyl carbacyclin ($\blacksquare$ 150, 300 and $600 \text{ ng kg}^{-1} \text{ min}^{-1}$), epoprostenol ($\circ$ 1.5, 3 and $6 \text{ ng kg}^{-1} \text{ min}^{-1}$) and placebo ($\bullet$). Values shown as means together with the standard error of the difference between treatments (s.e.d.). B represents baseline values, S the values before and after drug/placebo, 1, 2 and 3 the values during each dose of drug/placebo. **Significantly ($P < 0.01$) different from placebo.

Table 2 Cardiovascular variables during infusion of 9β-methyl carbacyclin (150, 300 and 600 ng kg⁻¹ min⁻¹), epoprostenol (1.5, 3 and 6 ng kg⁻¹ min⁻¹) and placebo. Values are shown as means together with standard error (s.e.) of the difference between separate treatments

	<i>Baseline</i>	<i>Saline</i>	<i>1</i>	<i>Dose</i> <i>2</i>	<i>3</i>	<i>Saline</i>	<i>s.e.</i>
<i>Heart rate (beats/min)</i>							
9β-methyl carbacyclin	61.7	58.3	59.0	62.5	65.7**	56.0	3.14
Epoprostenol	62.3	60.0	60.7	62.7	67.5**	56.5	
Placebo	60.0	55.2	54.8	54.8	55.2	57.7	
<i>Systolic BP (mm Hg)</i>							
9β-methyl carbacyclin	105.2	108.2	109.2	110.2	113.0	109.2	2.37
Epoprostenol	108.2	107.3	110.3	108.2	111.0	107.7	
Placebo	105.3	104.2	102.8	102.2	103.8	104.3	
<i>Diastolic BP (mm Hg)</i>							
9β-methyl carbacyclin	68.2	67.3	67.2	68.5	70.2	72.2	2.71
Epoprostenol	69.2	67.3	68.3	66.8	65.2	69.2	
Placebo	68.0	64.2	64.0	64.0	64.8	66.2	
<i>PEP (ms)</i>							
9β-methyl carbacyclin	117.3	115.3	118.7	112.5*	108.2**	128.5	4.37
Epoprostenol	120.0	119.8	120.8	111.8*	106.0**	127.7	
Placebo	121.7	119.8	122.2	127.5	127.7	128.0	
<i>LVET index (ms)</i>							
9β-methyl carbacyclin	381.5	384.0	381.0	382.8	385.2	372.7	4.28
Epoprostenol	377.0	379.2	378.2	385.8	384.2	373.5	
Placebo	375.8	378.3	377.2	375.3	375.0	369.3	
<i>PEP/LVET</i>							
9β-methyl carbacyclin	0.369	0.360	0.372	0.354*	0.346**	0.409	0.017
Epoprostenol	0.388	0.379	0.386	0.350*	0.339**	0.406	
Placebo	0.390	0.377	0.382	0.401	0.405	0.416	
<i>QT interval index (ms)</i>							
9β-methyl carbacyclin	393.0	399.5	398.3	400.0	407.5**	403.2	2.84
Epoprostenol	396.3	396.0	399.2	398.3	399.3†	400.2	
Placebo	393.0	395.8	392.8	394.7	396.0	399.5	
<i>QT_c (ms)</i>							
9β-methyl carbacyclin	288.2	293.7	291.5	296.2	307.8**	293.0	3.68
Epoprostenol	294.0	290.0	294.2	295.3	299.8**	289.3	
Placebo	288.7	289.3	283.5	286.8	288.0	292.2	

* and ** significantly different from placebo $P > 0.05$ and $P > 0.01$, respectively.

†Significantly different from 9β-methyl carbacyclin ($P > 0.05$).

PEP = pre-ejection period, LVET = left ventricular ejection time.

Heart rate (Figure 7, Table 2) was significantly ($P < 0.01$) increased at the highest dose of both drugs compared to placebo while values during post-treatment saline were similar for all treatments. Systolic and diastolic blood pressure (Table 2) were unchanged by any dose of drug compared to placebo. Pre-ejection period (PEP) (Table 2) was significantly reduced by both drugs compared with placebo at the middle ($P < 0.05$) and highest ($P < 0.01$) doses and the values for each of the two drugs were similar. Left ventricular ejection time index (LVET index) (Table 2) was unchanged by either drug compared to placebo and PEP/LVET (Table 2) showed changes similar to those observed with PEP for both drugs. PEP and PEP/LVET values during post-treatment saline were similar for both drugs and placebo. QS_2 index showed no significant change ($P > 0.05$) at any time. QT index (Table 2) was significantly increased ($P < 0.01$) by the highest dose of 9 β -methyl carbacyclin compared to placebo and QT_c (Table 2) was significantly increased ($P < 0.01$) by the highest dose of both drugs while during post-treatment saline values for both drugs were similar to placebo. There were no changes in PR interval, QRS duration or T wave height with either treatment compared to placebo.

Platelet aggregation responses to ADP are shown as ED_{50} ratio from baseline (Figure 8). Both drugs inhibited platelet aggregation to a comparable degree and values for both differed significantly from placebo values at

Figure 8 Platelet aggregation responses to adenosine diphosphate, as ED_{50} ratio to baseline values, during 9 β -methyl carbacyclin (■ 150, 300 and 600 ng kg $^{-1}$ min $^{-1}$) epoprostenol (○ 1.5, 3 and 6 ng kg $^{-1}$ min $^{-1}$) placebo (●). B represents baseline values, S the value before and after drug/placebo, 1, 2 and 3 the values during each dose of drug/placebo.

* and ** significantly different from placebo value $P < 0.05$ and $P < 0.01$ respectively.

Table 3 Kaolin activated clotting times in PRP in the presence of heparin (1 iu/ml). Values are means, together with standard error of the difference (s.e.) between treatments, during initial saline infusion and during 9 β -methyl carbacyclin (600 ng kg $^{-1}$ min $^{-1}$), epoprostenol (6 ng kg $^{-1}$ min $^{-1}$) and placebo infusions. There were no significant differences between the treatments.

	Saline	Drug/Placebo	s.e.
<i>PRP^{Heparin} (s)</i>			
9 β -methyl carbacyclin	103.3	120.5	
Epoprostenol	85.3	115.5*	11.5
Placebo	98.0	108.2*	

*Significantly different from saline value ($P < 0.05$).

dose 1 ($P < 0.05$), dose 2 ($P < 0.01$) and dose 3 ($P < 0.01$). Responses to both drugs had returned to similar values to placebo during the post-treatment saline infusion.

Bleeding time was significantly ($P < 0.01$) longer during infusion of epoprostenol (6.17 min) than either placebo (3.83 min) or 9 β -methyl carbacyclin (3.92 min). The standard error of the difference between treatments was 0.470.

There was no significant difference between the treatments on prothrombin time, PTTK, thrombin clotting time, fibrinogen titre, fibrinogen degradation products, euglobulin clot lysis time, or kaolin activated clotting time in PPP or PRP. However, in PRP with heparin there was an increase in kaolin activated clotting time, compared with the value during the initial saline infusion, after all treatments which achieved significance ($P < 0.05$) after epoprostenol (Table 3).

Discussion

The *in vitro* studies show epoprostenol and 9 β -methyl carbacyclin to exhibit similar effects on platelet function. In addition their anti-aggregation potencies were comparable in platelet rich plasma and in whole blood. The cAMP study showed that 9 β -methyl carbacyclin increased the platelet levels of cAMP as found previously with epoprostenol (Tateson *et al.*, 1977; Gorman *et al.*, 1977), presumably reflecting stimulation of adenylate cyclase activity. However, substantial changes were only detected at concentrations of epoprostenol and 9 β -methyl carbacyclin which caused maximal inhibition of ADP-induced platelet aggregation. It is likely that at lower concentrations, the small increase in cAMP is rapidly metabolized by platelet phosphodiesterase. This is consistent with the

finding of enhanced activity of low concentrations, of 9 β -methyl carbacyclin as an inhibitor of platelet aggregation *in vitro* in the presence of the potent phosphodiesterase inhibitor, IBMX.

The magnitude of change in the variables affected by the doses of both drugs given in the placebo controlled volunteer study was generally very similar, in particular for heart rate, pre-ejection period and inhibition of platelet aggregation. Epoprostenol produced significantly more facial flushing than 9 β -methyl carbacyclin at the lowest dose administered and significantly less headache at the highest dose. Given these similarities in response, the potency ratio between epoprostenol and 9 β -methyl carbacyclin in man is approximately 1:0.01. This is consistent with the potency ratio determined in the *in vitro* studies on platelets. The similarities in these latter studies between the responses of platelets in PRP and in whole blood to 9 β -methyl carbacyclin and to epoprostenol thus supports the use of platelet aggregation responses measured in PRP only, in these volunteer studies.

The duration of action of both drugs *in vivo* was comparable as, with the exception of headache in response to 9 β -methyl carbacyclin, all changes had reversed within 20 min of discontinuing drug infusion. Steady state cardiovascular and platelet inhibitory effects were reached within 15 min of starting infusion of a given dose of 9 β -methyl carbacyclin in the dose ranging study and this has previously been shown to be the case for epoprostenol also (O'Grady *et al.*, 1980).

The changes in heart rate and PEP and the lack of effect on LVET are consistent with those previously reported for epoprostenol alone (Warrington *et al.*, 1980). The changes in QS₂ index in the present study did not achieve significance with either drug as previously reported with epoprostenol alone (Hassan *et al.*, 1982). These STI changes together with facial flushing and headache are consistent with a predominantly arteriolar vasodilator effect of both drugs which would be expected to lower diastolic blood pressure and thus reflexly increase heart rate and contractility. However, in the present study the diastolic blood pressure did not fall in response to either drug and this is in contrast to some (O'Grady *et al.*, 1980; FitzGerald *et al.*, 1979; Pickles *et al.*, 1983) but not all (Data *et al.*, 1981) previous studies with epoprostenol alone. LVET was not affected by either drug probably because any abbreviation of LVET due to reduction in peripheral vascular resistance was offset by a reflex increase in stroke volume, which would prolong LVET. The lack of change in diastolic blood pressure

may in part reflect the relative insensitivity of the measurements as compared to measurement of heart rate and PEP. When this study was carried out the ambient temperature in the laboratory was 30°C. In the presence of generalized peripheral vasodilatation attributable to a high ambient temperature the vasodilator effects of both drugs might be less apparent. This may be the converse of the observation that in the presence of hypertension, presumably associated with increased peripheral resistance, small doses of epoprostenol may produce marked reductions in blood pressure (Webster *et al.*, 1980).

The mechanism whereby heart rate increases in response to these drugs in man is not clear, as β -adrenoceptor and parasympathetic blockade do not affect the heart rate response to epoprostenol (Hassan *et al.*, 1982) and minor direct effects of both drugs on heart rate and contractility cannot be excluded.

Though QT_c was significantly increased by the higher dose of both drugs, 9 β -methyl carbacyclin but not epoprostenol at the highest dose significantly increased QT index. The increase in QT_c with both drugs may result from overcorrection for the change in heart rate with the formula used

$$(QT_c = \frac{QT}{RR})$$

but increase in QT index (QT index = QT + 1.7 $\times$ heart rate) was confined to 9 β -methyl carbacyclin alone. The biological significance of these changes is unclear.

The prolongation of bleeding time by epoprostenol but not by 9 β -methyl carbacyclin is surprising given that both drugs produced similar inhibition of platelet aggregation and also of peripheral vasodilatation. However the effect of epoprostenol alone on the bleeding time has been variable, in some studies no change (O'Grady *et al.*, 1980) and in others an increase (Pickles *et al.*, 1983; Data *et al.*, 1981) has been reported. It is unlikely that the difference in response of the bleeding time to the two drugs can be explained on the basis of different qualitative effect on platelet function in the light of the *in vitro* studies and, in addition, since similar lack of effect of both drugs on platelet pre-coagulant activity in the volunteers was demonstrated.

Epoprostenol *in vivo* reduces or reverses the shortening of the recalcification time induced by kaolin and also, by preventing platelet activation, potentiates the anticoagulant effect of heparin (Bunting *et al.*, 1981). However these *in vitro* effects were only significant at epoprostenol concentrations of 5 ng ml⁻¹. The lack of effect of either drug on coagulation

variables in the present study probably reflects the less than maximal inhibition of platelet aggregation achieved by doses tolerated in healthy volunteers.

The side effects experienced with epoprostenol are similar to those reported previously (Pickles & O'Grady, 1982). Nasal stuffiness, presumably reflecting nasal vascular congestion is a feature of some other vasodilator drugs. It has not been reported previously with epoprostenol and the only subject who experienced it during epoprostenol did so during placebo infusion also. However, 9 β -methyl carbacyclin produced nasal stuffiness in two subjects who did not experience it on either epoprostenol or placebo. Interestingly the early human studies of BW245C, a hydantoin prostaglandin with vasodilator and platelet inhibitory properties (Orchard *et al.*, 1983; Shah *et al.*, 1984), have shown it to produce nasal stuffiness in a high

proportion of healthy volunteers. Prostaglandin D₂ has recently been reported to cause nasal congestion, in addition to facial flushing, when infused into man (Heavey *et al.*, 1984). If this observation with 9 β -methyl carbacyclin is confirmed in larger numbers of subjects it could indicate that some vascular beds are affected differently by epoprostenol and by 9 β -methyl carbacyclin.

The pharmacodynamic effects of 9 β -methyl carbacyclin and of epoprostenol are very similar and thus 9 β -methyl carbacyclin is a chemically stable analogue with considerable pharmaceutical advantages.

We are grateful to Mr Paul Lidbury for assistance with the platelet studies *in vitro* and to Ms Lotta Rosam for measurement of platelet cyclic AMP.

QAM and SH are supported by the Iraqi Government.

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(Received May 16, 1984,
accepted August 20, 1984)