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9β-Methylcarbacyclin, a stable prostacyclin analogue, in prevention of platelet losses during charcoal haemoperfusion

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The effect of 9β-methylcarbacyclin, a stable analogue of prostacyclin, as a platelet protective agent, has been investigated during in-vitro charcoal haemoperfusion with fresh heparinized human blood, a model of progressive platelet damage. There were no platelet losses over 3 h perfusion after an initial bolus (1 μg ml^{-1}) of 9β-methylcarbacyclin (101·6 ± s.e. 1·9% of initial value) whereas in the paired control circuit there was a significant reduction in the platelet level (73·5 ± 4·1%, $P < 0.05$). Prevention of rises in plasma thromboxane B_2 by 9β-methylcarbacyclin confirmed the lack of platelet damage and a significant, but less marked, effect on white cell losses was observed. In a second series of experiments a bolus of 9β-methylcarbacyclin in one circuit was compared with prostacyclin infusion in the other which demonstrated that this prostaglandin analogue was as effective as prostacyclin and on the basis of these initial results it would merit clinical evaluation.

Extracorporeal circulation of blood during haemoperfusion, haemodialysis and cardiopulmonary by-pass produces platelet activation as a result of blood contact with foreign materials which leads to platelet adhesion to surfaces, aggregation to form microemboli and release of platelet procoagulant activity, which can induce coagulation even in the presence of heparin. In charcoal haemoperfusion, the use of prostacyclin (PGI_2) as an antiplatelet agent was first evaluated in an in-vitro haemoperfusion circuit (Langley et al 1979) and then during haemoperfusion of patients with fulminant hepatic failure, where both platelet losses and damage, as assessed by release of the platelet protein β-thromboglobulin, were prevented (Gimson et al 1980). Haemoperfusion of longer duration requires dilute solutions of PGI_2 to be kept at room temperature (20 °C) for 10 h and although it is maintained at pH 10·5 as it is unstable at physiological pH, there is the danger of loss of activity.

In this study we have investigated the effects of 9β-methylcarbacyclin, a synthetic chemically stable PGI_2 analogue, during in-vitro charcoal haemoperfusion. This test system gives a model of progressive platelet activation and damage which could have wider application in the assessment of antiplatelet and antithrombotic drugs.

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Materials and methods

Haemoperfusion circuit. The paired in-vitro haemoperfusion circuit used has been described in detail (Hughes et al 1978) and consists of two identical circuits in parallel using standard blood lines (Leyland Medical, Preston, Lancs) connected to blood bags (300 ml Fenwal, Travenol Labs, Thetford, Norfolk). Fresh blood (500 ml) was collected immediately before use into heparin (4·5 u ml^{-1}, mucous, Leo Labs, Princes Risborough, Bucks) from patients undergoing repeated venesection for haemachromatosis, who had been screened for platelet count, platelet function and coagulation profile within normal limits. The blood was divided equally between the two circuits and was pumped (Watson Marlow, Falmouth, Cornwall) at 70 ml min^{-1} through two small haemoperfusion columns simultaneously for 3 h while kept at 37 °C. The plastic haemoperfusion columns (2 cm id., 7·2 cm length) contained polyhema-coated charcoal (17 g, Smith and Nephew Pharmaceuticals, Romford, Essex).

Prostaglandins. 9β-Methylcarbacyclin (ciprostene) was developed jointly by the Wellcome Foundation Ltd, London, and the Upjohn Company, Kalamazoo, USA. A stock solution of 9β-methylcarbacyclin in ethanol (2·5 mg ml^{-1}) was maintained at 4 °C and for use, an aliquot (250 μg) was dried under N_2 and dissolved in 1·25% sodium bicarbonate (2 ml) which was then added to one of the blood reservoirs as a bolus. In the other control circuit the same volume of sodium bicarbonate was added to the reservoir.

In a second series of experiments, the same dose of 9β-methylcarbacyclin was added to the blood reservoir of one circuit and in the other PGI_2 (epoprostenol, (10 μg ml^{-1}) Wellcome Foundation Ltd) in pH 10·5 glycine buffer was added to the blood reservoir (1·25 μg) followed by a continuous infusion (21 μg h^{-1}) into the circuit before the column inlet for 3 h.

Measurements. Blood samples were taken initially and during haemoperfusion after 30 min, 1, 2 and 3 h from the column inlet and at 10, 30 min, 1, 2, 3 h from the column outlet into EDTA tubes for determination of platelets and white blood cells by electronic counting (Model ZF Coulter Electronics, Luton, Bedfordshire) and haematocrit using a micro-haematocrit centrifuge. Blood was taken initially and then hourly from the

column outlet for measurement of the Swank screen filtration pressure to detect the presence of micro-aggregates (Swank 1968). Blood was also collected into tubes containing indomethacin (final concentration 20 μg ml^{-1}) initially and hourly from the column inlet for measurement of the plasma thromboxane B_2 level. Radioimmunoassay of thromboxane B_2 was performed as described by Salmon (1978) for other prostenoids with an antiserum raised in rabbits immunized with thromboxane B_2 conjugated to bovine serum albumin (Kirton et al 1972). Cross reactivity with 6-keto $PGF_{1\alpha}$, PGE_2, PGD_2 and $PGF_{2\alpha}$ and arachidonic acid was less than 0·1%. All samples were measured in the same assay with a within assay variation of 6 to 8% and a limit of detection of 8 pg ml^{-1}.

At the end of perfusion, the pressure across the column was measured using an electronic pressure transducer (Simonsen and Weel, Sidcup, Kent).

The results are given as mean ± 1 s.e. with statistical comparison by the Wilcoxon's test for pair differences.

Results

The mean platelet level at the column inlet remained unchanged in the circuit with 9β-methylcarbacyclin over 3 h haemoperfusion, whereas in the control circuit the platelet level fell rapidly during the first hour then more slowly to a final value of 73·5 ± 4·1% of the initial value, which is significantly lower than in the presence of 9β-methylcarbacyclin, 101·6 ± 1·9% ($P < 0·05$, Fig. 1). The mean platelet levels at the column outlet at 10 min were 101·9 ± 1·6% and 95·5 ± 1·4% with 9β-methylcarbacyclin and control, respectively, and no significant arteriovenous differences in platelets across the column were observed. For the white blood cells a similar loss was observed in both circuits during the first hour (Fig. 2). After this time the value in the 9β-methylcarbacyclin circuit remained at this level (92·9 ± 2·0% at 3 h) but continued to fall in the control circuit until 3 h (75·9 ± 2·3%, $P < 0·05$). No significant arteriovenous differences in the white blood cell levels were observed. There were no marked changes in the mean blood haematocrit in either circuit which with 9β-methylcarbacyclin was 36·8 ± 0·31% PCV initially and 35·3 ± 0·33% at the end of 3 h, with corresponding values of 36·7 ± 0·21% and 35·7 ± 0·21% in the control circuit. No significant rise in Swank screen filtration pressure was seen in either circuit at any of the sample times.

Plasma thromboxane B_2 levels did not rise during 3 h perfusion in the presence of 9β-methylcarbacyclin but in the control circuit there was a progressive increase in thromboxane B_2 (Fig. 3, 84 ± 34 pg ml^{-1} vs 9784 ± 2398 pg ml^{-1}, control at 3 h, $P < 0·05$). At the end of perfusion, the mean pressure drop across the column was 14 ± 0·9 mmHg in the 9β-methylcarbacyclin circuit and 25 ± 8·2 mmHg in the control circuit.

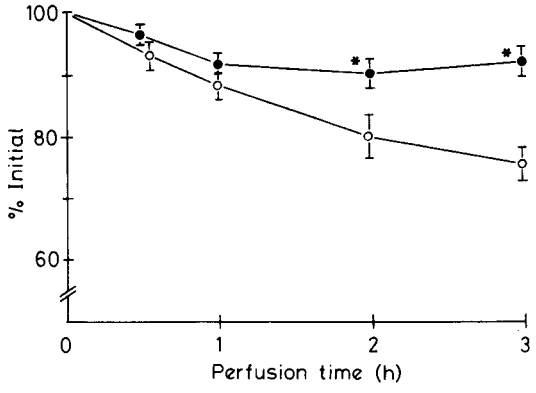

FIG. 2. White cell levels at the column inlet during in-vitro charcoal haemoperfusion in the presence of 9β-methylcarbacyclin (●) and in the control circuit (○). Mean ± s.e., n = 6, *$P < 0·05$.

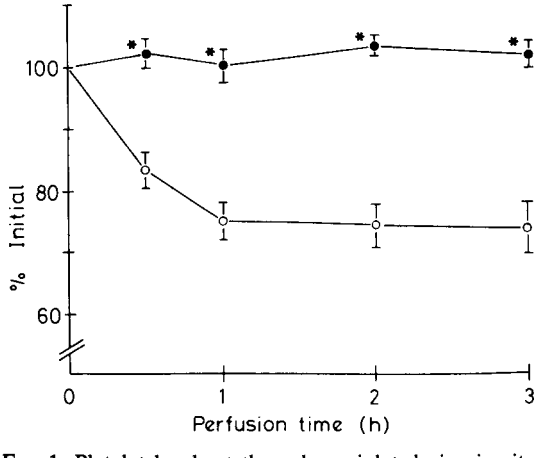

FIG. 1. Platelet levels at the column inlet during in-vitro charcoal haemoperfusion in the presence of 9β-methylcarbacyclin (●) and in the control circuit (○). Mean ± s.e., n = 6, *$P < 0·05$.

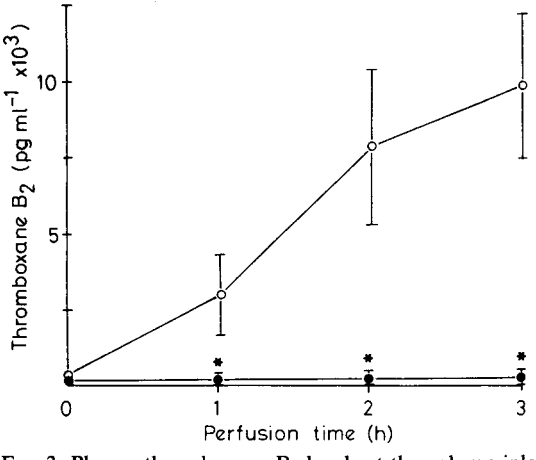

FIG. 3. Plasma thromboxane B_2 levels at the column inlet during in-vitro charcoal haemoperfusion in the presence of 9β-methylcarbacyclin (●) and in the control circuit (○). Mean ± s.e., n = 6, *$P < 0·05$.

In the second series of experiments 9β-methylcarbacyclin as a bolus was compared with PGI_2 infusion (Table 1). With platelets there were no significant changes in either circuit with 99·0 ± 1·6% of initial with 9β-methylcarbacyclin and 96·5% ± 1·1% for PGI_2. For the white blood cells some loss (20–30%) was observed in both circuits with the values tending to be lower with PGI_2, but this difference was not significant. There were no significant differences between the two circuits for either the screen filtration pressure, plasma thromboxane B_2 or pressure drop across the column at the end of perfusion.

Table 1. Platelet and white cell levels at the column inlet during in-vitro charcoal haemoperfusion with a bolus of 9β-methylcarbacyclin in one circuit and PGI_2 infusion in the other. Results are mean ± s.e., n = 6.

	Initial $\times 10^9$ litre^{-1}	30 min	1 h	2 h	3 h
			% Initial		
Platelets					
9β-Methylcarbacyclin	232·4 ±3·6	98·2 ±2·3	100·9 ±2·8	104·2 ±1·6	99·0 ±1·6
PGI_2	237·4 ±4·8	95·6 ±1·7	96·7 ±2·1	96·3 ±1·1	96·5 ±1·1
White blood cells					
9β-Methylcarbacyclin	5·77 ±0·17	97·9 ±1·9	93·4 ±1·0	86·2 ±1·6	82·9 ±1·8
PGI_2	5·89 ±0·12	93·8 ±0·9	87·3 ±0·7	79·3 ±2·7	74·6 ±2·4

Discussion

These in-vitro haemoperfusion experiments clearly demonstrated that 9β-methylcarbacyclin prevents both platelet losses and damage as assessed by the release of thromboxane B_2 and is in this respect as effective as prostacyclin. There was also a smaller but significant effect on the losses of white blood cells which may either be due to the direct effects of the prostaglandin on white cells or reduced entrapment by platelet clumps on the charcoal. The in-vitro biocompatibility circuit used in this study was first used with Amberlite XAD-7 resin, coated with albumin to prevent platelet adhesion (Hughes et al 1978) and proved reliable in predicting the good blood compatibility of this in repeated haemoperfusion of patients with fulminant hepatic failure (Bihari et al 1983). One of the advantages of the scaled-down haemoperfusion circuit is that fresh heparinized human blood is used, as in animal experiments species differences in blood clotting and platelet function (Mason & Read 1967, 1971) can be misleading. Although this circuit could be used to study platelet preservation with a variety of agents, one limitation of these in-vitro experiments is that potential in-vivo effects such as hypotension from vasodilatory drugs such as prostaglandins are not taken into account. One approach to prevent any systemic effects of the anti-platelet agent would be to immobilize it on the adsorbent to produce local platelet inhibition at the external surface.

The pharmacodynamics of 9β-methylcarbacyclin in normal healthy volunteers have been reported recently (O'Grady et al 1984). The data demonstrated that this compound has similar pharmacological activity to PGI_2 including the effects on platelet function and heart rate, though it is approximately 100 times less potent in man. Of major importance to the use of 9β-methylcarbacyclin in extracorporeal circulation was its duration of action which was short, being rapidly degraded in-vivo and thus during haemoperfusion any of the compound not adsorbed by the charcoal would not persist in the systemic circulation. On the basis of this and our results, 9β-methylcarbacyclin, which has similar biological properties to PGI_2, would merit clinical evaluation as a platelet protective agent during charcoal haemoperfusion. Stable PGI_2 analogues should have advantages in other clinical conditions where PGI_2 is used, such as acute thrombotic stroke and ischaemic peripheral vascular disease.

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