INHIBITION OF VASCULAR EPOPROSTENOL (PROSTACYCLIN, PGI₂) PRODUCTION *IN VITRO* BY PLASMA FROM HEALTHY SUBJECTS AND PATIENTS WITH SEVERE RENAL IMPAIRMENT

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- 1 The production of 6-oxo-prostaglandin F_{1α} (6-oxo-PGF_{1α}) and epoprostenol (prostacyclin, PGI₂) by fresh rat aortic rings were measured by radioimmunoassay (RIA). The effect of citrated platelet poor plasma (PPP) from subjects with renal failure (U-PPP) and healthy age/sex matched controls (C-PPP) on 6-oxo-PGF_{1α} production were compared.
- 2 No difference was detected between either 6-oxo-PGF_{1α} or PGI₂ concentration when rings were incubated with U-PPP or C-PPP for 4, 8 and 30 min for 6-oxo-PGF_{1α} ($P > 0.05$, $n = 8$); and 30 min for PGI₂ ($P > 0.05$, $n = 8$).
- 3 Consistently more 6-oxo-PGF_{1α} was produced in PPP that had been heated at 100°C for 5 min (H-PPP) than in C-PPP: mean 6-oxo-PGF_{1α} was increased by factors of 1.45, 1.61, 1.57 and 1.57 at 4, 8, 30 and 60 min respectively ($P < 0.005$ at each time, $n = 8$).
- 4 Similar amounts of 6-oxo-PGF_{1α} were produced by aortic rings incubated in H-PPP and in Tris buffer (50 mM, pH 7.5), $P > 0.05$ at all times ($n = 8$).
- 5 The half-life of PGI₂ in U-PPP was similar to that in C-PPP; 7.8 ± 0.6 min and 10.2 ± 1.9 min (mean $\pm$ s.e. mean) respectively ($P > 0.05$).
- 6 In separate experiments a comparison was made of 6-oxo-PGF_{1α} production by aortic rings incubated in C-PPP, H-PPP and H-PPP to which albumin had been added to restore its original concentration. It was confirmed that more 6-oxo-PGF_{1α} was produced in H-PPP than in C-PPP ($P < 0.05$, $n = 4$). This increment was abolished in the H-PPP to which albumin had been added.
- 7 It was concluded that the heat-labile inhibitor of vascular PGI₂ synthesis in human plasma is albumin.
- 8 The failure to demonstrate a stimulator of PGI₂ synthesis in *fresh* aortic rings by U-PPP does not support the hypothesis that the bleeding diathesis of renal failure is due to an excess of a PGI₂ stimulating factor in plasma.

Introduction

The clinical pharmacology of PGI₂ (FitzGerald *et al.*, 1979) makes it an attractive candidate for a role in protection against vascular disease. It is the major prostaglandin produced by blood vessels, and within the vessel wall it is the endothelium that has the greatest PGI₂ synthesising activity (Moncada *et al.*, 1977; MacIntyre *et al.*, 1978). Plasma is the physiological milieu of endothelium and it has been variously suggested that it contains factors that stimulate prostaglandin synthesis (Remuzzi *et al.*, 1978; MacIntyre *et al.*, 1978; Defreyn *et al.*, 1980) or conversely, factors that inhibit it (Saeed *et al.*, 1977; Herman *et al.*, 1978; Moore & Hoult, 1980). The studies demonstrating an inhibitory effect of plasma have employed homogenates or cultured cells and have not addressed the question of an effect on PGI₂

synthesis but rather demonstrated reduced synthesis of prostaglandin E₂ (PGE₂) and prostaglandin F_{2α} (PGF_{2α}). The studies demonstrating increased PGI₂ or its stable hydrolysis product (6-oxo-PGF_{1α}) have employed cultured cells (MacIntyre *et al.*, 1978) Defreyn *et al.*, 1980) or intact rings of rat aorta that have been 'exhausted' by incubation in Tris buffer (Remuzzi *et al.*, 1978). It seemed possible that the ability of such 'exhausted' rings subsequently to produce PGI₂ when transferred to plasma might reflect the restoration of more physiological conditions rather than the intervention of a specific stimulatory factor. Similarly the physiological relevance of increased PGI₂ production by cells in culture is also problematical.

Ritter *et al.* (1982) recently described a method for

studying 6-oxo-PGF_{1α} production by matched groups of rings of fresh rat aorta incubated in human plasma that gave reproducible results. We have now applied this method to determine whether human plasma inhibits or stimulates production of 6-oxo-PGF_{1α} by fresh aortic rings *in vitro*. In other systems it has been found that plasma from patients with renal failure has more PGI₂ stimulatory activity than plasma from normal subjects (Remuzzi *et al.*, 1978; Defreyn *et al.*, 1980) and it has been suggested that this may account for the bleeding diathesis of renal failure. Plasma from patients with severe renal impairment (U-PPP) was therefore included in the present study. Remuzzi *et al.* (1978) measured PGI₂ after 30 min, thus we also measured PGI₂ at this time in addition to 6-oxo-PGF_{1α}, to determine whether the increased PGI₂ that they observed could be attributed to reduced breakdown rather than increased synthesis. To pursue this further, the half-life of PGI₂ was measured in each sample of U-PPP and C-PPP. Some of these findings have been communicated in preliminary form (Lewis *et al.*, 1982; Orchard *et al.*, 1982).

Methods

Subjects

The characteristics of the patients are summarised in Table 1. None of the patients had received any form of dialysis when the blood was drawn. Each healthy control was matched for sex and age (± 1 year) with a subject with renal impairment.

Plasma

Blood was drawn by venepuncture and immediately added to 3.8% trisodium citrate (10 vol blood : 1 vol citrate for healthy controls; for subjects with renal impairment the volume of citrate was adjusted according to the haematocrit). The citrated blood was centrifuged immediately at 1000 g for 20 min at 4°C and the plasma (U-PPP or C-PPP) separated and

stored at -20°C until use. H-PPP was prepared by placing C-PPP in a glass vial in a boiling water bath for 5 min and expressing the liquid from the resulting coagulum through a nylon mesh.

Aortic rings

These were prepared from male CD rats (Charles River: Margate, U.K.) as described previously (Ritter *et al.*, 1982). A minor modification was that for experiments involving comparison of four incubation media, 1 mm rings from the aortas of three rats were combined to yield groups of 24 rings each, 8 rings in each group from each rat. Where three incubation media were compared, rings from the aortas of two rats were combined similarly. As before, each ring was individually allocated to a group in such a way as to minimise the difference between groups.

Incubations

Each group of rings was stored in balanced salt solution (Gey's solution, Gibco, Uxbridge, U.K.) on ice for less than 30 min before incubation. Incubations were started by adding a group of rings to 2 ml of fluid at 37°C in a shaking water bath. Simultaneous incubations were performed in Tris buffer (50 mM, pH 7.5), U-PPP, C-PPP and H-PPP. Aliquots were removed at predetermined times and stored at -20°C . They were subsequently analysed by RIA for 6-oxo-PGF_{1α} and in some instances PGI₂ (Ritter *et al.*, 1982; Orchard *et al.*, 1982).

Albumin determinations

The albumin concentration of C-PPP was measured by bromocresol green binding in a routine laboratory. The albumin concentration of H-PPP was determined by rocket immunoelectrophoresis (Laurell, 1972).

PGI₂ half-life determinations

These were performed by the method of Orchard & Robinson (1981).

Table 1 Characteristics of patients with renal impairment

Patient number	Age (years)	Sex	Primary diagnosis	Creatinine concentration (μM)
1	42	F	Hypertension	860
2	48	M	Polycystic kidneys	835
3	62	F	Hypertension	1040
4	41	M	Polycystic kidneys	818
5	54	F	Hypertension	1274
6	44	M	Glomerulonephritis	1496
7	59	F	Chronic pyelonephritis	1100
8	37	M	Glomerulonephritis	930

Materials

Human serum albumin (essentially fatty acid free) was obtained from Sigma (London, U.K.). PGI₂ was a generous gift from Wellcome Research Laboratories (Beckenham, Kent).

Statistical methods

Statistical comparisons were made using Student's paired 2-tailed *t*-test. Differences were considered significant when $P < 0.05$.

Results

In the first series of experiments groups of aortic rings were incubated simultaneously with Tris buffer (50 mM, pH 7.5), U-PPP, C-PPP and H-PPP. The amount of 6-oxo-PGF_{1α} produced under these conditions at various times, and of PGI₂ in U-PPP and C-PPP at 30 min were determined (Table 2). In every incubation more 6-oxo-PGF_{1α} was produced in H-PPP than in C-PPP or U-PPP ($P < 0.005$ for 4, 8 and 30 min, $P < 0.05$ at 60 min where fewer measurements were made). The mean production in H-PPP at 4, 8, 30 and 60 min exceeded that in C-PPP by factors of 1.45, 1.61 and 1.57 respectively. The production of 6-oxo-PGF_{1α} in H-PPP at 4, 8 and 30 min was similar to that in Tris buffer ($P > 0.05$, $n = 8$). No significant difference was detected between C-PPP and U-PPP at any time ($P > 0.05$, $n = 8$ at 4, 8 and 30 min). Similar amounts of PGI₂ were present at 30 min in U-PPP and in C-PPP: 0.69 ± 0.15 ng/mg compared with 1.28 ± 0.31 ng/mg ($P > 0.05$, $n = 8$).

The process of heating C-PPP at 100°C for 5 min and expressing the liquid from the proteinaceous coagulum so formed removes nearly all of the albumin: the concentration of albumin in a sample of H-PPP was found to be 0.2 g/l. In a separate series of experiments we studied the effect of restoring the albumin to its original concentration. Simultaneous incubations were performed in C-PPP, H-PPP and H-PPP in which human albumin had been dissolved to restore the original concentration. Four such

experiments were performed. The control albumin concentrations ranged from 43–49 g/l. The results are shown in Figure 1. As before, more 6-oxo-PGF_{1α} was produced in H-PPP than in C-PPP ($P < 0.05$, $n = 4$). The 6-oxo-PGF_{1α} produced in H-PPP with restored albumin was reduced compared with that in H-PPP ($P < 0.05$, $n = 4$). Thus the amount of 6-oxo-PGF_{1α} in H-PPP with restored albumin was similar to that produced in C-PPP ($P > 0.2$, $n = 4$).

The half-life of PGI₂ added *ex vivo* to each of the samples of C-PPP and U-PPP was measured at 37°C (pH 7.3–7.5). The mean half-life of PGI₂ in C-PPP was 10.2 ± 1.9 min (mean $\pm$ s.e. mean) and in U-PPP was 7.8 ± 0.6 min (mean $\pm$ s.e. mean); the difference was not significant ($P > 0.05$).

Figure 1 Albumin restores the inhibitory activity of H-PPP. Values represent 6-oxo-PGF_{1α} produced following incubation for 30 min in C-PPP, H-PPP and H-PPP to which albumin had been added so as to restore the original albumin concentration (mean $\pm$ s.e. mean, $n = 4$).

Table 2 6-oxo-PGF_{1α} and PGI₂ production by aortic rings in Tris buffer, U-PPP, C-PPP and H-PPP

	4 min ^a	6-oxo-PGF _{1α} (ng/mg)			PGI ₂ (ng/mg)
		8 min ^a	30 min ^a	60 min ^b	30 min ^a
Tris	1.13 ± 0.19	1.99 ± 0.41	4.39 ± 0.84	—	—
U-PPP	0.93 ± 0.20	1.57 ± 0.32	3.56 ± 0.80	—	0.69 ± 0.15
C-PPP	0.88 ± 0.19	1.47 ± 0.32	3.65 ± 0.93	3.20 ± 0.40	1.16 ± 0.35
H-PPP	1.28 ± 0.27	2.38 ± 0.51	5.45 ± 1.13	5.03 ± 0.79	—

^a Mean $\pm$ s.e. mean, $n = 8$, ^b Mean $\pm$ s.e. mean, $n = 5$

Discussion

The finding that more 6-oxo-PGF_{1α} is formed in H-PPP than in C-PPP is most simply explained by the proposal that plasma contains a heat labile inhibitor of PGI₂ synthesis. This accords with the work of Saeed *et al.* (1978). These workers described an endogenous inhibitor of prostaglandin synthesis in plasma, which inhibits PGE₂ and PGF_{2α} synthesis by bovine seminal vesicle homogenates. This endogenous inhibitor was subsequently shown to be largely due to the albumin content of plasma (Kendall *et al.* 1979). Herman *et al.* (1979) described a factor in human serum that inhibited PGE synthesis during co-culture of human thyroid cells and mononuclear cells. This inhibitor co-migrated with albumin on gel filtration and ion exchange chromatography. Our finding that replacing the original albumin concentration to H-PPP restored its inhibitory activity implies that albumin is the inhibitor of PGI₂ synthesis in our system. It is possible that albumin acts as a 'substrate trap' by binding free arachidonate, a mechanism that has been described for the inhibition of PGE₂ synthesis by cultured fibroblasts (Chandrabose *et al.*, 1980).

There is no evidence from these experiments with fresh aortic rings of a PGI₂ stimulating factor in plasma, either from healthy subjects or from patients with severe renal impairment of diverse aetiology (Table 1). This contrasts with the findings of Remuzzi *et al.* (1978). These workers studied aortic rings 'exhausted' by repeated washing in Tris buffer (Remuzzi *et al.*, 1978; Villa *et al.*, 1977; Moncada *et al.*, 1977). These rings produce PGI₂ when subsequently incubated with plasma. This effect was more marked with plasma from patients with uraemia. Our failure to demonstrate stimulatory activity by plasma is not attributable to the fresh aortic rings producing 6-oxo-PGF_{1α} maximally. This is because 6-oxo-PGF_{1α} production by fresh rings is markedly and significantly ($P < 0.005$) increased by

human serum (Lewis *et al.*, 1982).

Remuzzi *et al.* (1978) assayed PGI₂ after 30 min incubation. In the present study 6-oxo-PGF_{1α} was measured after hydrolysis of residual PGI₂. This is a true reflection of PGI₂ synthesis since it is unaffected by breakdown during incubation. (Ritter *et al.*, 1982). We therefore considered the possibility that the factor described by Remuzzi *et al.* (1978) was stabilising PGI₂ rather than increasing its production. However, the PGI₂ concentration after 30 min incubation in U-PPP was similar to that in C-PPP (Table 2). Direct measurement of the half-life of PGI₂ also failed to show a significant difference between C-PPP and U-PPP. Furthermore, Defreyne *et al.* (1980) using cultured endothelial cells and measuring 6-oxo-PGF_{1α} rather than PGI₂ obtained a result in agreement with that of Remuzzi *et al.* (1978). The disparity is thus likely to be due to differences in the preparations. It is possible that the rate-limiting enzyme is different in fresh and 'exhausted' rings or cultured cells. Thus phospholipase A₂ is rate-limiting in fresh rings (Panganamala *et al.*, 1981) while cyclooxygenase may be rate-limiting in exhausted rings or cultured cells. We are currently studying this possibility. Whatever the explanation, the behaviour of fresh aortic rings is probably more representative of the situation *in vivo* than is that of 'exhausted' rings. This is because fresh rings are maintained for the minimum possible time in physiologically balanced salt solution on ice. In contrast, 'exhausted' rings have been repeatedly washed in hypotonic buffer of low ionic strength and unphysiological pH at room temperature (Remuzzi *et al.*, 1978). Thus the present results do not support the hypothesis that the bleeding diathesis of uraemia is due to an excess of PGI₂ stimulating factor in plasma.

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