Distinction between prostaglandin E_2 and prostacyclin as inhibitors of granulomatous inflammation

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Prostaglandins (PGs) of the E series, are detectable at inflamed sites and exert pro-inflammatory actions, such as vasodilatation and potentiation of plasma exudation induced by other mediators (see Flower 1977). However, with animal models of chronic inflammation, high doses of PGEs exert anti-inflammatory actions in vivo (see Bonta & Parnham 1978). Recently, we observed that local administration of PGE_1, at 1000-fold lower doses than those used by other authors, during the early phase of sponge-induced granulomatous inflammation, enhances subsequent granuloma formation, whereas administration during the later phase inhibits granuloma formation (Bonta & Parnham 1979). In fact, in rats which were deficient in the fatty acid precursors of endogenous PGs, exogenous PGE_1 inhibited granuloma formation when given at the low daily dose of 50 ng. We now report similar pro- and anti-granuloma effects with low doses of PGE_2 (the most commonly detected PG at inflamed sites) and additionally show that the later inhibitory effects of PGE_2 on granuloma formation are capable of counteracting the early stimulatory effects. We have also compared the inhibitory action of PGE_2 with other PGs including prostacyclin (PGI_2), a novel PG (Moncada et al 1976) which has been found to exert similar actions to PGE_2, including vasodilatation and increased vascular permeability (Peck & Williams 1978; Higgs et al 1978b; Murota et al 1978).

Granulomatous inflammation was induced in rats, using subcutaneously implanted, carrageenan-soaked, polyether sponges (2 per rat) with indwelling cannulae (Bonta et al 1979), and the PGs were injected either with the 1 ml 2% Na carrageenan on implantation (day 1) and/or through the cannulae (in 0·5 ml) at different times, as described earlier (Bonta & Parnham 1979; Bonta et al 1979). Sponges in control animals were similarly treated with vehicle. PGE_2, $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ were injected in saline solution. PGI_2 was dissolved in Tris buffer (pH 8·4), which stabilized this PG to some extent (N. Whittaker, personal communication; Murota et al 1978) and sponges in the respective control animals were treated with this buffer. The buffer itself had no effect on granuloma formation (data not shown). While the other PGs were administered as single daily doses of 2 μg/sponge, PGI_2 was injected twice daily at a dose of 1 μg/sponge. Once again, this dose regime was intended to compensate for the rapid breakdown of PGI_2 in aqueous media (Moncada et al 1976). All rats were killed on day 8, sponges removed and granuloma formation expressed as the dry weight of tissue surrounding the sponges (Bonta et al 1979). In some experiments, both adrenals from each rat were removed and weighed together, their weights being expressed in terms of 100 g body weight.

Table 1 shows that, while injection of PGE_2 (2 μg) into sponges on implantation (day 1) had no effect on granuloma formation, measured after 8 days, the same daily dose administered on days 1–3 enhanced granuloma formation after 8 days. This action is similar to the granuloma stimulation produced by PGE_1 (1 μg), which was thought to be secondary to an initial vasodilating action, following injection on day 1 (Bonta & Parnham 1979). Furthermore, the inhibition of 8 day granuloma formation caused by administration of PGE_2 on days 4–7 (Table 1) was identical to the effect of PGE_1 administered over the same period (Bonta & Parnham 1979). It should be noted that the increase in control (saline-treated) granuloma weights with progressively later treatment periods, is coincidental, since, in a large number of control experiments, granuloma weight was unrelated to timing of saline treatment, despite considerable variation in granuloma weights (Bonta et al 1979). Furthermore, it was previously found that this variation in granuloma weights did not alter the effects of added PGE_1 (Bonta & Parnham 1979). Thus, PGE_1 injected on day 1 enhanced granuloma weight when control values ranged from 0·32 to 0·78 g and when injected on days 4–7, PGE_1 inhibited granuloma weight in experiments in which control values ranged from 0·35 to 1·03 g. In the present study PGE_2, administered on days 4–7, inhibited granuloma weight in two experiments in which

Table 1. Effects of prostaglandin E_2, administered at different time periods on experimental granuloma formation. Data shown are the mean dry weights ($\pm$ standard error) of granulomatous tissue surrounding the sponges, removed in every case 8 days after sponge implantation. Numbers in brackets indicate the numbers of observations. Significance of differences (control v. PGE_2-treated) was determined by one-tailed Mann-Whitney U test: * $P < 0·05$, ** $P < 0·01$.

Treatment (days)	Dry granulomata wt (g) Control	PGE_2 (2 μg)
1	0·29 ± 0·03 (10)	0·34 ± 0·03 (8)
1–3	0·53 ± 0·04 (10)	0·63 ± 0·03 (10)*
4–7	0·80 ± 0·03 (10)	0·68 ± 0·03 (10)**
1–8	0·54 ± 0·05 (10)	0·50 ± 0·04 (10)

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control values were 0·80 and 0·56 g (Tables 1 and 2), though in each case the inhibition was only 15%.

In contrast, daily treatment with PGE_2 on days 1–8 did not affect 8 day granuloma formation, indicating that the later inhibitory action of PGE_2 had reversed its early stimulatory effect (Table 1). The mechanism of the inhibition produced by PGE_2 is unknown. However, dietary-induced depletion of endogenous PGs, including PGE_2, in rats, leads to enhancement of granuloma formation, which is associated with increased collagen turnover (Bonta et al 1977; Parnham et al 1977). This indirectly suggests that the inhibitory effect of PGE_2 on granuloma formation somehow may involve an action on connective tissue metabolism.

Table 2 shows that, in contrast to the inhibitory effect of PGE_2, neither PGI_2, nor its metabolite 6-keto $PGF_{1\alpha}$, had any significant effect on 8 day granuloma formation, when administered on days 4–7. $PGF_{2\alpha}$ also failed to exert a significant effect (Table 2), though in higher doses $PGF_{2\alpha}$ inhibits protein, including collagen synthesis in carrageenan-induced granulomata (Ohuchi et al 1977). Despite the fact that PGI_2 is less stable than PGE_2, the vasodilating actions of these PGs last for approximately the same length of time (Peck & Williams 1978), and both PGs increase vascular permeability in carrageenan granulomata (Murota et al 1978). This suggests that the lack of effect of PGI_2 on granuloma formation was probably not due to its rapid breakdown. Certainly, the PGI_2 was present in a sufficiently high concentration to markedly suppress adrenal weight (Table 2), suggesting that the PG had diffused away from the injection site, which is commensurate with the postulated role of PGI_2 as a circulating hormone (Gryglewski et al 1978; Moncada et al 1978). The reduction by PGI_2 of adrenal weight is similar to the effect of $PGF_{2\alpha}$, while the PGI_2 metabolite, 6-keto $PGF_{1\alpha}$, enhanced the size of the adrenals (Table 2). Some doubt may be cast on the acceptability of this latter enhancement in the light of the considerable difference in the size of the adrenals in the 6-keto $PGF_{1\alpha}$ control group compared with that of the adrenals in the other control groups. It is possible that this variation in size was due to slight differences in the ages of the rats, a factor which markedly effects adrenal size in this species (Persellin et al 1972). In a recent report, 6-keto $PGF_{1\alpha}$ was shown to be 2–10 times more potent and PGI_2 100–1000 times more potent than PGE_2 in stimulating steroidogenesis in adrenocortical cells in vitro (Ellis et al 1978). The effects of PGI_2 on granuloma formation and adrenal weight, taken together, are clearly different from the effects of PGE_2 on these two parameters. Although it is possible that higher doses of PGI_2 may exert significant effects on granuloma formation, it is clear from the present data that there are differences between PGE_2 and PGI_2, at least in their relative potencies on different tissues. Moreover, the distinction between the effects of the two PGs on granuloma formation and adrenal weight suggests that PGE_2 did not inhibit granuloma formation indirectly, through an action on adrenal corticosteroid production. The differences in the effects of the PGs on granuloma formation are particularly interesting in the light of the observation that PGE_2 and PGI_2 are equipotent in increasing vascular permeability in carrageenan granulomata (Murota et al 1978). Other recent findings indicate that PGE_2 is also more potent than PGI_2 in potentiating carrageenan-induced paw oedema (Higgs et al 1978a), in relaxing the lamb ductus arteriosus (Clyman et al 1978) and in stimulating cyclic AMP accumulation in rat fat cells (Hjemdahl et al 1978).

Table 2. Effects of various prostaglandins, administered on days 4–7 after sponge implantation, on experimental granuloma formation and adrenal weights. PGs E_2, $F_{2\alpha}$ and 6-keto $F_{1\alpha}$ were administered at doses of 2 μg/sponge/day, PGI_2 at a dose of 1 μg/sponge twice daily. Data shown are the means (± standard error) of 10 (dry granuloma wt) or 5 (adrenal wt) observations made on day 8. Significance of differences (control v. PG-treated) was determined by one-tailed Mann-Whitney U test: $*P < 0.05$, $**P < 0.025$.

Treatment	Dry granuloma wt (g)		Adrenal wt (mg/100g body wt)	
	Control	Treated	Control	Treated
PGE_2	0·56 ± 0·04	0·48 ± 0·03*	33·6 ± 2·6	32·9 ± 0·6
$PGF_{2\alpha}$	0·56 ± 0·04	0·49 ± 0·05	33·6 ± 2·6	29·2 ± 0·9*
PGI_2	0·74 ± 0·04	0·69 ± 0·04	37·8 ± 2·3	29·9 ± 0·7**
6-keto $PGF_{1\alpha}$	0·59 ± 0·04	0·63 ± 0·04	21·2 ± 0·6	24·9 ± 1·6**

In conclusion, we have shown that PGE_2, like PGE_1, modulates granulomatous inflammation, depending upon the time of administration. Despite the similarities in the activities of PGE_2 and PGI_2 on some tissues, PGE_2 is more effective than PGI_2 as an inhibitor of granulation tissue formation. Since the effects observed were pharmacological, any conclusions regarding possible roles of endogenous PGs remain conjectural.

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Partition of cinoxacin and nalidixic acid in canine vascular and extravascular compartments

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Cinoxacin, 1-ethyl-1, 4-dihydro-4-oxo-(1,3)-dioxolo-(4,5-g) cinnoline-3-carboxylic acid, belongs to the same class of antimicrobial drugs as nalidixic acid and oxolinic acid, which are used in the treatment of infections of the genito-urinary system. While its antibacterial activity is similar to nalidixic acid and oxolinic acid in vitro, it may be superior in vivo against pyelonephritis in rats (Holmes et al 1974). Numerous antibiotics have been shown to be nephrotoxic. This toxicity has usually been related to high concentrations of the agent present in tissue fluid. Lymph fluid has been shown to be similar to interestitial fluid by Guyton (1963) and Mortillaro & Taylor (1976), therefore sampling lymphatic drainage from the kidney would be a means of determining if toxic concentrations of a drug were present in interestitial fluid when blood concentrations were at clinical values. To determine the relationship between blood and interstitial concentrations of cinoxacin and nalidixic acid, blood, thoracic duct lymph and renal hilar lymph were collected simultaneously over 5 h. Comparisons were made between the two drugs as well as between drug concentrations in various fluid compartments.

Thirty mongrel dogs of either sex, 16 ± 2 (s.e.) kg, were anaesthetized with pentobarbitone (25 mg kg^{-1}), then intubated with endotracheal tubes and ventilated via a Harvard Respirator. The femoral artery and vein were cannulated. The arterial cannula was connected to a Statham strain gauge pressure transducer and arterial blood pressure was recorded on a Beckman type RS Dynograph. The venous cannula was advanced into the inferior vena cava and connected to a saline monitor to measure pressure in the inferior vena cava. The measurements were made every 20 min.

The thoracic duct was exposed in the neck and cannulated as previously described (Szwed et al 1972). The left kidney was exposed and one renal hilar lymphatic identified and cannulated with P.E.-10 tubing for lymph collection. A catheter was placed into the bladder. Baseline fluid samples were collected for 30 min before drug administration. Cinoxacin and nalidixic acid were injected in doses of 5, 10 and 20 mg kg^{-1} as an intravenous bolus to separate groups of five dogs for each dose. No autologous fluid was returned to the animals to preclude reinfusion of antibiotic. A constant intravenous infusion of 0·5 ml min^{-1} of 5% dextrose in 0·9% NaCl saline was continued throughout the study. Blood, thoracic duct, renal lymph and urine samples were collected at 15, 30, 45, 60, 120, 180, 240, and 300 min after injection of the drugs. A fluorometric assay of cinoxacin (sensitivity 0·5 μg ml^{-1}) was carried out by the Eli Lilly Research Laboratories. Nalidixic acid was analysed by liquid chromatography (Schargel et al 1973). Statistical analysis was by analysis of variance for repeat measurements.

Samples of cinoxacin and nalidixic acid were similar in all body fluids at all times after the 5 and 10 mg kg^{-1} doses. With the 20 mg kg^{-1} dose, nalidixic acid concentrations in serum, thoracic duct lymph, and urine exceeded those of cinoxacin at all times ($P < 0{\cdot}01$) (Fig. 1A-C). Renal hilar lymph concentrations of nalidixic acid exceeded those for cinoxacin at 45, 60, and 300 min post injection ($P < 0{\cdot}05$) otherwise there were no differences (Fig. 1D). The serum concentrations of both drugs accurately reflected the concentration of the antibiotics in the renal hilar lymphatic tissue, i.e., renal interstitial fluid. Except for a difference in drug values after the 5 and 20 mg kg^{-1} doses of nalidixic acid during the vascular-extravascular 'mixing phase' occuring during the first 60 min after drug injection, all values for serum and renal hilar lymph were statistically identical (cf Fig. 1A and D). No significant changes in mean arterial blood pressures and inferior vena cava pressures were observed at anytime.

Interstitial fluid and lymphatic fluid are similar in

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