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Glucocorticoid protection against PAF-acether toxicity in mice

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1 Intravenous platelet activating factor (PAF-acether, 10 to 25 μg/kg body weight) produced dose-dependent mortality in both male and female mice.

2 Pretreatment with indomethacin (50 mg/kg), verapamil (40 mg/kg) or nifedipine (4 mg/kg) failed to inhibit the lethal effect of 20 μg/kg PAF-acether. This suggests that neither arachidonate cyclo-oxygenase products nor availability of extracellular Ca^{2+} mediate the toxic action.

3 In contrast, pretreatment with 100 mg/kg cortisone acetate (s.c.) daily for four days exerted a highly protective effect, i.e. 100% and 93% survival in males and females, compared to 13% and 7% respectively, in untreated animals.

4 PAF-acether-induced death may be a useful model for the *in vivo* evaluation of pharmacological agents in anaphylactic shock.

Introduction

Intravenous platelet activating factor (PAF-acether), 1-O-alkyl-2-acetyl-glyceryl-3-phosphorylcholine, induces an anaphylactic reaction in rabbits, baboons and guinea-pigs, characterized by thrombocytopaenia, hypotension and bronchoconstriction (McManus, Hanahan, Demopoulos & Pinckard, 1980; Chignard, Vargaftig, Benveniste & Le Couedic, 1980; Vargaftig, Lefort, Chignard & Benveniste, 1980; McManus, Pinckard, Fitzpatrick, O'Rourke, Crawford & Hanahan, 1981). At high doses, the anaphylactic reaction results in death (McManus *et al.*, 1980; Chignard *et al.*, 1980). In the rat, PAF-acether induces severe hypotension (Blank, Snyder, Byers, Brooks & Muirhead, 1979) but not bronchoconstriction or thrombocytopaenia. The latter may be due to the refractoriness of rat platelets to PAF-acether (Vargaftig, Chignard, Benveniste, Lefort & Wal, 1981).

PAF-acether-induced human platelet aggregation is inhibited *in vitro* by a variety of agents including chlorpromazine, indomethacin and prostacyclin, prostaglandin I_2 (PGI_2, Chesney, Pifer, Byers & Muirhead, 1982). Paw oedema induced by PAF-acether in the rat is attenuated by prednisolone and indomethacin and in addition by the calcium blocking agents nifedipine and verapamil (Bonnet, Loiseau, Orvoen & Bessin, 1981). Bronchoconstriction due to PAF-acether in the guinea-pig is inhibited by sulphinpyrazone (Chignard, Wal, Lefort & Vargaftig, 1982). However, no inhibitors of the generalized systemic reaction to intravenous PAF-acether have been reported. In this present study, we describe the acute toxicity of PAF-acether in mice, and evaluate the protective effects of a representative glucocorticoid and arachidonate cyclo-oxygenase inhibitor, as well as two calcium blockers.

Methods

Groups of 35 male and 35 female CD-1 mice (Charles River Breeding Laboratories) approximately 50 days old were anaesthetized with sodium amytal (100 mg/kg, i.p.) and injected via the jugular vein with 10, 15, 20 or 25 μg/kg semi-synthetic PAF-acether (Calbiochem). PAF-acether solutions were prepared in 0.9% w/v NaCl solution (saline) at concentrations such that the injection volume was 5 μl/g body weight. The incidence and time of mortality, as defined by cessation of respiration and lack of palpable heart beat, were recorded. Surviving animals were killed after 72 h. The mortality of male and female mice at specific PAF-acether doses was compared using the χ^2-test. The time to death, excluding surviving mice, was compared between male and female mice by Student's *t* test.

Additional groups of male mice were pretreated with cortisone acetate (Merck Sharp & Dohme),

verapamil hydrochloride (Knoll), nifedipine (Pfizer) or indomethacin (Sigma) prior to intravenous challenge with 20 μg/kg PAF-acether. Cortisone (saline suspension) was administered subcutaneously at a dose of 100 mg/kg daily for four days before challenge; verapamil (solution in saline) was given by gastric intubation 30 min before PAF-acether; nifedipine (solution in ethanol and polyethylene glycol) was also administered orally 30 min before PAF-acether; indomethacin (solution in buffered saline) was given orally 2 h before PAF-acether. These pretreatment regimens were selected on the basis of their high degree of efficacy in protecting against sudden death induced by arachidonic acid in mice (Penhos, Rabbani, Myers, Ramey & Ramwell, 1980; Rabbani, Myers, Ramey, Ramwell & Penhos, 1981; Myers, unpublished). Control groups received the appropriate vehicle pretreatment. The cortisone experiment was repeated in female mice due to the results of the experiment in males. The incidence of mortality in pretreatment and control groups was compared using the χ^2-test.

Results

PAF-acether when administered intravenously induced death preceded by respiratory depression in male and female mice in a dose-dependent manner (Figure 1). The time at which death occurred was inversely related to dose. The mortality rate of males tended to be higher than that of females, but between-sex comparisons were not statistically significant; males also tended to die sooner than females, and this difference was significant at the 25 μg/kg dose ($P<0.02$). From this dose-mortality study, a dose of 20 μg/kg PAF-acether was chosen for subsequent experiments.

Verapamil, nifedipine and indomethacin failed to protect male mice against the lethal effects of 20 μg/kg PAF-acether. Survival in groups of mice pretreated with these agents was 0 alive of 15 tested, 1 of 15 and 2 of 20, respectively, compared to 1 of 15, 4 of 15 and 2 of 20 in the control groups. Cortisone pretreatment completely protected male mice (15 of 15), compared to survival of 2 of 15 in controls ($P<0.001$). A similar degree of protection was obtained in females (14 of 15, compared to 1 of 15; $P<0.001$).

Discussion

PAF-acether produced a steep dose-mortality curve (Figure 1). Its potency in inducing death is approximately 2000 fold that of intravenous arachidonate and 4 fold that of the 9,11-methanoepoxy PGH_2 analogue (U46619), a thromboxane agonist (Myers, Penhos, Ramey & Ramwell, 1983). Sudden death induced by arachidonate or U46619 is thought to result from occlusive pulmonary thrombosis. However, in the case of PAF-acether-induced sudden death in mice, platelet aggregation may only play a minor role, since like rat platelets (Vargaftig *et al.*, 1981), mouse platelets are refractory to PAF-acether (Myers, unpublished).

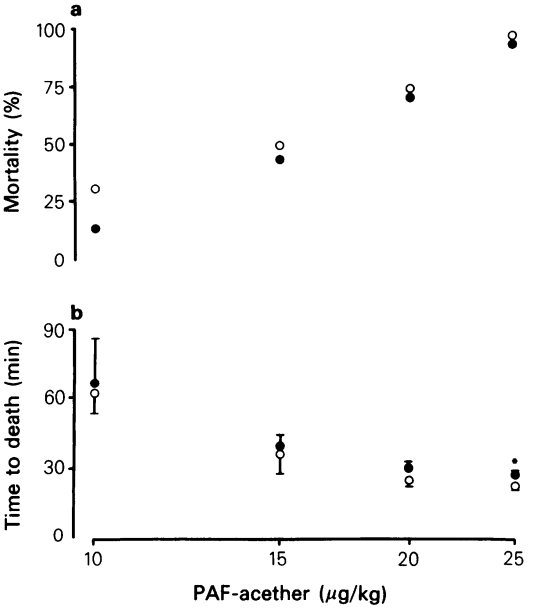

Figure 1 Mortality induced by intravenous PAF-acether in mice. Ordinates: (a) percentage mortality; (b) time to death, in min. Abscissa scale: i.v. dose of PAF-acether, log scale. (○) Male mice; (●) female mice ($n=35$ for each sex). Bars represent one standard error. * $P<0.02$ for the between-sex comparison.

The failure of indomethacin to inhibit the lethal effect of PAF-acether suggests that cyclo-oxygenase products of arachidonic acid do not mediate PAF-acether toxicity. Further, the lack of protection by either nifedipine or verapamil against PAF-acether challenge suggests that availability of extracellular Ca^{2+} is unnecessary for the toxic effects. This is in contrast to arachidonate-induced sudden death, in which calcium channel blockers (Okamatsu, Peck & Lefer, 1981), cyclo-oxygenase inhibitors (Kohler, Wooding & Ellenbogen, 1976), a thromboxane synthetase inhibitor (Myers, Rabbani, Penhos, Ramey & Ramwell, 1981) and a thromboxane receptor antagonist (Myers *et al.*, 1983) all confer protection.

In contrast, cortisone was highly protective against PAF-acether-induced sudden death, as are glucocorticoids in a variety of challenge models including

arachidonate-induced sudden death in mice (Penhos, Montalbert-Smith, Rabbani, Ramey & Ramwell, 1979) and IgE-mediated lung anaphylaxis induced by ovalbumin in guinea-pigs (Andersson & Brattsand, 1982). Indeed, IgE-induced anaphylaxis itself may be mediated by PAF-acether (Pinckard, Halonen, Palmer, Butler, Shaw & Henson, 1977). Cortisone may protect against PAF-acether toxicity by inhibiting the release of arachidonic acid and thereby its subsequent metabolism to lipoxygenase products, which induce anaphylaxis-like effects (Piper, Samhoun, Tippins, Williams, Palmer & Peck, 1981). An alternative explanation is that glucocorticoids might induce the release of potential anti-inflammatory plasma factors (Saeed, McDonald-Gibson, Cuthbert, Copas, Schneider, Gardiner, Butt & Collier, 1977; Moore & Hoult, 1980). Such factors inhibit the formation of prostaglandins (Saeed et al., 1977; Moore & Hoult, 1980) and lipoxygenase products (Saeed, Drew & Collier, 1980) from arachidonic acid in *in vitro* systems. The protective effect of cortisone against intravenous PAF-acether-induced death suggests that this model may provide a useful test system for the development of protective agents against anaphylactic reactions.

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