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The effect of 6-oxo-prostaglandin E_1 on human platelet aggregation in whole blood in-vitro

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The effect of PGI_2, 6-oxo-PGE_1 and PGE_1 on ADP-induced human platelet aggregation has been assessed in whole blood and in blood centrifuged to prepare platelet-rich plasma (PRP). PGI_2 was the most potent anti-aggregatory agent in both media. The concentration of PGI_2 required to produce 50% inhibition of platelet aggregation was approximately 0·3 ng ml^{-1} in each case. In contrast both E series prostaglandins exhibited significantly greater (400–700%) anti-aggregatory activity when tested in whole blood than when tested in PRP. Since whole blood presumably represents a truer reflection of platelet reactivity in-vivo, we believe that the potency of 6-oxo-PGE_1 (and PGE_1) as inhibitors of platelet aggregation has been underestimated in previous experiments using PRP. In human whole blood 6-oxo-PGE_1 has approximately 40% the anti-aggregatory activity of PGI_2. The reasons for the increased anti-aggregatory potency of E series prostaglandins in whole blood is not known. We suggest that 6-oxo-PGE_1 and PGE_1 (but not PGI_2) may prevent the release of pro-aggregatory ADP from red blood cells thereby enhancing their ability to inhibit platelet aggregation.

6-Oxo-prostaglandin E_1 (6-oxo-PGE_1) is a metabolite of prostacyclin (PGI_2) formed by the enzyme 9-hydroxyprostaglandin dehydrogenase (9-PGDH). In recent years, the potent biological activity of 6-oxo-PGE_1 in different test systems has been unequivocably established (see Moore & Griffiths 1983 for review). 6-Oxo-PGE_1 mimics PGI_2 (but has greater potency) as a renin and erythropoietin secretagogue (Jackson et al 1981; Nelson et al 1983), bronchodilator (Spannhake et al 1981), spasmogen on gut smooth muscle (Griffiths et al 1982), inhibitor of noradrenaline release from electrically stimulated sympathetic nerve terminals (Griffiths & Moore 1983) as a fibrinolytic agent (Korbut et al 1983). For this reason, 6-oxo-PGE_1 which, unlike PGI_2, is chemically stable at body temperature and pH, may be of potential clinical use in the treatment of conditions like asthma and as a thrombolytic drug. Although 6-oxo-PGE_1 also inhibits human platelet aggregation in-vitro, reports of its potency relative to PGI_2 have varied widely. Originally it was reported to be equipotent with PGI_2 (Wong et al 1979), later research revealed that PGI_2 was either 3× (Schwertschlag et al 1982), 18× (Griffiths et al 1982) or even 20× (Miller et al 1980) more potent than 6-oxo-PGE_1. However, in each of these publications anti-aggregatory activity was assessed in centrifuged blood devoid of erythrocytes and leucocytes which, by releasing agents like ADP, adenosine and even PGI_2 itself, may influence in-vitro platelet reactivity. Clearly, experiments of this type are not representative of the physiological situation in-vivo. For this reason we have now re-examined the ability of PGI_2, 6-oxo-PGE_1 and PGE_1 to prevent platelet aggregation in a more physiological environment using whole blood and measuring platelet aggregation by the technique of electronic impedence aggregometry.

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Methods

Blood (18 ml) was collected by clean venepuncture from adult, male volunteers and anti-coagulated with 2 ml trisodium citrate (2·0% w/v) containing 2 U ml^{-1} heparin. Platelet aggregation was measured in 1 ml aliquots of blood using a Chronolog impedence aggregometer, model 540VS (Coulter Electronics Ltd). For these experiments, blood was warmed to 37 °C and stirred by means of a Teflon stir bar rotating at 1000 rev min^{-1}. Platelet aggregation was induced with ADP at a concentration (5–10 μM) just sufficient to produce an irreversible platelet aggregation. The extent of platelet aggregation was measured after 8 min. Dilute concentrations of 6-oxo-PGE_1 and PGE_1 were prepared on the day of use in 50 mM Tris-HCl buffer (pH 7·4) from ethanol master stocks (1 mg ml^{-1}) maintained at −20 °C. In contrast, PGI_2 which was stored (1 mg ml^{-1}) in 0·1 M NaOH (pH 12) at −20 °C was diluted in 50 mM Tris HCl buffer (pH 8·4) before use. All prostaglandins were kept on ice until added in volumes less than 10 μl to the cuvette 1 min before the injection of ADP. Tris-HCl buffer (pH 7·4 and 8·4) did not affect the whole blood platelet aggregation response to ADP when added to the cuvette in volumes up to 20 μl.

In some experiments, the anti-aggregatory activity of PGI_2, 6-oxo-PGE_1 and PGE_1 was also determined in human platelet rich plasma (PRP). For this purpose, blood was collected, anti-coagulated (1:9 v/v) with trisodium citrate (3·8% w/v) and centrifuged at 200*g* in an MSE bench top centrifuge (room temperature (20 °C), 10 min). The resulting PRP was removed and a small aliquot (0·2 ml) recentrifuged (1000 *g*, 10 min) to prepare platelet poor plasma (PPP). Platelet aggregation to ADP (5–10 μM) was assessed turbidometrically in 0·1 ml samples PRP using a Payton dual channel aggregometer (37 °C, 1100 rev min^{-1}) as described elsewhere (Moore 1979) Tris-HCl buffer volumes (<5 μl) did not affect ADP induced platelet aggregation in human PRP.

Results

All prostaglandins tested inhibited ADP-induced aggregation of human platelets suspended either in plasma or in whole blood. In each case the rank order of potency was PGI_2 > 6-oxo-PGE_1 > PGE_1. However, the absolute anti-aggregatory potency of the three prostaglandins did vary between PRP and whole blood as shown in Fig. 1. The concentrations of prostaglandins required for half maximal inhibition of ADP-induced platelet aggregation (EC50) in human PRP (whole blood in parentheses) were as follows: PGI_2, 0·30 ng ml^{-1} (0·38 ng ml^{-1}), 6-oxo-PGE_1 6·50 ng ml^{-1} (0·85 ng ml^{-1}) and PGE_1 36·0 ng ml^{-1} (8·50 ng ml^{-1}) respectively.

Discussion

Although the effect of PGI_2 and PGE_1 on platelet aggregation in both blood and PRP has been well documented, there have been no published reports of the effect of the prostacyclin metabolite, 6-oxo-PGE_1, on platelet reactivity in intact, uncentrifuged blood. Of the three prostaglandins tested, PGI_2 not surprisingly, was found to be the most powerful inhibitor of ADP-induced human platelet aggregation. The log dose-effect curves for PGI_2, assessed using blood and PRP, were parallel and, measured in terms of its EC50 value, PGI_2 had similar potency in both media. Compared with 6-oxo-PGE_1 and PGE_1, PGI_2 was some 21 and 120 times more potent an inhibitor of ADP-induced human platelet aggregation determined in PRP. These potency ratios are in good agreement with previous reports from this and other laboratories (Griffiths et al 1983; Miller et al 1980). However, a novel finding of the present study is that prostaglandins of the E series (but not PGI_2) are more potent inhibitors of platelet aggregation in blood than they are in PRP. When heparinized and citrated blood were used, PGI_2 was only 2·2 and 22 times more potent than 6-oxo-PGE_1 and PGE_1 respectively. The reasons why 6-oxo-PGE_1 and PGE_1 have greater anti-aggregatory potency in blood than PRP are not clear. It seems reasonable to assume that E-type prostaglandins exert an additional anti-platelet action in whole blood which does not occur in PRP, and which is not shared by PGI_2. In this context, it may be of relevance that prostaglandins of the E series reduce haemolysis of human red blood cells exposed to hypertonic saline solution by 'stabilizing' the erythrocyte membrane (Kury et al 1974). Thus, we propose that PGE_1 and 6-oxo-PGE_1 (but not PGI_2) prevent the efflux of pro-aggregatory ADP from erythrocytes thereby augmenting their anti-aggregatory effect in whole blood.

Whatever the precise mechanism of action of 6-oxo-PGE_1, these results may have consequences for the role of this prostaglandin in the control of platelet function

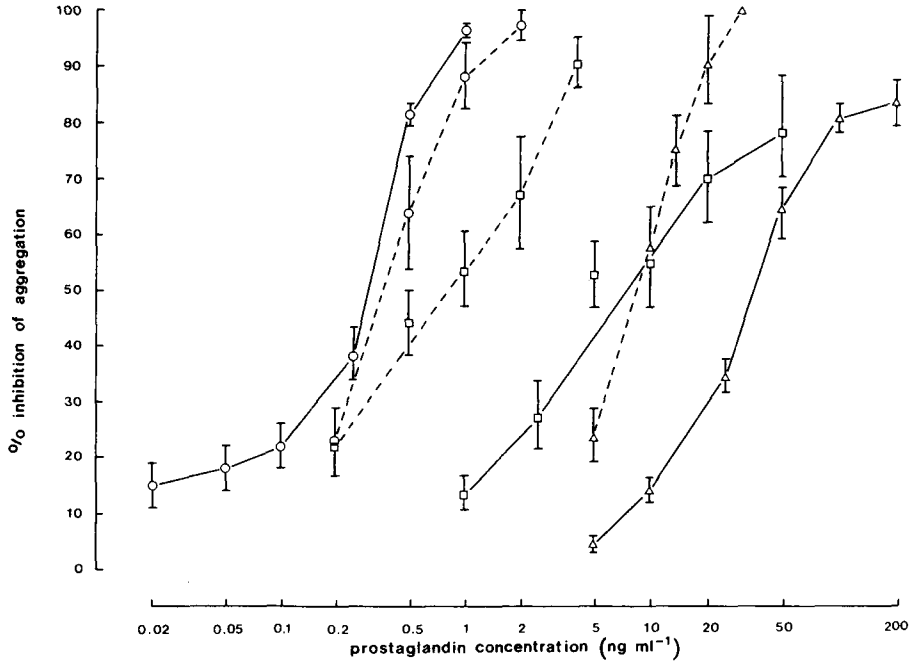

FIG. 1. Inhibition of ADP-induced human platelet aggregation by PGI_2 (○—○), 6-oxo-PGE_1 (□—□) and PGE_1 (△—△) determined in whole, uncentrifuged blood (broken lines) and PRP (continuous lines). Results show mean ± s.e.m., n = 6–16.

in-vivo. Until now, most researchers have assumed that the concentration of 6-oxo-PGE_1 in plasma required to exert a threshold platelet anti-aggregatory effect in man is ca 1 ng ml^{-1}. This is based on experiments in which the anti-aggregatory potency of this prostaglandin was assessed in PRP prepared from man and animals. In the light of the present results, it is clear that much lower concentrations of 6-oxo-PGE_1 prevent platelet aggregation in human blood, a medium that must be considered a better indication of platelet function in-vivo than experiments using PRP. In our hands, even concentrations of 6-oxo-PGE_1 as low as 200 pg ml^{-1} significantly inhibited (by 17–28%) ADP-induced human platelet aggregation.

Even though 6-oxo-PGE_1 prevents platelet aggregation in human blood at subnanogram concentrations, it is unlikely that this prostaglandin exerts a significant effect on platelet function in healthy subjects in-vivo since human plasma contains less than 30 pg ml^{-1} 6-oxo-PGE_1 (Jackson et al 1982). However, we should not ignore the possibility that higher concentrations of this prostaglandin (perhaps sufficient to prevent platelet aggregation) do occur locally in the vicinity of a platelet plug formed from PGI_2 and/or 6-oxo-$PGF_{1\alpha}$ by platelet cytoplasmic 9-PGDH. Furthermore, elevated plasma 6-oxo-PGE_1 has been observed in patients with Barrter's syndrome and may be responsible for the defect in platelet aggregation and increased bleeding time which characterizes this condition. We suggest that the results of the present study be borne in mind when interpreting the relevance of plasma 6-oxo-PGE_1 levels in healthy human volunteers and in patients with clinical disease.

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Cocaine-like action of diphenhydramine in cat cerebral arteries

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Diphenhydramine ($5{\cdot}3 \times 10^{-7}$ M) significantly reduced the tritium efflux evoked by 10^{-7} M tyramine from cat cerebral arteries preloaded with [^{3}H]noradrenaline but not that brought about by 50 mM KCl. These results indicate the ability of diphenhydramine to block the amine neuronal uptake.

Histamine has the property of releasing noradrenaline from sympathetic nerve endings present in the walls of cat and human cerebral arteries (Marco et al 1980; Balfagón et al 1984; Marco et al 1984). Most of the available data suggest that it achieves this effect by means of an exocytotic process after entering the nerve terminals through the amine uptake system (Balfagón et al 1984). This action of histamine shows a strong dependence on external calcium and appears inhibited in the presence of cocaine or colchicine. Nevertheless, such a conclusion seems to be obscured by the fact that diphenhydramine also blocks the tritium release induced by histamine from cat cerebral arteries preloaded with [^{3}H]noradrenaline, which would indicate the possible activation of a presynaptic receptor by this amine (Marco et al 1980). In the present communication we try to elucidate the way in which diphenhydramine is able to interfere with the release of noradrenaline evoked by histamine in this kind of vessel.

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Methods

Cats of either sex, 1·5–4 kg, were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.) and killed by bleeding. The brain was removed and the circle of Willis with its branches was dissected out. The vessels were cleaned to remove traces of blood and surrounding tissue and incubated for 1 h in Krebs-Henseleit solution