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EFFECTS OF SULPHINPYRAZONE AND ASPIRIN ON PROSTAGLANDIN I_2 (PROSTACYCLIN) SYNTHESIS BY ENDOTHELIAL CELLS

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Synthesis of prostaglandin I_2, (PGI_2, prostacyclin) by vascular endothelium (assayed by the ability of cultured endothelial cells to inhibit platelet aggregation) was inhibited by aspirin. At 100 μmol/l aspirin completely blocked measurable PGI_2 production, but endothelial cells had substantially recovered their ability to synthesize PGI_2 24 h after removal of the drug. In contrast, the effect of 1 mmol/l aspirin was still evident 24 h after drug withdrawal. Sulphinpyrazone also inhibited PGI_2 synthesis, but was about 100 fold less potent than aspirin, and the effect of the drug was lost within 24 h of its addition, even when endothelial cells were left in contact with the drug during this period.

Introduction Aspirin and sulphinpyrazone have both been tested as anti-thrombotic agents in relation to the prevention of subsequent coronary attacks after an initial myocardial infarction (Elwood, Cochrane, Burr, Sweetnam, Williams, Welsby, Hughes & Renton, 1974; Sherry *et al*, 1978). Aspirin irreversibly blocks the cyclo-oxygenase enzyme (Roth, Stanford & Majerus, 1975) in the prostaglandin synthetic pathway in platelets that generates the pro-aggregatory compounds thromboxane A_2, prostaglandin G_2 (PGG_2) and PGH_2. The site of action of sulphinpyrazone, which has not yet been unequivocally established, may well be the same as aspirin (Ali & McDonald, 1977).

PGI_2 (prostacyclin), a powerful inhibitor of platelet aggregation, is produced by vascular tissue (Moncada, Gryglewski, Bunting & Vane 1976; Gorman, Bunting & Miller, 1977), mainly by the endothelium (Moncada, Herman, Higgs & Vane, 1977; MacIntyre, Pearson & Gordon, 1978).

Aspirin is apparently about ten fold more potent than sulphinpyrazone as an inhibitor of platelet function (Ali & McDonald, 1977; Baenziger, Dillinger & Majerus, 1977) but no such comparison has been attempted for vascular tissue. We now report on the potencies of these drugs as inhibitors of endothelial prostacyclin production.

Methods Porcine aortic endothelial cells were isolated and cultured by methods described by Pearson, Carleton, Hutchings & Gordon (1978). The production of PGI_2 by endothelial cells was assessed by measurement of the inhibitory effect of samples of endothelial cell suspensions on adenosine diphosphate (ADP)-induced primary platelet aggregation, using 0.1 ml samples of human citrated platelet-rich plasma (Born, 1962; Gordon & Drummond, 1974). Details of the method, and the rationale for its use as an assay for prostacyclin production have been published (Macintyre *et al*., 1978). Aggregation responses in the absence of endothelial cells were not influenced by aspirin or sulphinpyrazone, because ADP-induced primary aggregation does not involve the platelet prostaglandin synthesis pathway.

Aspirin (acetylsalicyclic acid powder, dissolved at 10 mmol/l in 154 mmol/l NaCl) and sulphinpyrazone (from Geigy Pharmaceuticals, dissolved at 0.3 mol/l in 0.05 mol/l NaOH) were stored at −20°C and diluted freshly in cell culture medium as required. For experiments to test the short-term effects of either drug, suspensions of endothelial cells in serum-free tissue culture medium were incubated at 37°C with drug before testing the effect of these cell suspensions on platelet aggregation. For long-term experiments, confluent monolayers of endothelial cells were maintained in tissue culture medium with 5% foetal calf serum (changed daily) in the presence or absence of drugs for various times as described below, before suspension of the cells in serum-free medium for testing.

Results Preliminary experiments indicated that both aspirin and sulphinpyrazone inhibited PGI_2 production when incubated with suspensions of endothelial cells, and that their effects were maximal within 30 to 60 min. Under these conditions aspirin was approximately 100 times more potent than sulphinpyrazone. The concentration of aspirin required to reduce by 50% the inhibitory effect of a fixed number of endothelial cells varied between 10 μmol/l and 30 μmol/l, depending on the batch of endothelial cells used. The dose-response curves for aspirin and sulphinpyrazone in a typical experiment are shown in Figure 1a.

When endothelial cells were maintained in culture in the presence of 1 mmol/l aspirin for 24 h immedi-

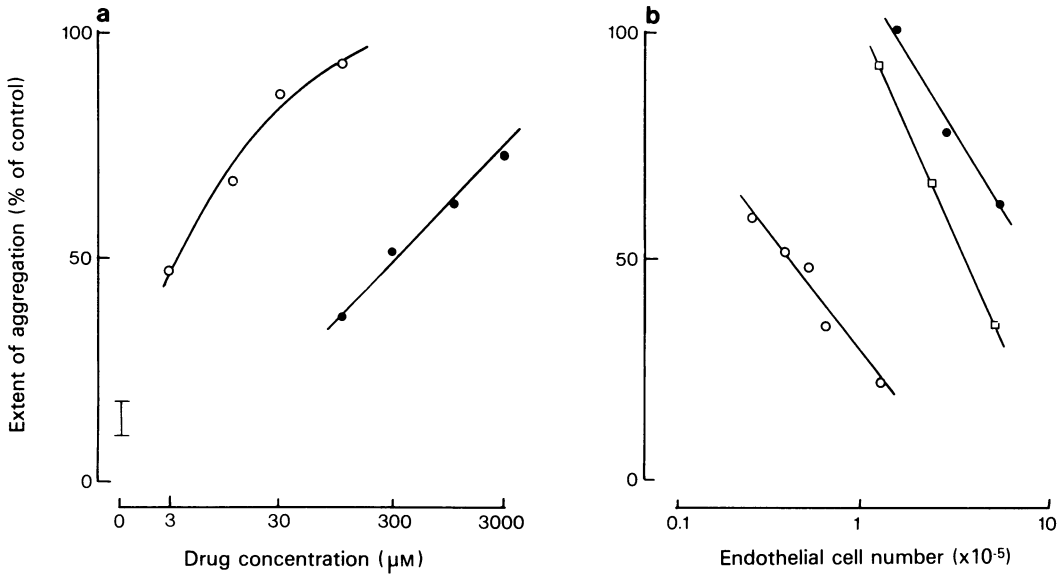

Figure 1 Inhibition of prostacyclin production by aspirin and sulphinpyrazone. (a) Endothelial cells were preincubated alone or with drug for 60 min at 37°C before testing their ability to inhibit ADP-induced primary aggregation. The points are the mean of duplicates in the presence of drug and the vertical bar shows the range of 4 replicates obtained with cells alone. The range of control responses to ADP varied by <5%. (○) Aspirin; (●) sulphinpyrazone. (b) (○) Endothelial cells alone; (●) cells cultured with 1 mmol/l aspirin for 24 h; (□) cells cultured with 1 mmol/l aspirin for 24 h and then without drug for 24 h before testing. The points are the means of 2 or 3 observations. The range of control responses to ADP varied by <5%.

ately before testing, PGI_2 synthesis was virtually abolished. Over 5×10^5 aspirin-treated endothelial cells were less effective at inhibiting platelet aggregation than 2.5×10^4 control cells (Figure 1b); ID_{50} for control cells $= 4 \times 10^4$ cells. If the endothelial cells were cultured for 24 h in the presence of 1 mmol/l aspirin and then for a further 24 h without aspirin before testing, PGI_2 synthesis recovered slightly, but ten times more aspirin-treated cells ($ID_{50} = 4 \times 10^5$) than control cells were still needed to achieve comparable inhibition of platelet aggregation (Figure 1b). Cells cultured in the presence of 100 μmol/l aspirin for 24 h, like those treated with 1 mmol/l aspirin, synthesized very little PGI_2 (control cells $ID_{50} = 2 \times 10^4$; drug-treated cells $ID_{50} > 10^5$. Unlike the cells treated with the higher dose of aspirin, however, those incubated with 100 μmol/l aspirin and then for a further 24 h without the drug inhibited platelet aggregation almost as well as non-treated cells (control cells $ID_{50} = 2 \times 10^4$; drug-treated cells $ID_{50} = 5 \times 10^4$). Sulphinpyrazone (1 mmol/l), in contrast to aspirin, had no significant effect on PGI_2 synthesis even when present for 24 h before testing (control cells $ID_{50} = 1.5 \times 10^4$; drug-treated cells $ID_{50} = 1.3 \times 10^4$).

Discussion Aspirin irreversibly inhibits fatty acid cyclo-oxygenase by acetylation (Roth *et al.*, 1975) and tests of platelet function and prostaglandin synthesis show inhibition for about a week (the platelet lifespan) after ingestion of two tablets of aspirin (Kocsis, Hernandovich, Silver & Smith, 1973). However, we have shown here that the effects of 100 μmol/l aspirin on confluent monolayers of endothelium (where cell turnover is very slow) are substantially reversed within 24 h of removing the drug, demonstrating that there is significant synthesis of new cyclo-oxygenase within this time. With a higher dose of aspirin (1 mmol/l) there was much less recovery in 24 h, which suggests that the initial acetylation of cyclo-oxygenase was greater, and that insufficient enzyme was synthesized in 24 h to restore PGI_2 production to normal.

Although sulphinpyrazone inhibited PGI_2 production in short-term experiments, we found no inhibition after incubation of endothelial cells for 24 h with 1 mmol/l sulphinpyrazone immediately before testing. It seemed possible that this might be due to instability of sulphinpyrazone in culture medium, but 1 mmol/l sulphinpyrazone was not inactivated by 24 h incubation in culture medium without endothelium, although its potency was somewhat reduced, presum-

ably due to oxidation by atmospheric oxygen. It therefore appears that endothelial cells may metabolize sulphinpyrazone: this possibility merits further investigation.

Any extrapolation from results obtained in experiments such as these must be made with caution because of the artificial conditions of the *in vitro* system used but, given this caveat, the following conclusions can be drawn. First, therapeutic levels of aspirin and sulphinpyrazone can inhibit PGI_2 production by endothelium but aspirin is much more active than sulphinpyrazone in this respect: the peak plasma levels of sulphinpyrazone in man after therapeutic doses are only about 50 μmol/l (Dieterle, Faigle, Mory, Richter & Theobald, 1975), which, on the basis of our results, would have little effect on endothelial PGI_2 synthesis. Secondly, aspirin is effective after prolonged incubation with endothelium, while sulphinpyrazone is not, and with a high dose of aspirin the inhibition is measurable for at least 24 h after drug withdrawal. Thus, drugs that inhibit platelet prostaglandin synthesis (and are therefore candidates for trials as anti-thrombotics) may have variable effects on endothelial PGI_2 synthesis. Moncada & Korbut (1978) recently emphasized that large doses of aspirin can prevent the synthesis of PGI_2 and hence negate the anti-thrombotic effects of drugs such as dipyridamole. Our results suggest that sulphinpyrazone is less likely than aspirin to exert this potentially dangerous effect.

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