DAZOXIBEN EXAMINED FOR PLATELET INHIBITORY EFFECT IN AN ARTIFICIAL CIRCULATION

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- 1 Platelet inhibitory therapy may protect patency in prosthetic arterial grafts.
- 2 The effect of the thromboxane synthetase inhibitor dazoxiben (UK 37248) on ^{113}In -labelled platelet deposition on Dacron arterial grafts was examined in an artificial circulation containing human blood which mimics the haemodynamics of femoropopliteal bypass. The influence of this drug on thromboxane production and platelet reactivity was measured during the experiment. The study was controlled and performed in two parts to assess *in vitro* and *ex vivo* drug effect.
- 3 Both *in vitro* and *ex vivo* dazoxiben reduced plasma thromboxane by about 80% and in citrated *ex vivo* blood increased mean ($\pm$ s.e. mean) platelet aggregation threshold to collagen from control values of 4.8 ± 1.7 mg/ml to 10.6 ± 3.3 mg/ml ($P < 0.01$). Dazoxiben, however, had no effect on platelet deposition on the arterial grafts.
- 4 Selective thromboxane inhibition failed to exert an antithrombotic effect in this model.

Introduction

ARTIFICIAL surfaces incorporated in the circulation attract blood platelets with subsequent thrombus formation (Salzman, 1978). Hence in vascular surgery thromboembolic events may complicate the technically successful implantation of a prosthetic material. Such thrombi on heart valves may embolize with particular risk of cerebral infarction. The equivalent phenomena on peripheral arterial prostheses may result in thrombotic occlusion with subsequent threat to limb viability. Following femoropopliteal bypass grafting with a prosthetic, one year patency rates may be as low as 50% (Christenson & Eklof, 1979; Clyne *et al.*, 1979). Although platelet inhibitory drugs have reduced embolic episodes following heart valve replacement (Sullivan *et al.*, 1971; Dale *et al.*, 1977), the results of randomized studies on their effect on arterial graft patency have yet to be reported.

Recent interest has centred on the possibility that drugs acting on prostaglandin metabolism and in particular thromboxane synthesis may have antithrombotic effect (Editorial, *Lancet*, 1981). For this reason dazoxiben (UK 37248), a specific thromboxane synthetase inhibitor, offers considerable potential as an antiplatelet drug. We have therefore examined the effect of dazoxiben on thromboxane production in human blood and on platelet deposition on Dacron arterial grafts in an artificial circulation which mimics the haemodynamics of a femoropopliteal bypass (Hamlin *et al.*, 1978), and which has been used previously to evaluate platelet inhibitory drugs (McCollum *et al.*, 1980; Aukland *et al.*, 1982).

Methods

The artificial circulation was essentially as described by Hamlin *et al.* (1978). It consisted of a circuit of silicon rubber tubing into which had been inserted, using tapered plastic connectors, a 15 cm length of 6 mm pre-clotted woven Dacron (USCI, DeBakey). Pre-clotting, to seal the interstices of the graft, was performed by first injecting the stretched prosthesis with donor blood. Following irrigation with fresh whole blood the graft was stripped between fingers to remove luminal clot, mounted in the circuit, and flushed with 250 ml normal saline. Blood (300 ml) from the same donor, heparinized (mucous heparin, Weddel Pharmaceuticals) to a final concentration of 2.5 IU/ml and maintained at 37°C, was circulated by a roller pump (Sarns Inc 5500) with a pulsatile flow of 140 ml/min at a pressure of 120/80 mm Hg. Graft perfusion was maintained for 30 minutes.

The study was performed in two parts to evaluate the effect of dazoxiben both *in vitro* and *ex vivo*.

In vitro

Eight healthy male volunteers aged 22–39 yr who had avoided any medication for 7 d each donated 600 ml blood into one bag of a standard twin collection pack (Travenol) containing heparin. A 20 ml sample was removed and the platelets labelled with ^{113}In indium oxine by the method described by Hakwer *et al.* (1980) for ^{111}In oxine, with no modifications required to accommodate either the alternative isotope or the use of heparin rather than citrate. The ^{113}In oxine (half-life 99.5 min) was prepared in the laboratory

from generator eluted ^{113}In chloride (Amersham International) mixed with a solution of oxine and buffered to a pH of 6.4 using HEPES buffer (Flow Laboratories). Platelets labelled in this way have virtually unchanged *in vitro* function with negligible release of free isotope.

Following thorough mixing of the labelled platelets with the blood donation, samples were withdrawn for the measurement of plasma thromboxane, platelet aggregation threshold to collagen and radioactivity levels. Thereafter the blood was divided equally between the two packs which were to act as test and control. Dazoxiben 10 mg in 5 ml isotonic saline was added to the test and an equal volume of saline to the control. Both packs were incubated for 15 min before being simultaneously introduced into identical paired circulations. After perfusing the grafts for 30 min the circulation was stopped and repeat blood samples were taken. Each circuit was drained and the grafts rinsed gently with 250 ml normal saline to remove non-adherent labelled platelets. The central 12 cm portion was excised and the radioactivity of the graft and the pre- and post-perfusion blood samples were simultaneously measured in a scintillation well crystal.

Ex vivo

A further 10 healthy male volunteers aged 22–39 yr again taking no other medication, each gave two 300 ml donations for evaluation in the circuit. One was after taking dazoxiben 100 mg twice daily for 48 h and the other after no drug. These donations were in random order and separated by an intervening 4 weeks on no therapy. The last dose of dazoxiben was given with a light breakfast 1.5 h before blood was taken. Following venesection into heparin, an additional 9 ml was taken into 1 ml 3.8% sodium citrate and a further 8 ml of native blood was used to measure platelet adhesion to glass beads in a commercially available kit (Adeplat S). Platelets were labelled with ^{113}In oxine and then the blood perfused through the circuit containing preclotted Dacron as described in the *in vitro* study.

Evaluation of drug effect

Thromboxane levels were measured at the beginning and end of each 30 min perfusion. Samples (5 ml) were taken into EDTA/indomethacin and centrifuged on ice to obtain platelet-poor plasma which was stored at -20°C for subsequent measurement of plasma thromboxane by radioimmunoassay (Randall *et al.*, 1981).

Platelet deposition on the grafts (graft activity) at the end of each perfusion was expressed as the ratio of emissions from the graft to emissions from the 5 ml

pre-perfusion blood samples counted at the same time. By using this ratio, comparisons can be made between individual circuit results without the need to correct for the initial isotope activity or isotopic decay. As platelets are deposited on the graft they are removed from circulating blood. This loss of labelled platelets was derived from the difference between simultaneous scintillation counts in 5 ml blood samples obtained before and after perfusion and expressed as a percentage of radioactivity in pre-circulation blood.

Blood samples for platelet aggregation were centrifuged at 200 g for 10 min to obtain platelet rich plasma and platelet counts adjusted to a count of $30 \times 10^9/\text{l}$ with platelet poor plasma. Aggregation threshold response to collagen (Horm Chemi; 1 mg/ml) was then determined in a Payton aggregometer.

Statistical comparisons between test and control results were made with the paired Wilcoxon signed rank test.

Results

In vitro

After graft perfusion the mean ($\pm$ s.e. mean) plasma thromboxane level in the active circuits at 354.3 ± 46.3 pg/ml was significantly lower than that in the control circuits of 2013 ± 301 pg/ml ($P < 0.05$) with both post-perfusion circuits having elevated levels compared with the mean pre-circuit value of 79.1 ± 28.7 pg/ml (Figure 1). Mean graft activities of 1.11 ± 0.35 in the active circuits and 1.32 ± 0.36 in the control circuits showed no important difference (Figure 2). Similarly no significant difference was found between the mean percentage fall in circulating labelled platelets in the active circuits at $6.39 \pm 2.4\%$ and the control circuits with a fall of $8.49 \pm 3.2\%$ (Figure 3).

Ex vivo

In pre-perfusion samples dazoxiben had reduced mean plasma thromboxane to 89.3 ± 21.7 pg/ml compared with a control value of 320 ± 65.5 pg/ml ($P < 0.05$) (Figure 4). Following graft perfusion, thromboxane levels rose in all circuits with a substantially higher mean of 1896 ± 275.7 pg/ml in the control blood compared with 391 ± 85.7 pg/ml in blood from volunteers on dazoxiben ($p < 0.05$). However, mean graft activity of 3.02 ± 0.35 in the active circuits was similar to that of 2.88 ± 0.41 found in the controls. Neither was there any important difference between the mean percentage fall in circulating labelled platelets of active and control circuits at $15.12 \pm 2.5\%$ and $13.6 \pm 2.6\%$ respectively.

In *ex vivo* volunteers mean platelet aggregation threshold to collagen when performed in heparin was

Figure 1 IN the *in vitro* circuits dazoxiben (D) prevented the massive post-perfusion rises in thromboxane which occurred in the controls (C) but failed to restrict thromboxane to pre-perfusion levels.

5.1±1.6 mg/ml and 4.8±1.7 mg/ml in citrate. Following treatment with dazoxiben, these thresholds increased to 10.9±2.56 mg/ml and 10.6±3.3 mg/ml respectively, which achieved statistical significance only in citrate ($P<0.01$). Adeplat S measurements of platelet adhesion to glass beads showed a mean percentage retention in the column of 52.3±8.6% of platelets using control blood which was little changed after dazoxiben to 68.4±7.5%.

Discussion

In this experimental system, which mimics the haemodynamics and physiological conditions present immediately following femoropopliteal bypass with Dacron, the successful reduction of circulating thromboxane by almost 80% failed to influence platelet accumulation on the grafts. Since early platelet and thrombus deposition appears to influence the likelihood of subsequent graft failure (Yates *et al.*, 1975; Christenson *et al.*, 1981), this has

Figure 2 Both *in vitro* and *ex vivo* graft activities in the dazoxiben circuits (D) were similar to those found in the controls (C). Since the volunteers and type of woven Dacron used in the *ex vivo* experiments were not the same as in the *in vitro* experiment, comparison between studies is not possible.

direct clinical relevance. Inhibition of thromboxane synthetase by dazoxiben was unable to prevent small rises in plasma thromboxane levels during perfusion of the grafts. Hence as even low levels of thromboxane may be sufficient to support platelet aggregation (Butler *et al.*, 1982) merely inhibiting thromboxane production may be inadequate to prevent platelet activity. Furthermore, there are alternative pathways of platelet activation, independent of prostaglandin metabolism which could be equally relevant in this thrombosis model and in clinical arterial bypass.

Dacron grafts preclotted with whole blood are a source of thrombin which can directly provoke platelet activation. Although in the model the heparin would deactivate thrombin this would often not be given clinically. In the same way artificial surfaces can initiate platelet reactions similar to those induced by adding thrombin or ADP to platelet-rich plasma (Salzman, 1978). Furthermore, ADP itself, arising either from platelets or damaged red cells is capable of provoking platelet aggregation in the

Figure 3 The percentage fall in circulating labelled platelets was not influenced by dazoxiben in either the *in vitro* or *ex vivo* circuits. The relatively higher falls in the *ex vivo* series are in keeping with the increased platelet deposition (graft activity) found on these more tightly crimped grafts.

presence of thromboxane although platelet release may be blocked (Best *et al.*, 1980). Our finding that thromboxane inhibition reduces platelet aggregation to collagen supports Randall *et al.* (1981) but conflicts with others (Butler *et al.*, 1981; Walter *et al.*, 1981).

Although dazoxiben seems effectively to prevent platelet aggregation in response to arachidonic acid (Vermeylen *et al.*, 1981), inhibition of thromboxane is less successful in affecting platelets challenged by ADP and thrombin, substances capable of simultaneously activating several pathways (Butler *et al.*, 1981; Walter *et al.*, 1981). This suggests that thromboxane inhibition may only prevent platelet activation in selected *in vitro* systems where the arachidonic acid pathway has assumed an importance which may not exist *in vivo*. Moreover, it is notable that in previous experiments using this artificial circulation both in our laboratory (Aukland *et al.*, 1982) and elsewhere (McCollum *et al.*, 1980) platelet deposition was best prevented by ticlopidine and a

Figure 4 Dazoxiben (D) reduced plasma thromboxane when compared with controls (C) both before and following perfusion in the *ex vivo* circuit. It did not, however, completely block thromboxane production in the active circulation.

combination of aspirin and dipyridamole, whose known pharmacological actions are far more diverse than thromboxane inhibition.

In this study dazoxiben successfully reduced thromboxane production and yet thromboxane inhibition in isolation did not demonstrate a useful antithrombotic effect. We believe this casts further doubt on the role of thromboxane as an essential mediator of platelet reactions and challenges an antithrombotic approach limited to manipulation of prostaglandins alone.

The absence of a source for prostacyclin generation in the model differs from the clinical situation. Although local conditions within the graft would be essentially similar, the presence of vascular endothelium in the patient, even at a distance, might permit a shift of prostaglandin metabolism towards prostacyclin. In addition, any accumulation of endoperoxide intermediates, themselves pro-aggregatory, would be less likely to occur. Consequently it is

possible that the potential of dazoxiben may have been underestimated and further experiments using prosthetic grafts in animals are being undertaken.

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