USE OF A THROMBOXANE SYNTHETASE INHIBITOR, DAZOXIBEN, DURING HAEMODIALYSIS

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- 1 We have assessed the thromboxane synthetase inhibitor, dazoxiben (UK 37248), during dialysis in a double-blind, placebo-controlled crossover study.
- 2 Thromboxane generation was markedly inhibited, and there was an increase in serum 6-keto prostaglandin $F_{1\alpha}$ levels during active treatment studies.
- 3 We were unable to demonstrate any reduction of platelet activation, or dialyser fibrin deposition, nor were heparin requirements altered by the drug.
- 4 Dazoxiben had no adverse effects.

Introduction

HAEMODIALYSIS is a valuable model for investigation of the potency and mode of action of antiplatelet agents. During standard dialysis with heparin anticoagulation the arterial platelet count usually falls by 10–15%, subsequently rising to pre-dialysis values or higher after 3–4 hours. There is a concomitant rise in plasma levels of the specific platelet release protein β -thromboglobulin (BTG) (Turney *et al.*, 1980). Platelet loss occurs by adhesion and aggregation onto the dialyser membrane, with associated fibrin deposition (Lindsay *et al.*, 1972a), and by removal of platelets from the circulation within the patient (probably in the pulmonary vascular bed and spleen). Lindsay *et al.* (1972b) have also reported that aspirin and dipyridamole protected the platelets and reduced dialyser blood loss. We have reported platelet sparing activity and reduced dialyser fibrin deposition with sulphinyprazole (Woods *et al.*, 1979), and more recently shown that prostacyclin can minimize platelet loss and reduce heparin requirement during regular haemodialysis (Turney *et al.*, 1980).

Surface contact activation of platelets in the extra-corporeal circulation may lead to an accumulation of prostacyclin endoperoxides, PGG_2 and PGH_2 , which are then converted to thromboxane A_2 (TXA_2) by the enzyme thromboxane synthetase, promoting platelet aggregation. Thromboxane generation would be unopposed by prostacyclin synthesis in the extra-corporeal circuit, due to the absence of endothelium. Thus thromboxane synthetase inhibition may be of benefit in reducing platelet aggregability, and may allow redirection of prostaglandin metabolism so that endoperoxides are preferentially converted to other prostaglandins, including anti-aggregatory PGD_2 , and in the presence of vascular endothelium, PGI_2 .

In this paper we report the effects of a specific

thromboxane synthetase inhibitor, dazoxiben (UK 37248) administered intravenously during routine haemodialysis.

Methods

Six patients on long term haemodialysis for more than 2 months entered the study. Informed consent was obtained and the study was approved by the King's College Hospital Ethical Committee. None of the patients were taking aspirin or other drugs known to affect platelet function. Each patient underwent two study dialyses separated by 3 weeks, and was allocated to receive active drug, or placebo, in a randomized double-blind crossover trial design. Dialyses studies were standardized using Nephros 1600 hollow fibre cuprophane dialysers, 38 mmol/l acetate buffered dialysate, blood flow rate of 200–300 ml/min, dialysate flow rate of 500 ml/min and a 4 h dialysis duration. A bolus dose of heparin (Leo porcine mucosal batch No. 13T81) at 50 units/kg was given at the start of dialysis, and an infusion of 30 units/kg for the first 3 h of dialysis. Dazoxiben (or placebo vehicle) was administered at a dose of 100 mg intravenous bolus, 30 min before dialysis, and as a continuous infusion of 25 mg/h into the dialyser inlet blood line during the 4 h study period. (Dazoxiben was provided in solution in normal saline adjusted to pH 8.8 with sodium hydroxide. Placebo vehicle was normal saline.)

Blood samples were taken from a peripheral vein before the bolus dose of dazoxiben (–30 min), from the “arterial” needle or arterial shunt limb at the start of dialysis (0 min) and from the dialyser inflow blood line at intervals during dialysis. Measurements included platelet count on a Coulter ZF, β TG and platelet factor 4 (PF4) by radioimmunoassay,

Figure 1 Mean values for TXB₂ and 6-keto-PGF_{1α}, platelet count (expressed as a percentage of pre-dialysis value), βTG and PF4, during active (a) and placebo dialyses (b).

activated whole blood clotting time (AWBCT, Hemochron), thrombin clotting time, plasma fibrinogen (Klauss), serum TXB₂ and serum 6-keto-PGF₁₁ by radioimmunoassay (whole blood collected into glass tubes, incubated at 37°C, rimmed to release clot at 30 min and centrifuged after 1 h at 3000 r.p.m. for 15 min; serum stored at -20°C until assayed). Fibrinopeptide A (FPA) and early plasmin cleavage product β15-42 antigen were measured by radioimmunoassay of samples collected into trasylol and heparin. Dialyser fibrin deposition was measured by the method of Woods *et al.* using commercial ¹²⁵I-fibrinogen injected as a bolus 30 min before

dialysis (thyroid protection was achieved with 100 mg oral sodium iodide daily for 2 weeks after each study dialysis). Plasma and dialyser standards were prepared for every vial of ¹²⁵I-fibrinogen.

Results

Dazoxiben was tolerated by all patients with no side-effects. There was a significant sustained reduction in serum TXB₂ levels when dazoxiben was administered, accompanied by a rise in serum 6-keto-PGF_{1α} levels (Figure 1). During placebo dialyses serum

Figure 2 Mean values for thrombin time, activated whole blood clotting time (AWBCT; expressed as a percentage of pre-dialysis value), FPA, $\beta 15-42$ antigen, fibrinogen and standardized plasma radioactivity (^{125}I -fibrinogen; expressed as a percentage of pre-dialysis value), during active (a) and placebo dialyses (b).

TXB₂ and 6-keto-PGF_{1 α} levels were unchanged. There were no significant differences in platelet loss between the two treatment regimes. β TG rose progressively during dialysis, confirming platelet activation, while PF4 showed an early rise probably as a result of heparin bolus administration, followed by a fall to pre-dialysis values. The response was similar on active or placebo treatment (only four placebo and four active dialyses were assessed). Results of coagulation studies are shown in Figure 2. Activated whole blood clotting times and thrombin

clotting times confirmed adequate heparinization and were unaffected by the drug.

FPA levels were higher at the start of dialysis (0 min) than at the time of the first blood sample before dazoxiben bolus. This difference is probably because the -30 min sample was venous while the 0 min sample was arterial. Subsequent low levels of FPA confirm adequate heparinization. The elevation of $\beta 15-42$ antigen levels, with no significant change during dialysis, is thought to be due to the fact that this fragment normally undergoes renal catabolism

(Lane, 1982) and does not in this context reflect enhanced fibrinolysis. Plasma fibrinogen levels rose progressively throughout dialysis in both treatment groups. Plasma radioactivity (^{125}I -fibrinogen) fell slightly after 15 min dialysis and then returned to pre-dialysis values after 1 h, remaining at this level throughout dialysis. Thus no measurable consumption of labelled fibrinogen occurred and the rise in plasma fibrinogen must represent release of unlabelled fibrinogen into the circulation. Fibrin deposition in the dialysers was similar on dazoxiben or placebo: mean value $\pm$ s.d.; active 3.2 mg (1.7); placebo 2.6 mg (2.4).

Discussion

Our results confirm that dazoxiben is an effective inhibitor of thromboxane synthesis, and that it may be administered safely to patients, without side-effects. The finding of elevated serum 6-keto-PGF $_{1\alpha}$ levels demonstrates that thromboxane synthetase inhibition results in a potential increase in prostacyclin synthesis, presumably due to the availability of endoperoxides no longer acting as substrate for thromboxane synthetase. The *in vivo* significance of this "redirection" is uncertain since serum, not plasma, levels were measured. However, an elevation of plasma 6-keto-PGF $_{1\alpha}$ levels has previously been documented in normal volunteers who received dazoxiben (Vermynen *et al.*, 1981).

It is interesting that whole blood, when clotted in the presence of a thromboxane synthetase inhibitor should generate 6-keto-PGF $_{1\alpha}$; if this product accurately reflects prostacyclin synthesis, this rise must be due to the presence of leukocytes containing prostacyclin synthetase. The rise in 6-keto-PGF $_{1\alpha}$ levels is small (470 pg/ml) compared to the fall in TXB $_2$ levels (120 mg/ml); however, *in vivo* with abundant endothelial prostacyclin synthetase available this discrepancy may not occur and the result may simply reflect the unphysiological nature of the environment in which 6-keto-PGF $_{1\alpha}$ and TXB $_2$ are being generated. It is also possible that other prostaglandins may be synthesized from the accumulating endoperoxides, including anti-aggregatory PGD $_2$ (Smith *et al.*, 1974) and pro-aggregatory PGE $_2$ (Kadowitz *et al.*, 1975). Depending on the relative proportion of these, and other synthetic products, the net effect of dazoxiben could be either pro, or anti-aggregatory, and this may vary from one subject to another.

Despite achieving significant inhibition of thromboxane synthesis we were unable to demonstrate any significant effect on platelet count, platelet release, or

coagulation parameters. However, it is important to note that the fall in the platelet count was much less than we have previously observed (Turney *et al.*, 1980). This may be related to the type of dialyser and perhaps to the particular batch of heparin used. Nevertheless, platelet activation did occur as demonstrated by the rise in βTG levels. It may be that TXA $_2$ generation is not essential for platelet activation during haemodialysis; indeed we have in a preliminary study been unable to demonstrate a rise in plasma TXB $_2$ levels on either side of the dialyser, despite a substantial fall in the platelet count.

Further appraisal of dazoxiben will require detailed characterization of the secondary "redirection" effects in a variety of clinical contexts. The importance of thromboxane and its spectrum of physiological activity remains uncertain.

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