SELECTIVE INHIBITION OF THROMBOXANE SYNTHESIS DURING EXPERIMENTAL ENDOTOXEMIA IN THE GOAT: EFFECTS ON PULMONARY HAEMODYNAMICS AND LUNG LYMPH FLOW

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1 The effects of a selective thromboxane synthetase inhibitor, dazoxiben (UK 37248), on haemodynamics, eicosanoid levels, and lung vascular permeability were assessed in three unanesthetized goats with chronic lung lymph fistulae following infusions of *E. coli* endotoxin (1 µg/kg). Each animal received an infusion of endotoxin in both a control and treatment trial. In the control trial endotoxin alone was infused. In the treatment trial endotoxin administration was preceded by a bolus intravenous infusion of dazoxiben 25 mg/kg followed by a maintenance infusion of 10 mg/kg/h.

2 In animals receiving endotoxin alone the peak elevation in pulmonary artery and wedge pressures, the initial increase in lymph flow, and the fall in cardiac output correlated with the peak elevation in plasma thromboxane B₂ (TXB₂) concentration at 30 min after endotoxin. Although TXB₂ levels had returned to baseline by 3 h, lung lymph flow remained elevated for at least 6 h after endotoxin. The lymph/plasma protein ratio (L/P) did not fall.

3 In dazoxiben-treated animals the rise in plasma concentrations of TXB₂ after endotoxin was prevented and, in concert, the increase in pulmonary artery and wedge pressures, the fall in cardiac output, and the initial increase in lymph flow were greatly attenuated. The increase in lymph flow at 2–6 h after endotoxin, however, was not prevented in dazoxiben-treated animals, and again L/P did not change.

4 Plasma 6-keto-prostaglandin F_{1α} concentrations were not significantly altered by dazoxiben treatment except for an earlier peak.

5 We conclude that selective total inhibition of thromboxane synthesis can be achieved by dazoxiben. The inhibition ameliorates many of the adverse haemodynamic consequences of experimental endotoxaemia and reduces, but does not prevent pulmonary oedema formation. This seems to be due to an increase in lung microvascular permeability which is not mediated by TXA₂.

Introduction

INFUSION of endotoxin in experimental animals is known to produce large elevations of plasma thromboxane A₂ (TXA₂) and prostacyclin (PGI₂) (Demling *et al.*, 1981). Because these eicosanoids have potent but opposing vasomotor effects, their relative amounts may be an important factor in the genesis of the haemodynamic and permeability changes of endotoxaemia. Pharmacological studies using phospholipase or cyclo-oxygenase inhibitors cannot differentiate the effects of the individual eicosanoids since they inhibit the synthesis of both prostaglandins and thromboxanes. Recent studies with selective thromboxane synthetase inhibitors have demonstrated that TXA₂ mediates most of the pulmonary hypertension following endotoxin infusion (Watkins *et al.*, 1982), but the role of TXA₂ in the permeability and other haemodynamic changes remains uncertain. For this reason, we have examined the effects of dazoxiben (UK 37248), a selective thromboxane synthetase inhibitor, on haemodynamic and permeability changes following infusion of *E. coli* endotoxin in goats.

Methods

Three goats with chronic lung lymph fistulae were prepared as previously described (Winn *et al.*, 1980; Stothert *et al.*, 1981). Experiments were performed 48 to 72 h later, at which time the awake goats were lightly restrained with a neck collar attached to a stanchion, allowed to stand or lie, and given free access to food and water. A Swan-Ganz flow-directed catheter was placed in the pulmonary artery to measure pulmonary artery (PPA), pulmonary wedge (PW), and central venous (PV) pressures using a strain gauge pressure transducer (Statham P23 Db). Aortic pressure (PA) was measured via a catheter that was placed in the aorta at the time of thoracotomy. Cardiac output (CO) was computed by standard thermodilution technique using an Edwards cardiac output computer. Lymph samples were collected for 15-min intervals in tared heparinized tubes and volumes were determined by weight. Blood samples for protein determination were drawn every 30 min from the central venous line of the flow-directed catheter. Lymph and plasma protein were determined by a modified biuret technique (Failing *et*

al., 1960). Blood samples for eicosanoid measurements were drawn from the aorta into 3 ml syringes containing 0.5 ml acid-citrate-dextrose solution (NIH Formula A) with 5 mM aspirin. Samples were then transferred to polypropylene tubes and spun at 35,000g for 10 min at 4°C. Plasma was decanted, frozen, and maintained at -70°C. TXB₂ and 6-keto-PGF_{1α} concentrations were determined by radioimmunoassay using labelled tracers and antibodies from New England Nuclear Corporation, Boston.

After control conditions were documented for a period of 2 h, endotoxin (*E. coli* 055:B5, Sigma, St Louis) was dissolved in sterile 5% dextrose and infused over 30 min to a final dose of 1 µg/kg. Two infusions were performed in each goat, separated by 48 hours. In control trials (three), animals received only an endotoxin infusion; in treatment trials (three) the same animals received a bolus infusion of dazoxiben 25 mg/kg in 50 ml sterile saline immediately before endotoxin and a maintenance infusion of dazoxiben 10 mg/kg/h in 50 ml/h of sterile saline for 5.5 h following endotoxin infusion. The sequence of these trials was alternated between animals. Dazoxiben alone at this dose produced no effect on eicosanoid, haemodynamic, or permeability measurements (unpublished data).

The results obtained following endotoxin infusion were compared with the immediately preceding baseline values and significance was assessed using the paired *t* test at *P*<0.05 unless otherwise indicated. Differences between the group means of control and dazoxiben-treated animals were tested for significance using unpaired two-tailed *t*-test, also at *P*<0.05.

Results

Plasma concentrations of TXB₂ were markedly elevated for 1-2 h in untreated control animals following the infusion of endotoxin, whereas in dazoxiben-treated animals plasma concentrations of TXB₂ were never significantly elevated above baseline (Figure 1a). On the other hand, plasma concentrations of 6-keto-PGF_{1α} were significantly elevated in both control and dazoxiben-treated animals following endotoxin (Figure 1b).

The effects of dazoxiben on endotoxin-induced hemodynamic changes are summarized in Figure 2. PPA was very significantly elevated in endotoxin control animals with a peak mean value of 40.6±5.13 cm H₂O at 30 min (*P*<0.01) and remained elevated over the subsequent 5.5 hours. In dazoxiben-treated animals PPA was significantly elevated only at 75 min after endotoxin (27.0±0.01 cm H₂O, *P*<0.01). The difference between the peak values was also highly significant (*P*<0.01). PW was elevated significantly in control endotoxin animals, with a peak value of 25.0±10.8 cm H₂O at 30 min, whereas in the dazoxiben-treated endotoxin animals PW did not increase above baseline at any time. CO fell significantly in control animals from a baseline of 4.0 to 4.5 l/min to a nadir of 2.7±1.02 l/min at 30 min after endotoxin infusion and remained depressed at around 3.5 l/min for several hours. In dazoxiben-treated animals the decrease in cardiac output was transient, being significantly decreased from baseline (4.5-5.0 l/min) only at 30 min after endotoxin infusion (3.75±0.46 l/min). Systemic arterial pressure fell substantially in both

Figure 1 Effect of dazoxiben on plasma concentrations of TXB₂ and 6-keto-PGF_{1α}. Plasma TXB₂ (a) and 6-keto-PGF_{1α} (b) levels were determined by radioimmunoassay following infusion of either endotoxin alone (●) or endotoxin along with dazoxiben (○). Values represent means from three animals.

Figure 2 Effect of dazoxiben on haemodynamic measurements following endotoxin infusion. The first 2 h represent baseline conditions. Pulmonary artery pressure (PPA), pulmonary wedge pressure (PW), aortic pressure (PA) and cardiac output (CO) were measured in animals receiving infusions at time zero of endotoxin alone (●) or endotoxin along with dazoxiben (○). Values represent means from three animals.

control and dazoxiben-treated endotoxin animals to lows of 65–70 torr. Central venous pressure did not change significantly from baseline in either group.

Lymph flow increased significantly in both control and dazoxiben-treated endotoxin animals (Figure 3), but with quite different time courses. The peak in control endotoxin animals (20.9 ± 5.2 ml/h) occurred at 30 min, whereas the peak in dazoxiben-treated endotoxin animals (12.68 ± 7.2 ml/h) was broadly spread over 1.5–3 h and remained high for the next 3 hours. In control endotoxin animals the early increase in lymph flow corresponded to peak PPA, but lymph flow then remained significantly elevated after PPA had returned toward baseline. Although the early increase in lymph flow did not occur in dazoxiben-treated endotoxin animals, the delayed increase in lymph flow was still observed and was not different from control endotoxin animals. L/P did not change significantly in either the control or dazoxiben-treated endotoxin animals (Figure 3).

Figure 3 Effect of dazoxiben on lung lymph flow following endotoxin infusion. Lung lymph flow and lymph/plasma protein ratios (L/P) were measured following infusion at time zero of endotoxin alone (●) or endotoxin along with dazoxiben (○). The initial 2 h of record are baseline. Values represent means from three animals.

Discussion

These studies demonstrate that dazoxiben can completely and selectively inhibit TXA_2 synthesis following endotoxemia in goats. The limited number of trials prevents resolution of the question regarding possible “shunting” of endoperoxides into PGI_2 synthesis in dazoxiben-treated endotoxin animals, although the earlier peak of 6-keto- $\text{PGF}_{1\alpha}$ would be consistent with this effect.

Inhibition of TXA_2 synthesis by dazoxiben markedly attenuates the increase in PPA and PW and the fall in CO following endotoxin infusion in goats. Similar results following endotoxin infusion have been observed in sheep receiving a selective thromboxane synthetase inhibitor (Watkins *et al.*, 1982). These results imply that TXA_2 mediates most of the pulmonary vasoconstriction of endotoxemia. The role of TXA_2 in the systemic haemodynamic changes has remained uncertain. In our series, although inhibition of thromboxane synthesis by dazoxiben does not prevent the fall in PA, it prevents the prolonged decrease in CO following endotoxin infusion suggesting that TXA_2 might contribute to myocardial dysfunction. Further studies including measurement of left ventricular end-diastolic pressures will be required to clarify this possibility.

The marked attenuation of the early increase in lymph flow by dazoxiben is almost certainly due to its ability to prevent most of the pulmonary vasoconstriction seen after endotoxin. Previous studies have demonstrated that this early phase of increased lymph flow is due to increased pulmonary microvascular pressure resultant in an increased

filtration rate (Brigham *et al.*, 1979; Demling *et al.*, 1981). By preventing the TXA₂-mediated pulmonary hypertension and the resultant increase in pulmonary microvascular pressures, dazoxiben reduces filtration pressure and lymph flow.

The delayed increase in lymph flow appearing 1–2 h after endotoxin in both groups probably represents a true permeability change since PPA had returned toward baseline and L/P remained stable (Brigham *et al.*, 1979; Demling *et al.*, 1981). Similarly, in dazoxiben-treated endotoxin animals lymph flow also increased significantly above baseline at a time when PPA was normal and L/P was stable. This result suggests that TXA₂ may not mediate the permeability changes following endotoxin infusion.

The observations that thromboxane synthetase inhibitors reduce mortality in experimental endotoxic shock (Wise *et al.*, 1980) together with the salutary effects of dazoxiben that we have observed on pulmonary haemodynamic and permeability changes following experimental endotoxaemia suggest a potentially important role for thromboxane synthetase inhibitors in clinical sepsis.

This work was supported by grants GM29853, GM24990, HL29036 and HL25706 from the National Institute of Health and a grant from R. J. Reynolds Industries Inc. It was performed during the tenure of a Clinician Scientist award of the American Heart Association with funds contributed in part by the Washington Heart Association.

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