EFFECTS OF DAZOXIBEN, AN INHIBITOR OF THROMBOXANE SYNTHETASE, ON FOREARM VASOCONSTRICTION IN RESPONSE TO COLD STIMULATION, AND ON HUMAN BLOOD VESSEL PROSTACYCLIN PRODUCTION

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- 1 In healthy male volunteers dazoxiben (UK 37248), an inhibitor of thromboxane synthetase, abolished the arterial and venous vasoconstriction produced in the forearm by cold stimulation.
- 2 Aspirin alone had no effect on this vasoconstriction but negated the effect of dazoxiben.
- 3 *In vitro* dazoxiben increased the production of prostacyclin by human arteries and veins.
- 4 By reducing the synthesis of thromboxane A₂, a potent vasoconstrictor, and by increasing the synthesis of prostacyclin, a potent vasodilator, dazoxiben may have a therapeutic role in conditions associated with abnormal vasoconstriction.

Introduction

IMMERSION of part of a limb in a mixture of ice and water causes vasoconstriction. This is thought to be due to an increase in sympathetic activity as reflected by an increase in circulating catecholamine levels (Robertson, 1979). In addition, there is evidence that circulating levels of thromboxane A₂ (TXA₂), which is synthesized by platelets and vascular endothelial cells, and prostacyclin (PGI₂), which is synthesized by white blood cells and blood vessel walls, also increase in response to cold stimulation (Serner, 1981). Since TXA₂ is a potent vasoconstrictor and PGI₂ is a potent vasodilator, alteration of the balance of these in favour of TXA₂ may contribute to the vasoconstrictor response. If so, an alteration of the synthesis of either would change this response.

PGI₂ and TXA₂ are synthesized from arachidonic acid in the cell membrane. The arachidonic acid is first converted via a cyclo-oxygenase into the prostaglandin endoperoxides, prostaglandin G₂ and prostaglandin H₂. These are then converted by either prostacyclin synthetase or thromboxane synthetase into PGI₂ and TXA₂ respectively. Dazoxiben (UK 37248) is a selective inhibitor of thromboxane synthesis (Tyler, 1981; Vermeylen, 1981). Aspirin by its action on cyclo-oxygenase inhibits both PGI₂ and TXA₂ production.

We have investigated the effects of dazoxiben alone and in combination with aspirin on cold-induced vasoconstriction in man and on human vascular PGI₂ synthesis *in vitro*.

Methods

Nine healthy male volunteers aged 23–35 yr, who had taken no medication during the preceding week,

were studied on 5 consecutive days. Forearm blood flow (five subjects) or venous tone (five subjects) was measured at rest and in response to cold stimulation. Room temperature was maintained between 22°C and 24°C throughout the period of testing. Cold stimulation was produced by immersing the left hand and forearm in a mixture of ice and water at 0–2°C for 2 minutes. Two cold stimulation tests (separated by 60 min) were performed each day.

The schedule used for dazoxiben and aspirin was:

- Day 1: Familiarization with the technique
- Day 2: Control day – no drugs
- Day 3: Dazoxiben 200 mg orally immediately after the first test
- Day 4: Aspirin 1800 mg orally during the 24 h before the test
Dazoxiben 200 mg orally immediately after the first test
- Day 5: Aspirin 1800 mg orally during the 24 h before the test.

Forearm blood flow was measured in the right arm by venous occlusion plethysmography using a mercury in rubber strain gauge (Greenfield, 1963). Systemic arterial pressure was measured in the left arm by auscultation (diastolic pressure was taken at phase V). Measurement were made at 30 s intervals for 1 min before cold stimulation, during the 2 min of cold stimulation, and for 2 min after. Forearm vascular resistance, in arbitrary units, was calculated according to the formula (Bennett, 1979):

$$\frac{1/3 \times (\text{systolic} + 2 \times \text{diastolic blood pressure})}{\text{forearm blood flow}}$$

Forearm venous tone was measured in the right arm by the method of Wood modified by Robinson (Robinson, 1968). A cuff at the wrist was inflated above arterial systolic pressure to exclude the hand from measurements. Changes in forearm circumference are then representative of changes in forearm volume (Greenfield, 1963). A venous occlusion cuff was inflated around the upper arm to produce an intravenous pressure measured via a superficial vein of 30 mm Hg. This was held constant for 2 min until the forearm circumference, measured by a mercury in rubber strain gauge and intravenous pressure, were also constant. The cuff was then slowly deflated over 90 s and the forearm circumference was noted at pressures of 25 and 5 mm Hg. These measurements were taken to represent differences in forearm volume between these pressures, as an indicator of venous tone (ml/100 ml forearm volume over 25–5 mm Hg). Venous tone was measured at rest, and in response to cold stimulation applied 30 s before cuff deflation, and maintained over the period of cuff deflation.

Measurements of vascular prostacyclin production

Human venous tissue was obtained from four patients at operation for removal of varicose veins, and arterial tissue was dissected from the amputated legs of two patients who had a lower limb amputation for peripheral vascular disease.

The vessels were sectioned longitudinally and 6 mm discs were punched out and stored on ice in 0.05 M Tris – HCl buffer, pH 7.5.

Two discs of the arterial or venous tissue were incubated for 30 min at 37°C in either 1 ml McEwen's solution or 1 ml McEwen's solution containing a range of concentrations of dazoxiben prepared from a stock solution (5×10^{-4} M) dissolved in water. For two subjects venous tissue was also incubated in McEwen's solution containing aspirin (1×10^{-3} M) and dazoxiben. After incubation the supernatants were decanted and stored at -20°C .

The supernatants were assayed for 6-keto-prostaglandin $F_{1\alpha}$ (the stable hydrolysis product of PGI_2) by radioimmunoassay using an antibody (a gift

Figure 1 Changes in forearm vascular resistance on day 2 (a) and day 5 (b) (mean $\pm$ s.e.m.). ●, first cold stimulation test; ○, second cold stimulation test.

from Dr N.L. Poyser, Edinburgh) and technique described previously (Poyser, 1980). All samples for each subject were measured in the same assay in triplicate at two dilutions (1:5 and 1:10) with assay buffer. The sensitivity of the assay for 6-keto-PGF_{1α} was approximately 40 pg/ml.

The interassay coefficient of variation of this assay is 13.5%.

The results are the mean obtained from the two dilutions expressed as ng 6-keto-PGF_{1α} produced by two discs of vessel.

Results

Table 1 shows mean values for vascular resistance and venous tone at rest on each day of the study. There was little difference in these values either between different days or different tests on the same day.

Figures 1 and 2 illustrate the changes obtained in forearm vascular resistance in five subjects during the

study. Results are expressed as the mean percentage change ($\pm$ s.e.m.) of the resting value, itself a mean of three values. Results are shown at 30 s intervals throughout the test period.

Figure 1a illustrates the results obtained on the control day. Forearm vascular resistance increased in response to cold stimulation both during the first test (solid line) and the second test (broken line). Figure 1b illustrates similar results obtained on day 5 following ingestion of aspirin 1800 mg in the previous 24 hours.

Figure 2a plots the results obtained on day 3. Sixty minutes after dazoxiben 200 mg (broken line), the response to cold stimulation was abolished. Figure 2b shows the results from day 4, and shows that when the subjects had taken aspirin 1800 mg over the preceding 24 h, the effect of dazoxiben had been negated.

The effect of cold stimulation on venous tone on each of the four study days is illustrated in Figure 3. Results are expressed as mean percentage increase ($\pm$ s.e.m.) of the resting value. The venous response to cold stimulation was abolished 60 min after

Figure 2 Changes in forearm vascular resistance on day 3 (a) and day 4 (b) (mean $\pm$ s.e.m.). ●, first cold stimulation test; ○, second cold stimulation test.

Figure 3 Changes in forearm venous tone on the four study days (mean ± s.e.m.).

Figure 4 Effect of dazoxiben on synthesis of 6-keto-PGF_{1α} by human vascular tissue. Open symbols represent venous tissue and closed symbols arterial tissue. Broken lines indicate samples incubated in McEwan's solution which contained aspirin.

dazoxiben 200 mg, and again pretreatment with aspirin negated this effect of dazoxiben on day 4.

Dazoxiben caused a marked increase in synthesis of 6-keto-PGF_{1α} by both arterial and venous tissue in all subjects and this increase was substantially reduced by the presence of aspirin (Figure 4).

Discussion

Dazoxiben abolished both the arterial and venous vasoconstriction in the forearm in response to cold stimulation. Aspirin alone had little effect but negated the effect of dazoxiben. Neither drug altered resting vascular tone.

Both dazoxiben and aspirin, in the doses used, are known to inhibit TXA₂ synthesis (Tyler, 1981; Smith, 1971). However, as these drugs had different effects on cold-induced vasoconstriction, simple inhibition of TXA₂ synthesis cannot account for these results. Aspirin also inhibits PGI₂ synthesis (Hanley, 1981), whereas there is some evidence that thromboxane synthetase inhibitors increase vascular PGI₂ synthesis, at least in a system in which platelets are present (Needleman, 1979). The mechanism for this increase may be that inhibition of thromboxane synthetase makes more of the platelet derived prostaglandin endoperoxides, PGG₂ and PGH₂, available for conversion to PGI₂ (Figure 5). The results of our *in vitro* studies, using isolated human vascular tissue, suggest that this redirection of synthesis may occur in vessel wall, and therefore that TXA₂ is

Figure 5 Effects of aspirin and dazoxiben on prostaglandin pathways.

produced by human vascular tissue. However, we are unable to exclude the possibility that the endoperoxides were derived from platelets adherent to the vessels.

As aspirin had no effect on cold induced vasoconstriction it seems unlikely that PGI_2 or TXA_2 are normally involved in this response. However, by manipulating the $\text{TXA}_2/\text{PGI}_2$ pathways, by thromboxane synthetase inhibition, the increase in vessel wall PGI_2 synthesis may reduce the sensitivity of the vessels to cold-induced vasoconstrictor stimuli. The negation of the effect of dazoxiben, by aspirin, provides further evidence that its mechanism of action is due to alteration of cyclo-oxygenase derived products. The lack of effect of dazoxiben on resting vascular tone would suggest that the increase in PGI_2 synthesis was insufficient to cause vasodilatation at rest.

These experiments were confined to the modulation of cold-induced vasoconstriction in the forearm. Whether similar effects occur in other vascular beds is unknown. However, increased levels of TXA_2 have been measured in patients with angina; and furthermore, indomethacin, another cyclo-oxygenase inhibitor, has been demonstrated to decrease coronary

artery blood flow in man (Friedman, 1981). Therefore, if prostaglandins are important in modulating coronary artery tone, this drug may theoretically be beneficial in patients with coronary artery disease. Clinical trials are needed to evaluate this and also its efficacy as a potential antithrombotic agent.

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Table 1 Resting values of forearm venous tone and vascular resistance (mean $\pm$ s.e.m.).

	Vascular resistance (arbitrary units)		Venous tone (ml/100 ml forearm volume/25.5 mmHg)	
	First test	Second test	First test	Second test
Day 2	30 $\pm$ 2	31 $\pm$ 3	2.7 $\pm$ 0.3	2.8 $\pm$ 0.4
Day 3	34 $\pm$ 5	34 $\pm$ 6	2.6 $\pm$ 0.2	2.3 $\pm$ 0.3
Day 4	32 $\pm$ 5	33 $\pm$ 5	2.8 $\pm$ 0.5	2.3 $\pm$ 0.3
Day 5	33 $\pm$ 4	32 $\pm$ 5	2.8 $\pm$ 0.3	2.8 $\pm$ 0.3

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