

## Endogenous prostacyclin biosynthesis and platelet function during selective inhibition of thromboxane synthase in man.

G A FitzGerald, ... , J A Oates, A K Pedersen

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### Research Article

The consequences of inhibiting the metabolism of prostaglandin G<sub>2</sub> to thromboxane A<sub>2</sub> in man were studied by using an inhibitor of thromboxane synthase, 4-[2-(1H-imidazol-1-yl)ethoxy] benzoic acid hydrochloride (dazoxiben). Single doses of 25, 50, 100, and 200 mg of dazoxiben were administered to healthy volunteers at 2-wk intervals in a randomized, placebo-controlled, double-blind manner. Serum thromboxane B<sub>2</sub> and aggregation studies in whole blood and platelet-rich plasma were measured before dosing and at 1, 4, 6, 8, and 24 h after dosing. Both serum thromboxane B<sub>2</sub> and the platelet aggregation response to arachidonic acid (1.33 mM) were reversibly inhibited in a dose-dependent manner. Aggregation induced by 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine (0.4 and 4.0 microM) in platelet-rich plasma as well as both aggregation and nucleotide release induced by collagen (95 micrograms/ml) in platelet-rich plasma and whole blood were unaltered by dazoxiben. Additional evidence for a platelet-inhibitory effect of the compound was a significant prolongation of the bleeding time at 1 h after administration of the highest dose (200 mg) of dazoxiben. Endogenous prostacyclin biosynthesis was assessed by measurement of the major urinary metabolite of prostacyclin, 2,3-dinor-6-keto-PGF<sub>1</sub> alpha (PGI-M). PGI-M excretion was increased by dazoxiben; it rose a mean 2.4-fold from predosing control values at 0-6 h after administration of the highest dose studied (200 mg).

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# Endogenous Prostacyclin Biosynthesis and Platelet Function During Selective Inhibition of Thromboxane Synthase in Man

GARRET A. FITZGERALD, ALAN R. BRASH, JOHN A. OATES, and  
ANDERS K. PEDERSEN, *Division of Clinical Pharmacology,  
Departments of Medicine and Pharmacology, Vanderbilt University,  
Nashville, Tennessee 37232*

**ABSTRACT** The consequences of inhibiting the metabolism of prostaglandin  $G_2$  to thromboxane  $A_2$  in man were studied by using an inhibitor of thromboxane synthase, 4-[2-(1H-imidazol-1-yl)ethoxy] benzoic acid hydrochloride (dazoxiben). Single doses of 25, 50, 100, and 200 mg of dazoxiben were administered to healthy volunteers at 2-wk intervals in a randomized, placebo-controlled, double-blind manner. Serum thromboxane  $B_2$  and aggregation studies in whole blood and platelet-rich plasma were measured before dosing and at 1, 4, 6, 8, and 24 h after dosing. Both serum thromboxane  $B_2$  and the platelet aggregation response to arachidonic acid (1.33 mM) were reversibly inhibited in a dose-dependent manner. Aggregation induced by 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (0.4 and 4.0  $\mu$ M) in platelet-rich plasma as well as both aggregation and nucleotide release induced by collagen (95  $\mu$ g/ml) in platelet-rich plasma and whole blood were unaltered by dazoxiben. Additional evidence for a platelet-inhibitory effect of the compound was a significant prolongation of the bleeding time at 1 h after administration of the highest dose (200 mg) of dazoxiben. Endogenous prostacyclin biosynthesis was assessed by measurement of the major urinary metabolite of prostacyclin, 2,3-dinor-6-keto-PGF $_{1\alpha}$  (PGI-M). PGI-M excretion was increased by

dazoxiben; it rose a mean 2.4-fold from predosing control values at 0–6 h after administration of the highest dose studied (200 mg).

## INTRODUCTION

Thromboxane  $A_2$ , a potent vasoconstrictor and stimulus to platelet aggregation, is a major oxygenated metabolite of arachidonic acid in the platelet (1). Because of the potential importance of such a compound in the mediation of vascular occlusive events in vivo, there has been a considerable interest in the pharmacologic effects attendant to the inhibition of thromboxane biosynthesis in man (2, 3). In particular, attempts have been made to clarify the human pharmacology of aspirin, an irreversible inhibitor of the enzyme cyclooxygenase (4). This enzyme converts arachidonic acid to unstable endoperoxides which are further transformed to prostaglandins and thromboxanes. Thromboxane  $A_2$  is the predominant endoperoxide product in the platelet, whereas in vascular endothelium, the major product is prostacyclin, a potent vasodilator and inhibitor of platelet aggregation (5).

Cyclooxygenase inhibitors, such as aspirin and sulfinpyrazone, result in a reduction in prostacyclin generation coincident with that of thromboxane  $A_2$  (6, 7, 8). Inhibition of thromboxane synthase has certain theoretical attractions in human syndromes of platelet activation (9). Inhibition of thromboxane formation at this step in its biosynthesis would preserve the capacity to generate prostacyclin, which is a potentially important endogenous inhibitor of platelet aggregation. Furthermore, accumulation of the endoperoxide substrate for thromboxane synthase is likely to occur in the platelet. This might permit donation of this labile intermediate to vascular prostacyclin synthase, and thereby actually increase prostacyclin biosynthesis

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coincident with inhibition of thromboxane formation *in vivo*.

Three classes of selective thromboxane synthase inhibitors have now been developed: imidazole and pyridine derivatives and endoperoxide/thromboxane A<sub>2</sub> structural analogues. Although sharing the property of inhibiting thromboxane synthase, these compounds differ in their actions on platelets. Whereas the endoperoxide analogues reliably inhibit arachidonic acid-induced platelet aggregation, the results with the other compounds have been much more variable (9a, 10, 11). Dazoxiben, 4-[2-(1H-imidazol-1-yl) ethoxy] benzoic acid hydrochloride and OKY-1581 (sodium-E-3-[4-(3-pyridylmethyl) phenyl]-2-methyl-2-propenoate) have undergone initial clinical investigation (12, 13, 14) and result in a dose-dependent depression of immunoreactive thromboxane B<sub>2</sub> formation *ex vivo*. Additional mechanisms by which these compounds may influence platelet function include stimulation of platelet adenylate cyclase (11), either directly or via enhanced prostaglandin (PG)<sup>1</sup>D<sub>2</sub> production, and thromboxane receptor antagonism (15, 16). However, it has been suggested that redirection of accumulated endoperoxide substrate towards prostacyclin biosynthesis (17, 18) may represent the dominant mode of action of these compounds (11). Biochemical evidence consistent with such a mechanism occurring *in vivo* is lacking. We report a randomized, double-blind, placebo-controlled study of the effects of dazoxiben, an imidazole-analogue thromboxane synthase inhibitor, on platelet function and endogenous prostacyclin biosynthesis in man.

## METHODS

**Study design.** Seven healthy male volunteers (aged 21–36 yr; wt 68–75 kg) participated in the study which was approved by the Committee for the Protection of Human Subjects of Vanderbilt University Medical Center. The study was performed double-blind. Identical, unlabeled, white oval capsules that contained either placebo or dazoxiben in doses of 25, 50, 100, or 200 mg were provided by Dr. P. Urquilla of Pfizer, Inc., Groton, CT. Each placebo capsule contained lactose, 218.75 mg, maize starch, 126.0 mg, and magnesium stearate (90%)/sodium laurel sulfate (10%), 5.25 mg. The subjects were randomized in blocks as to the order in which they received the drug and the double-blind code was not broken until the biochemical and statistical analyses were completed.

Study days were separated from each other by at least 10 d. This interval would preclude carry-over effects as previously published studies indicate that the biological half-life of the drug (recovery of inhibition of serum thromboxane B<sub>2</sub>) approximates 4–6 h (9a). The subjects fasted from at least

10 h before dosing to 4 h after drug administration. Urine was collected for prostacyclin metabolite determination for the 24 h before each study day and for 0–6 h, 6–12 h, and 12–24 h postdosing. Blood (30 ml) was drawn from a fresh venipuncture for platelet function studies before dosing and at 1, 4, 6, 8, and 24 h postdosing. Bleeding time was measured before dosing and at 1 h postdosing. Blood pressure and heart rate were measured after the subjects had lain supine for 10 min, sat for 5 min, and stood for 2 min. These measurements were performed just prior to blood withdrawal at each time point on the study days. Physical examinations of the volunteers were performed before entry into the study and 1 mo after its completion. Safety tests (full blood count and platelet count, SMA-12, and urinalysis) were performed at the time of the physical examinations, and before dosing and 24 h postdosing on each study day. These were unaltered throughout the study.

**Platelet function studies.** Aggregation studies in platelet-rich plasma were performed according to the light transmission method of Born (19) by using a dual channel aggregometer (Payton Associates, Buffalo, NY) in the manner which we have previously described (6). Briefly, blood was withdrawn via a 19-gauge butterfly needle and K50 extension tube by the two syringe method. The 2 ml that was drawn into the initial syringe was discarded and blood was drawn into a second syringe that contained 0.11 M citrate buffer, pH 5.0, to make a final blood/buffer ratio of 9:1. The platelet count in platelet-rich plasma was adjusted to 300,000/ml before the aggregation studies by using platelet-poor plasma prepared from autologous blood, which had been spun down at 8,000 *g* for 2 min. The aggregation base line (10% light transmission) was set by using platelet-rich plasma and buffer that were added in concentrations equivalent to the test system. Full transmission (100%) was set by using platelet-free plasma. Aggregating agonists were obtained as follows: 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (AGEPC or platelet-activating factor) from Supelco, Inc. (Bellafonte, PA) and collagen from Bio/Data Corp. (Horsham, PA). A stock solution of 50 mg/ml arachidonic acid in ethanol was used. Before the studies, 100  $\mu$ l of this solution was evaporated under a stream of nitrogen and dissolved in 0.05 M Na<sub>2</sub>CO<sub>3</sub> for addition to platelet-rich plasma.

Aggregation studies in whole blood and in platelet-rich plasma using impedance aggregometry (see below) were performed in a whole blood aggregometer (Chrono-log Corp., Havertown, PA). Platelet nucleotide release was monitored in both whole blood and platelet-rich plasma by a chemiluminescence method (20).

The lag time after addition of a platelet agonist to platelet-rich plasma to 50% of the maximal alteration in light transmission which occurred in the ensuing 4.5 min was expressed as the LT<sub>50</sub>. If no alteration in light transmission occurred within that period, 4.5 min was taken as a minimal estimate of the LT<sub>50</sub>.

**Thromboxane B<sub>2</sub> measurements.** Samples were obtained via the same venipuncture as those for the aggregation studies. They were incubated at 37°C for 45 min, and then at 20°C for 2 h. Then, the samples were centrifuged at 2,000 *g* for 10 min, and the serum was frozen and stored for analysis. Immunoreactive thromboxane B<sub>2</sub> was measured by a previously described method (21).

**Quantitation of 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub>  (PGI-M).** PGI-M was measured by a stable isotope dilution assay that used negative ion-chemical ionization gas chromatography-mass spectrometry. This is a modification of a previously published method (22). Briefly, 5 ng of a deuterated internal standard was added to a 5-ml aliquot of urine. After ex-

<sup>1</sup> Abbreviations used in this paper: AGEPC, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; PG, prostaglandin; PGI-M, 2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub> .

traction and back extraction under alkaline and acidic conditions, the sample is derivitized as the methoxime, pentafluorobenzyl ester. After further purification by thin-layer chromatography, derivitization was completed by formation on the trimethylsilyl ether derivative. Quantitation was accomplished by stable isotope dilution using a Hewlett Packard 5980 instrument that operated in the negative ion mode, monitoring  $m/z$  (mass/charge ratio) 586 for endogenous PGI-M and  $m/z$  590 for the deuterium-labeled internal standard.

**Bleeding time.** The forearm template bleeding time (Simplat General Diagnostics, Morris Plains, NJ) was measured before and 1 h postdosing.

**Statistical analysis.** Data were analyzed by nonparametric methods (23, 24); thereby, assumptions as to the distributions of the variables involved were avoided. Data were subjected to one-way analysis of variance by the method of Kruskal and Wallis and subsequent pairwise comparison with control values by the Lord U test.

## RESULTS

**Serum thromboxane  $B_2$ .** A dose-related inhibition of thromboxane  $B_2$  formation in serum occurred following administration of dazoxiben (Fig. 1). Peak inhibition was measured at 1 h after dosing, although it may have actually preceded this blood withdrawal or fallen between 1 and 4 h after dosing. Serum thromboxane  $B_2$  did not alter significantly from the predosing value (mean  $\pm$  SD) of  $435 \pm 70$  ng/ml after placebo administration, but fell from a predosing value of  $463 \pm 87$  to  $25.6 \pm 7.0$  ng/ml at 1 h after administration of 200 mg of dazoxiben. Both the peak inhibition and duration of inhibition of thromboxane  $B_2$  formation appeared to be more marked after 200 mg of dazoxiben than after the lower doses. Serum thromboxane  $B_2$  measurements that were made before the various

doses of dazoxiben administration were not significantly different from those obtained before placebo.

**Platelet aggregation studies.** Placebo administration caused no significant alteration in the lag time before 50% maximal aggregation ( $LT_{50}$ ) induced by arachidonic acid (1.33 mM). However, dazoxiben resulted in a dose- and time-dependent increase in  $LT_{50}$  (Fig. 2). Peak prolongation was measured at 1–4 h after dosing; both the degree and duration of inhibition of arachidonic acid-induced aggregation were more pronounced after 200 mg than after other doses of dazoxiben.

Dazoxiben was without effect on the aggregation of platelets when AGEPC (0.4 and 4.0  $\mu$ M) was employed as agonist. Studies that used collagen as an agonist employed impedance aggregometry (25), in contrast to the preceding studies, which measured platelet aggregation by the light transmission method (19). This method permits the rapid study of aggregation in whole blood. Detection of an effect of dazoxiben on aggregation in whole blood but not in platelet-rich plasma would be consistent with an effect mediated by a labile inhibitor of aggregation, such as prostacyclin. A slight inhibitory effect of dazoxiben on collagen-induced platelet aggregation and nucleotide release was related to both dose and time, but did not achieve statistical significance. Collagen-induced whole blood aggregation and nucleotide release were also unaltered by dazoxiben.

**Bleeding time.** Bleeding time was approximately doubled at 1 h after administration of 200 mg of dazoxiben. This increase attained statistical significance after 50 and 200 mg of dazoxiben (Fig. 3). Bleeding

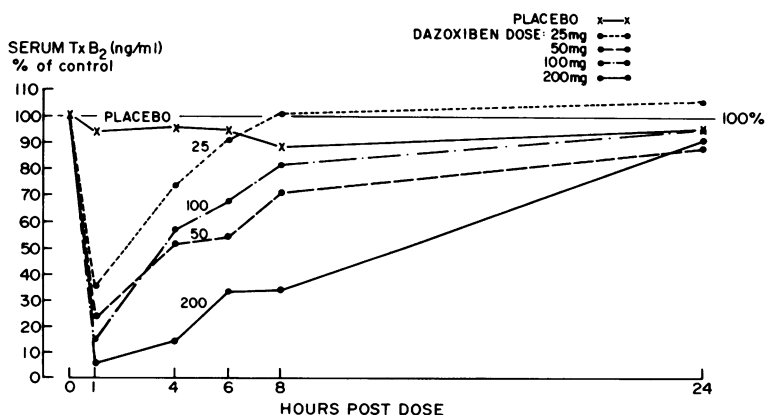


FIGURE 1 Inhibition of serum thromboxane  $B_2$  formation after administration of dazoxiben or placebo. When compared with corresponding values postplacebo, significant depression of serum thromboxane  $B_2$  occurred 1 ( $P < 0.01$ ) and 4 h ( $P < 0.05$ ) after 25 mg of dazoxiben; 1, 4 ( $P < 0.01$ ), and 6 h ( $P < 0.05$ ) after 50 and 100 mg of dazoxiben; and 1, 4, 6, and 8 h (all  $P < 0.01$ ) after 200 mg of dazoxiben.

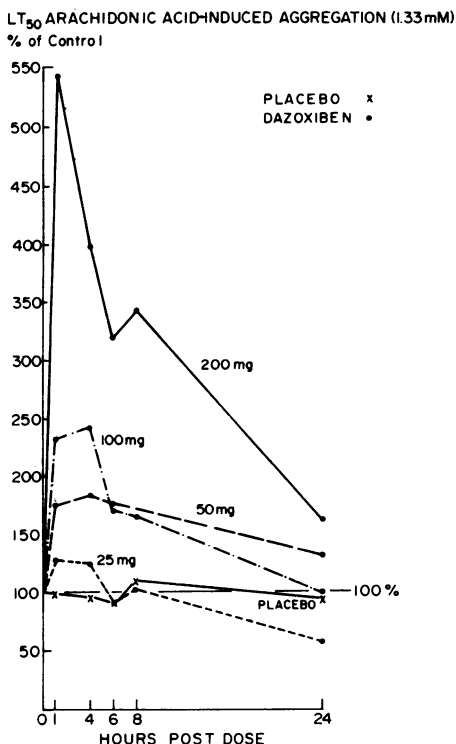


FIGURE 2 Platelet aggregation ex vivo after administration of dazoxiben and placebo. Mean values for the LT<sub>50</sub> (see text) for arachidonic acid-induced aggregation are shown as a percentage of the corresponding predose control. Significant prolongation occurred 1 and 4 h after 25 mg of dazoxiben ( $P < 0.05$ ); 1, 4, and 6 h after 50 mg of dazoxiben ( $P < 0.05$ ); and 1, 4, 6, and 8 h after 100 and 200 mg of dazoxiben ( $P < 0.01$ ). Aggregation studies in two individuals were not performed at 8 h after 50 mg of dazoxiben.

time was prolonged in five of the seven patients who received dazoxiben (100 mg) but failed to change significantly for the group as a whole.

**PGI-M excretion.** The rate of PGI-M excretion in the three timed aliquots after placebo administration (0–6, 6–12, and 12–24 h) did not differ significantly from that in the 24 h before dosing ( $12.8 \pm 1.8$  ng/h). Furthermore, PGI-M excretion did not differ significantly between the control days that preceded each drug administration (Fig. 4). Urinary volumes within each of the collection periods did not differ significantly between treatment days. Dazoxiben in doses of 25, 50, and 100 mg resulted in a modest, but significant increase ( $P < 0.05$ ) in PGI-M excretion at 0–6 h after dosing. This increase had declined at 6–12 h after dosing and had returned to control values at 12–24 h after dazoxiben administration (Fig. 1). The increase in PGI-M excretion after 200 mg of dazoxiben was more marked; it rose from  $14.96 \pm 2.2$  ng/h before dosing to

$35.2 \pm 10.7$  and  $36.2 \pm 11.6$  ng/h in the 0–6 and 6–12 h aliquots after drug administration. PGI-M had fallen to  $18.4 \pm 7.0$  ng/h at 12–24 h after dosing.

## DISCUSSION

The consequences of inhibiting the metabolism of prostaglandin endoperoxides by thromboxane synthase in man have been addressed in the present study. This was achieved by relating the dose of a specific inhibitor of this enzyme to platelet function and the biosynthesis of thromboxane A<sub>2</sub> and prostacyclin.

We employed several approaches to quantitate the effects of dazoxiben on platelet function, including measurement of platelet aggregation and nucleotide release in platelet-rich plasma and in whole blood ex vivo, and determination of the bleeding time. Previous studies have defined the ability of cyclooxygenase inhibitors, such as aspirin, to influence both platelet aggregation and the platelet release reaction, which reflect stimulated platelet function ex vivo (2, 3). The bleeding time is a "provocative" test of platelet-vascular function in vivo. It is important to stress that measurements were performed double-blind because this index is particularly liable to the observer's bias. This may have occurred despite these precautions as the individual who was performing the test was aware whether it preceded or followed dosing, although he was not cognizant of the medication administered. Given these limitations, the bleeding time is a measurement of platelet-vascular homeostasis in vivo; it also reflects the platelet-inhibitory action of aspirin-like drugs in man (2, 3).

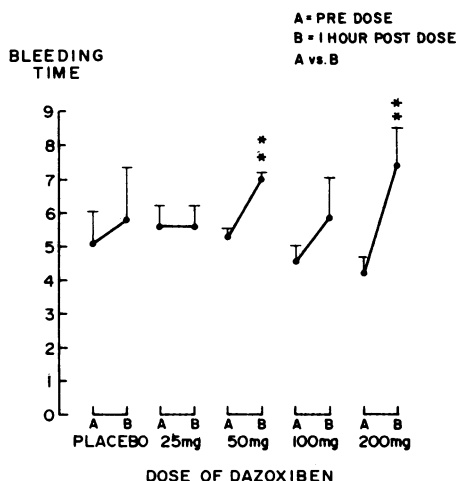
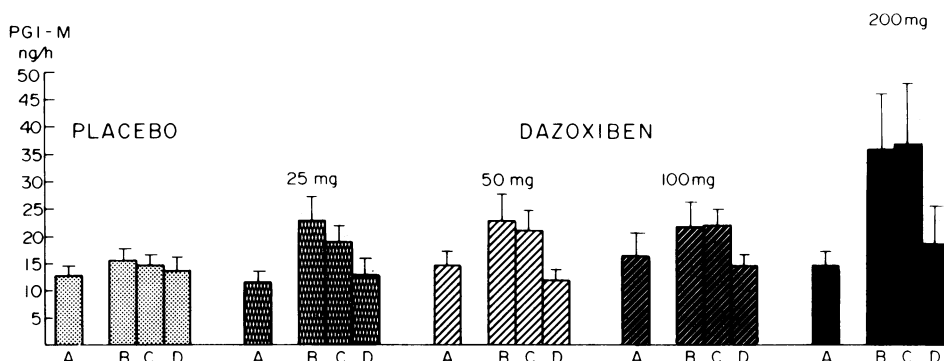


FIGURE 3 Bleeding time (min) before and 1 h after dosing with dazoxiben or placebo. \*\*,  $P < 0.01$ .



**FIGURE 4** Endogenous prostacyclin biosynthesis after administration of dazoxiben and placebo. Urinary PGI-M is expressed in nanograms per hour on the ordinate in the 24-h period before dosing (column A) and in three timed aliquots after administration of single doses of dazoxiben or placebo (columns B, C, and D). Urinary volumes within each of the collection periods did not differ significantly between treatment days. PGI-M excretion increased significantly 0–6 and 6–12 h after dosing with 25, 50, and 100 mg (all  $P < 0.05$ ), and 200 mg ( $P < 0.01$ ) dazoxiben as compared with corresponding placebo values. PGI-M excretion had returned to control values at 12–24 h after all doses of dazoxiben. Control: A, 0–24 h. Postdose: B, 0–6 h, C, 6–12 h, D, 12–24 h.

Previous groups have failed to demonstrate a consistent effect of imidazole-analogue thromboxane synthase inhibitors on platelet aggregation *ex vivo* (10, 13, 16). Indeed, the aggregation response of human platelets to arachidonic acid, ADP, and collagen *in vitro* is often preserved, despite inhibition of thromboxane formation by such drugs (10, 15, 16, 26). This may result from a direct, thromboxane-independent, proaggregatory action of the drug *in vitro*. For example, platelets possess an actin-myosin system and imidazoles contract glycerol-extracted muscle fibers *in vitro* (15). However, these observations have also raised the possibility that the proaggregatory effects of accumulated endoperoxides might fully substitute for the effects of thromboxane  $A_2$  *in vivo*. We found that administration of dazoxiben resulted in a dose- and time-related inhibition of the  $LT_{50}$  for arachidonic acid-induced aggregation by dazoxiben *ex vivo*. Arachidonic acid-induced platelet aggregation is the most sensitive indicator of cyclooxygenase-dependent inhibition of platelet aggregation *ex vivo*. In particular, the lag time before the onset of aggregation represents a sensitive quantitative measurement of inhibition of arachidonic-induced platelet activation (27, 28). Weak cyclooxygenase inhibitors, such as sulfinpyrazone and its sulfide and sulfone metabolites, consistently inhibit aggregation induced by arachidonic acid, but not by other platelet agonists, such as collagen (27). The effects of dazoxiben on this index of stimulated platelet function *ex vivo* are consistent with its ability to prolong the bleeding time. The mean prolongation of bleeding time after 200 mg of dazoxiben (1.8-fold) is

similar to that induced by 325 mg of aspirin (1.6-fold) in our hands under similarly blinded conditions.

We found no effect of dazoxiben on AGEPC or collagen-induced aggregation, although the data suggest a slight inhibition of the latter at the higher doses of the drug. Collagen can induce thromboxane-independent platelet aggregation (29). The failure to detect an effect of dazoxiben may also reflect the insensitivity of impedance aggregometry (30). However, we have found that aspirin (325 mg) results in 50–60% inhibition of both impedance-dependent aggregation and nucleotide release induced by the dose and preparation of collagen employed in these studies (31). Dazoxiben did not significantly alter collagen-induced whole blood aggregation. Agonist was added to the samples within 30 s of blood withdrawal in the hope of detecting the inhibitory effects of labile platelet-active compounds such as prostacyclin. Although the absence of such an effect may reflect the insensitivity of the approach, it is also consistent with a failure of prostacyclin to attain platelet-active concentrations in the systemic circulation after dosing with dazoxiben.

Finally, we determined the effects of dazoxiben on endogenous prostacyclin biosynthesis by measuring the major urinary metabolite of prostacyclin. Previous experiments *in vitro* suggest that enhancement of prostacyclin synthesis by a thromboxane synthase inhibitor requires use of accumulated platelet endoperoxide substrate by a tissue source of prostacyclin synthase, such as vascular endothelium or neutrophils (17, 32, 33). The formation of substantial quantities of thromboxane  $B_2$  (300–400 ng/ml) render serum an optimal

system for the detection of endoperoxide redirection by dazoxiben. However, comparable amounts of thromboxane are unlikely to be formed in vivo in healthy volunteers. Plasma measurements of 6-keto-PGF<sub>1α</sub> have been used as an index of prostacyclin biosynthesis after dazoxiben administration in man (13). Although an increase from ~50 pg/ml to 400 pg/ml was reported 1 h after dosing with 200 mg of dazoxiben, it is now known that normal concentrations of 6-keto-PGF<sub>1α</sub> in plasma are in the low picogram range (34); thus, the earlier data were rendered uninterpretable. PGI-M excretion in healthy volunteers reflects the rate of prostacyclin biosynthesis when the interaction of platelets with the vessel wall proceeds at a physiological rate (35); this index is increased in patients with severe atherosclerosis and with evidence of platelet activation in vivo (36). These observations are consistent with a role for endogenous prostacyclin as a homeostatic regulator in the presence of platelet activation in vivo. Cyclooxygenase inhibitors, such as aspirin and sulfinpyrazone, depress prostacyclin biosynthesis in healthy volunteers (7, 8). In contrast, dazoxiben did not diminish prostacyclin biosynthesis coincident with a dose-related inhibition of thromboxane B<sub>2</sub> formation in serum. Indeed, an increase in prostacyclin generation was evident, particularly after the highest dose was administered. Thus, the drug does act as a "selective" inhibitor of thromboxane synthase in man.

Our present results suggest that 200 mg of dazoxiben results in a mean 2.4-fold increase in prostacyclin biosynthesis. The other doses of dazoxiben (25, 50, and 100 mg) resulted in less marked increases; this suggests their close approximation on the dose-response curve relating dazoxiben to prostacyclin metabolite excretion. The relationship of such increases in prostacyclin biosynthesis to the inhibition of platelet function observed in the present study must remain speculative. Certainly, such an increment is unlikely to result in plasma concentrations consistent with prostacyclin functioning as a circulating hormone in vivo. This increment in the physiological secretion rate (~0.09 ng/kg per minute) of prostacyclin (35) into the bloodstream would still be considerably less than the concentrations of infused prostacyclin (2–4 ng/kg per minute) required to inhibit platelet function (37, 38). Comparison of the log dose-response characteristics for dazoxiben with those previously published for aspirin (39) indicate that ~300 mg of dazoxiben and 120 mg of aspirin would be expected to result in maximal inhibition of thromboxane B<sub>2</sub> formation in serum (Fig. 5). Chronic administration of this dose of aspirin would result in either a minor reduction or no change in en-

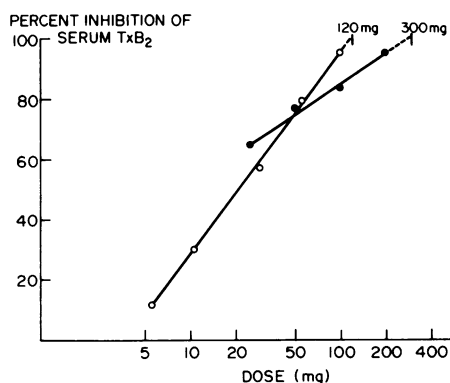


FIGURE 5 Log-dose response relationship for aspirin (data obtained from reference 38) and dazoxiben on serum thromboxane B<sub>2</sub> formation. Data have been extrapolated to indicate doses at which maximal suppression of serum thromboxane B<sub>2</sub> would be anticipated. O, aspirin; ●, dazoxiben.

dogenous biosynthesis of prostacyclin, together with inhibition of thromboxane synthesis (7).

The mechanism by which dazoxiben increases endogenous prostacyclin biosynthesis is not addressed by the present study. This could represent use of the accumulated endoperoxide substrate by a tissue source prostacyclin synthase, such as endothelial "steal" of platelet endoperoxides (32). Alternatively, the increase in prostacyclin formation may result from a direct, hitherto undescribed action of the drug in vivo. Finally, it is possible that endoperoxide redirection occurred in the present study in tissues that can synthesize both prostacyclin and thromboxane, such as the lung (40).

In conclusion, we selected an imidazole analogue, dazoxiben, as a model compound to elucidate the pharmacologic consequences of inhibiting thromboxane synthase in man. A dose-dependent inhibition of arachidonic acid-induced platelet aggregation *ex vivo* was detected, although, unlike aspirin, dazoxiben failed to modify platelet aggregation induced by other agonists. Additional evidence for the platelet-inhibitory action of dazoxiben in vivo was its ability to prolong the bleeding time. Prostacyclin biosynthesis increased ~2.4-fold coincident with inhibition of thromboxane formation, which is consistent with "selective" inhibition of thromboxane synthase by dazoxiben in vivo. The biochemical effects and biologic potency of such drugs in the presence of abnormal platelet-vascular homeostasis remain to be defined in man.

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