AH23848*: a thromboxane receptor-blocking drug that can clarify the pathophysiologic role of thromboxane A₂


ABSTRACT Despite numerous suggestions in the literature that thromboxane A₂ is involved in a variety of occlusive vascular diseases, no definitive evidence is available. Arguments have been presented to support the view that such evidence can only come from clinical studies with a highly specific thromboxane receptor-blocking drug. We have now identified such a drug, AH23848, in our laboratories. Preliminary experiments with AH23848, ([1α(Z), 2β,5α]-(-)-7-[5-[(1,1'-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid), show that it is a potent, specific thromboxane receptor-blocking drug that is orally active and has a long duration of action. It should be a valuable tool in elucidating any physiologic or pathologic role of thromboxane A₂.


THROMBOXANE A₂ (TxA₂), a product of arachidonic acid metabolism in blood platelets, is an exceptionally potent inducer of platelet aggregation and of contraction of vascular and respiratory smooth muscle.¹ Together with prostacyclin, it may play a physiologic role in the maintenance of vascular homeostasis and may also contribute to the pathogenesis of a variety of vascular disorders.²,³ As yet, however, there is no rigorous proof that TxA₂ plays a critical role in any disease process. Furthermore, such proof is unlikely to be obtained by observing the consequences of inhibition of the synthesis of TxA₂. If this is achieved by inhibition of fatty acid cyclooxygenase, the formation of other biologically active prostanoids such as prostacyclin and prostaglandin E₂ is also inhibited and, if by inhibition of TxA₂ synthetase, the production of the other products of the cyclooxygenase pathway may be enhanced. Thus, in neither case is it possible to relate the end result solely to a deficiency of TxA₂. Clearly a specific TxA₂ antagonist would, in principle, be a better tool for investigating any putative role of TxA₂ in disease states because it should affect only the actions of TxA₂.

Until recently specific thromboxane receptor-blocking drugs were not available. One of the first available was AH19437 but this, like most subsequently described thromboxane receptor-blockers, such as EP045 and BM 13,177, is relatively weak.⁴,⁵ We now describe the pharmacology of AH23848 (figure 1), which is chemically related to AH19437, but is about 100 times more potent. The high potency and selectivity of action of AH23848 make it a valuable tool for blocking thromboxane receptors. Preliminary studies in both animals and man provide further support for the view that TxA₂ plays a key pathologic role in some types of cardiovascular disorders. However, more detailed and wider ranging clinical studies are needed with drugs like AH23848 before we can be sure about the importance of TxA₂ in various diseases.

Materials and methods

Isolated smooth muscle. Isolated vascular smooth muscle samples from the rat and dog were prepared as spiral strips and set up in a modified Krebs solution gassed with 95% oxygen and 5% carbon dioxide for recording changes in isometric tension, as described previously.⁶,⁷ The human blood vessels were removed from macroscopically normal lung pieces obtained from surgical resection 12 to 48 hr earlier from patients with carcinoma of the lung. The arteries were cut into spiral strips and set up as described for the rat and dog vessels.

Other isolated smooth muscle preparations examined were the guinea pig ileum, guinea pig fundus, dog fundus, chick ileum, cat iris, and dog iris. These were prepared and set up in
Platelets. Human platelets from healthy male volunteers were examined in platelet-rich plasma with the use of a standard technique of preparation and aggregation was monitored with a photometric aggregometer. Except when examining the effects of collagen and arachidonic acid, aspirin (2.5 \times 10^4 mol/liter) was added to the platelet-rich plasma to prevent secondary aggregation resulting from TxA2 production.

Platelet aggregation was also examined in human whole blood with a novel technique expressly devised for this study. Aliquots of blood (treated with aspirin, 2.0 \times 10^{-3} mol/liter) were equilibrated at 37°C with a 95% air and 5% carbon dioxide mixture to maintain normal blood gas pressures and pH. Aggregation was measured by monitoring the peak percentage of aggregation was measured by monitoring the peak percentage of aggregation to show the expected activity of known inhibitors, namely indomethacin (1 \times 10^{-5} mol/liter), UK 34787 (1.0 \times 10^{-5} mol/liter)\(^{16}\) or 1-butyl-imidazol (5.0 \times 10^{-4} mol/liter), tranylcypromine (3 \times 10^{-3} mol/liter),\(^{17}\) and isobutylmethylxanthine (IBMX; 1 \times 10^{-5} mol/liter), respectively.

Biosynthesis of TxA2. TxA2 was biosynthetically generated from prostaglandin endoperoxide, PGH2, and a suspension of indomethacin-treated human platelet microsomes in 0.1 mol/liter Tris buffer (pH 7.5).\(^{13}\),\(^{18}\) The PGH2 was prepared by incubating arachidonic acid with rat seminal vesicle microsomes as described by Gorman et al.\(^{19}\) and stored at -70°C in anhydrous diethyl ether until needed.

Platelet deposition. In-labeled human platelets were prepared by the method of Hawker et al.\(^{20}\) and then resuspended in autologous blood, which was circulated over everted deendothelialized segments of rabbit aorta at 160 ml/min for 10 min at 37°C in a Baumgartner chamber.\(^{21}\) With the use of fresh everted aortic segments the blood was recirculated after addition of different concentrations of AH23848 (1.0 \times 10^{-9} - 1.0 \times 10^{-4} mol/liter). The number of platelets deposited was calculated by measurement of radioactivity in the aortic segments and reference to the platelet count and specific activity of the blood. Comparison of the inhibitory effect of AH23848 was made with the effect of prostacyclin and aspirin at different concentrations.

Anesthetized animals. Guinea pigs were anesthetized with sodium pentobarbital (60 mg/kg ip) and blood pressure and tracheal inflation pressure were monitored. Platelet counts were obtained in arterial blood samples with an Ultra-Flo 100 platelet counter. AH23848, aspirin, or vehicle were administered intravenously as bolus injections to animals before administration of collagen (0.1 mg/kg iv) or ADP (15 μg/kg/min iv). Each animal received only one treatment and one aggregatory challenge. The peak fall in platelet count, change in blood pressure, and tracheal inflation pressure after administration of the aggregating agent were compared with values immediately preceding the challenge.

Beagle dogs were anesthetized with sodium barbital and prepared so that blood pressure, heart rate, tracheal inflation pressure, and mesenteric arterial vascular resistance could be measured as described elsewhere. Dose-response curves for increases in mesenteric arterial vascular resistance were constructed for U-46619 administered intravenously close to the bed. When constant, curves were determined after administration of increasing intravenous doses of AH23848.

Platelet studies ex vivo. Platelet aggregation induced by collagen and ADP was measured ex vivo in whole blood from conscious dogs before and at intervals after oral administration of AH23848. Similar studies were performed in human volunteers, but the aggregating agents used in these studies were U-46619 and ADP. In both series of experiments platelet aggregation was measured with the Ultra-Flo whole blood platelet counter as described above.

Analysis of data

Estimates of antagonist potency. The interaction of agonists and antagonists on isolated smooth muscle preparations and on isolated platelets in whole blood was examined by the method of Arunlakshana and Schild with the use of established experimental protocols.\(^8\),\(^9\) Agonist concentration ratios were determined by dividing the concentration of agonist required to produce 50% of its maximal effect in the presence of antagonist by the dose or concentration of agonist required to produce the same response in the absence of antagonist. Schild analysis enabled determination of pA2 values, which are the negative logarithms of the concentration of antagonist required to produce a twofold rightward shift in the agonist concentration-effect curve. More importantly, if (1) the shifts of the agonist concentration-effect curve are parallel over a reasonable antagonist concentration range, (2) there is no reduction in the maximum response to the agonist, and (3) the Schild plot represents a straight line with a slope of unity, then the antagonist can be considered to be acting competitively. If so and there are no complicating factors the pA2 value can be shown to be equivalent to the negative logarithm of the antagonist dissociation constant for the receptor involved.\(^{24}\),\(^{25}\)

Statistical analysis. Where appropriate all data have been presented as the mean ± SEM of n observations.

Drugs used. The drugs used and their suppliers were as follows: aspirin (BDH), AH23848 (Glaxo Group Research), arachidonic acid (Sigma), (\(-\))adrenaline (Sigma), collagen (Hormon Chemie), 5-hydroxytryptamine (5-HT; Koch-Light), indomethacin (Merck, Sharpe & Dohme), IBMX (Aldrich Chemical Co.), prostaglandin D2 (PGD2; Glaxo Group Research), prostaglandin E2 (PGE2; Ono), prostaglandin F2a (PGF2a; tromethamine salt; Upjohn), prostaglandin I2 (PGI2; Glaxo Group Research), tranylcypromine (Smith, Kline & French), UK 34787.
[(2-isopropyl)-3-(1-imidazolylmethyl) indole; Pfizer]; U-46619 (11,9-exopxymethano-PGH_{2}; Glaxo Group Research).

All except the following compounds were prepared as stock solutions in distilled water. ADP was dissolved in 5 × 10^{-2} mol/liter Tris HCl, pH 6.5. Aspirin was prepared immediately before use in 1 × 10^{-3} mol/liter Tris HCl, pH 8.5. PG_{1} was dissolved in ice cold 5 × 10^{-2} mol/liter Tris HCl, pH 8.0. PG_{2}, U-46619, and indomethacin were each dissolved in 10% weight/volume (wt/vol) sodium bicarbonate. AH23848 was dissolved in ethanol (6% wt/vol) followed by dilution to stock concentrations in saline or 10% wt/vol sodium bicarbonate. Collagen was dissolved in a commercially supplied buffer (pH 2.8) or in 1.5 × 10^{-1} mol/liter sodium chloride. Arachidonic acid was dissolved in 5% wt/vol sodium carbonate followed promptly by dilution with 10% wt/vol sodium bicarbonate. UK 34787 was dissolved in 1 × 10^{-1} mol/liter Tris HCl, followed by dilution to stock concentrations with 1.5 × 10^{-3} mol/liter sodium chloride. Epinephrine was dissolved in 1.5 × 10^{-3} mol/liter sodium chloride or water, both of which contained 5 × 10^{-3} mol/liter ascorbic acid. For all compounds, further dilutions to working concentrations were made with 1.5 × 10^{-1} mol/liter sodium chloride or with physiologic buffers or by direct addition to plasma or blood. In all platelet studies, concentrations refer to those in platelet rich plasma or blood. During experiments all drug solutions were kept on ice.

**Results**

**Isolated smooth muscle.** AH23848 (6.0 × 10^{-8} to 6.0 × 10^{-6} mol/liter) was assessed as an antagonist of the contractile actions of the stable TXA_{2} mimetic U-46619 in isolated vascular smooth muscle. In human pulmonary arteries U-46619 produced sustained concentration-dependent changes in tension to a maximum tension of 0.48 ± 0.02 g at 1.0 × 10^{-6} mol/liter (n = 31). The concentration-effect curve for U-46619 was shifted to the right by AH23848 in a concentration-dependent manner with little or no reduction in the maximum response (figure 2, A). The pA_{2} value for AH23848 was found to be 7.8 and the slope of the Schild plot was close to unity, indicating competitive antagonism. There was a similar interaction between U-46619 and AH23848 on vascular preparations from rat and dog (table 1). In some experiments in the human pulmonary vessels and dog saphenous vein, but not in the rat aorta, AH23848 by itself produced weak transient contractions (up to about 15% of the maximum response to U-46619), but these waned within minutes.

The antagonistic action of AH23848 was very specific since in isolated preparations of rat aorta it did not antagonize contractions induced by 5-HT or potassium chloride, even at concentrations as high as 10^{-3} mol/liter (table 1). Similarly, AH23848 (1.0 × 10^{-6} mol/liter) did not inhibit contractions induced by PGE_{2} or PGF_{2α} in nonvascular smooth muscle preparations such as guinea pig ileum and fundus, dog fundus, chick ileum, and cat and dog iris, which are highly sensitive to PGE_{2} or PGF_{2α}, but relatively much less so to TXA_{2} or U-46619.

Confirmatory evidence that AH23848 is a specific antagonist of TXA_{2} per se was obtained with the use of dog saphenous vein preparations suspended for cascade superfusion. AH23848 (1.0 × 10^{-7} mol/liter) almost abolished the contractions produced by TXA_{2}, but was without effect on contractile responses to 5-HT (figure 3). AH23848 appeared to be about as potent an antagonist of TXA_{2} as of U-46619, but the instability of TXA_{2} precluded accurate pA_{2} determinations.

**Platelets.** In human platelet-rich plasma AH23848 selectively inhibited aggregation caused by submaximal concentrations of TXA_{2}, U-46619, PGH_{2}, arachidonic acid, or collagen (table 2). The antagonistic action of AH23848 at platelet thromboxane receptors was specific since even concentrations as high as 1.0 × 10^{-4} mol/liter did not inhibit the primary phase of aggregation caused by epinephrine, 5-HT, or ADP (table 2). AH23848 (1.0 × 10^{-7} to 1.0 × 10^{-5} mol/liter) did not cause any change in platelet shape or aggregation on its own nor did it affect the antiaggregatory actions of prostacyclin or PGD_{2}. In addition, AH23848 (1.0 × 10^{-5} mol/liter) had no significant action on the activity of fatty acid cyclooxygenase, thromboxane synthetase, prostacyclin synthetase, or cyclic AMP phosphodiesterase (data not shown).

In human whole blood AH23848 (3.0 × 10^{-4} to 3.0 × 10^{-7} mol/liter) was a specific antagonist of U-46619–induced platelet aggregation, producing a concentration-dependent rightward shift of the U-46619 concentration-effect curve (figure 2, B). The pA_{2} value obtained for AH23848 of 7.8 was similar to those obtained with the vascular preparations, although unlike in the vascular preparations, the slope of the Schild plot was significantly greater than unity (table 1). AH23848 had no effect on ADP-induced aggregation, even at concentrations as high as 1.0 × 10^{-4} mol/liter (table 1).

With the use of a modified Baumgartner technique, AH23848 was found to inhibit deposition of autologous platelets from human whole blood onto isolated deendothelialized rabbit aorta. The maximum achievable degree of inhibition was about 70% of the total platelet deposition, which was similar to the maximum inhibition achieved with prostacyclin and aspirin (figure 4). AH23848 produced 50% of its own maximal inhibition at a concentration (IC_{50}) of 5 × 10^{-4} mol/liter being about 17 times weaker than prostacyclin (IC_{50} = 3 × 10^{-4} mol/liter) and 180 times more potent than aspirin (IC_{50} = 3 × 10^{-3} mol/liter).

**Studies in whole animals.** In the anesthetized guinea pig ileum, AH23848 produced dose-related contractions in a concentration-dependent manner. AH23848 (1.0 × 10^{-6} mol/liter) produced responses almost identical to those of U-46619 (1.0 × 10^{-5} mol/liter), with AH23848 causing a greater degree of contraction than U-46619 at high concentrations (1.0 × 10^{-5} mol/liter).

AH23848 (1.0 × 10^{-6} to 1.0 × 10^{-4} mol/liter) was suspended in 5% wt/vol sodium bicarbonate and administered intravenously.
pig intravenous injection of collagen (0.1 mg/kg) produced a marked thrombocytopenia (40 ± 4% fall in circulating platelets), a biphasic effect on diastolic blood pressure with a small depressor response (Δ 6 ± 2 mm Hg) followed by a pressor response (Δ 9 ± 4 mm Hg), and bronchoconstriction (114 ± 17% increase in tracheal inflation pressure, n = 8). AH23848, at doses as low as 0.03 mg/kg iv, substantially reduced the thrombocytopenia and almost completely abolished the diastolic pressor response and the bronchoconstriction induced by collagen (figure 5). The thrombocytopenia produced by ADP (15 μg/kg/ min iv) was not inhibited by AH23848 at doses as high as 1.0 mg/kg iv.

In the anesthetized dog, AH23848 (0.01 to 0.3 mg/kg iv) had little or no effect on blood pressure or mesenteric arterial blood flow, but specifically antagonized the vasoconstriction produced by close intra-arterial injection of U-46619 into the bed. U-46619 (0.003 to 0.03 μg/kg intra-arterially) produced a dose-dependent decrease in mesenteric blood flow and a mean decrease in vascular conductance of 68% (range 54% to 86%, n = 7) at a dose of 0.03 μg/kg intra-arterially. At a dose of 0.1 mg/kg iv AH23848 caused a
mean rightward shift in the U-46619 dose-effect curve of 6.5-fold (range 3- to 13-fold, n = 4), while in separate control experiments the spontaneous shift was less than twofold (n = 3).

In the conscious dog orally administered AH23848 (1 mg/kg) produced a marked inhibition of collagen-induced platelet aggregation in whole blood ex vivo. There was a 23-fold rightward shift (mean peak effect, range 11 to 43, n = 5) of the collagen concentration aggregation curve and a residual effect (twofold shift) was still evident after 11 hr. In the same experiments there was no inhibition of ADP-induced aggregation ex vivo.

Studies in man. The effects of orally administered AH23848 (0.125 to 1.0 mg/kg) in eight healthy volunteers were qualitatively similar to those in the conscious dog. Platelet aggregation induced by U-46619 (but not ADP) ex vivo was inhibited for at least 8 hr after doses of 0.5 mg/kg or greater (figure 6). No cardiovascular or other effects that could be attributed to the drug were observed.

Discussion

The data presented in this study show that AH23848 is a highly specific thromboxane receptor-blocking drug in vitro and in vivo and as such should be a valuable drug tool in defining the physiologic and pathologic roles of TxA2. In isolated smooth muscle the surmountable nature of the antagonism against the TxA2 mimetic U-46619 and the linear Schild regression with a slope not significantly different from unity indicate a competitive drug-receptor interaction (see Methods). The anomalously high slope of the Schild regression for human platelets may have resulted from a nonspecific effect of the agonist at the high concentrations required in the presence of AH23848. Thus, high concentrations of U-46619 appear to raise intracellular cyclic AMP levels in platelets, presumably by a mechanism independent of its TxA2 mimetic action. Such an action by U-46619 would lead to an antiaggregatory effect that would physiologically antagonize its aggregatory action at thromboxane receptors and might explain the apparent reduction in the maximum response in the presence of 3.0 \times 10^{-7} \text{ mol/liter} AH23848 and the high slope of the Schild plot. The antagonism produced by AH23848 in platelets and smooth muscle was evidently mediated via thromboxane-receptor blockade per se since the actions of agonists acting via other receptors (e.g., 5-HT or ADP) were not inhibited, thus excluding any possible non-

![FIGURE 3. Dog isolated superfused saphenous vein. Original tracing showing the specific antagonism by AH23848 (1 \times 10^{-7} \text{ ml/liter}) of TxA2 biosynthetically generated and administered to the superfusion medium. Note that the contractile response to TxA2 was markedly inhibited while that to 5-HT was unaffected.]

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TABLE 2
Potency of AH23848 as an inhibitor of human platelet aggregation in platelet-rich plasma

<table>
<thead>
<tr>
<th>Agonist (concentration)</th>
<th>IC_{50} (× 10^{-7} mol/l)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen (1 μg/ml)</td>
<td>6.3 ± 1.2</td>
<td>15</td>
</tr>
<tr>
<td>Arachidonic acid (10^{-3} mol/l)</td>
<td>2.95 ± 0.8</td>
<td>4</td>
</tr>
<tr>
<td>PGF_{2α}/PGH_{2} (10^{-6} mol/l)^{a}</td>
<td>1.05 ± 0.45</td>
<td>4</td>
</tr>
<tr>
<td>TxA_{2} (from 10^{-6} mol/l PGH_{2})^{a}</td>
<td>2.62 ± 1.61</td>
<td>3</td>
</tr>
<tr>
<td>U-46619 (10^{-6} mol/l)^{a}</td>
<td>1.62 ± 0.8</td>
<td>4</td>
</tr>
<tr>
<td>ADP (10^{-6} mol/l)^{a}</td>
<td>&gt;1000^{b}</td>
<td>4</td>
</tr>
<tr>
<td>5-HT (5 × 10^{-6} mol/l)^{a}</td>
<td>&gt;1000^{b}</td>
<td>4</td>
</tr>
<tr>
<td>Epinephrine (10^{-6} mol/l)^{a}</td>
<td>&gt;1000^{b}</td>
<td>4</td>
</tr>
</tbody>
</table>

Platelet aggregation studied in platelet-rich plasma with the use of a Born aggregometer. Each value is the mean ± SEM of n determinations.

IC_{50} = molar concentration of AH23848 causing 50% inhibition of aggregation produced by a submaximal concentration of aggregating agent.

^aPlatelets pretreated with 2.5 × 10^{-4} M aspirin.
^bNo significant effect at 10^{-4} mol/l.

specific effects of AH23848, such as activation of adenylate cyclase or phosphodiesterase inhibition.

The weak contractions produced by AH23848 that were seen occasionally in some isolated vascular preparations (see Results) were transient and did not complicate the measurement of its antagonistic potency since this was always measured in the absence of any agonistic activity 30 min after its administration. The agonistic activity also exhibited marked tachyphylaxis, which was absent if a second dose of AH23848 was administered subsequently.* The mechanism involved is under investigation and will be the subject of a later report. It may result from a degree of partial agonistic activity at thromboxane receptors, but its weak and ephemeral nature makes it unlikely to have any clinical consequence. Indeed, AH23848 had no effect on blood pressure in human volunteers and no proaggregatory effects have ever been observed on platelets.

In human platelet-rich plasma AH23848 potently and specifically antagonized the aggregation induced not only by U-46619 and TxA_{2}, but also PGH_{2}, collagen, and arachidonic acid, each of which is known to produce its effects either directly or indirectly via thromboxane-receptor activation.\(^{9,27}\) AH23848 also inhibited the deposition of \(^{111}\)In-labeled human platelets onto isolated deendothelialized rabbit aorta and was as effective as prostacyclin in this respect. The deposition of platelets is believed to result from both adherence (platelet monolayer bound to subendothelium) and aggregation (platelets bound to other platelets).\(^{28,29}\) The inhibitory effect of AH23848 presumably resulted from inhibition of aggregation per se induced by thromboxane-receptor activation since AH23848 was effective at the same concentrations as those necessary to prevent collagen- or U-46619-induced platelet aggregation in human whole blood. Neither AH23848 nor prostacyclin had any effect on the residual 25% to 30% of platelet deposition, which probably represents platelet adherence, a phenomenon known to be prostacyclin-insensitive.\(^{29}\)

In the anesthetized guinea pig, AH23848 inhibited the bronchoconstrictor action of collagen, which has been shown to be platelet dependent and like a substantial component of the thrombocytopenic effect to result from the formation of a prostaglandin cyclooxygenase product.\(^{30}\) The potent inhibition of these responses by AH23848 is consistent with this and indicate that the particular prostanooid involved is TxA_{2}. The vasoconstrictor effect of collagen was also selectively abolished by AH23848, suggesting that this too results from the endogenous release of TxA_{2}, which is presumably platelet derived and produces vasoconstriction resulting in an increase in total peripheral resistance. In the anesthetized dog AH23848 was shown to be a potent antagonist against the vasoconstrictor actions of U-46619. Furthermore, the vasoconstrictor responses to endogenously released TxA_{2} in the guinea pig were inhibited by doses of AH23848 similar to those necessary to inhibit vasoconstrictor responses to the exogenous synthetic TxA_{2} mimetic U-46619 in the dog. These findings are consistent with the view that AH23848 blocks vascular thromboxane receptors in vivo.

Evidence has also been provided that AH23848 will block platelet thromboxane receptors in vivo. Thus, AH23848 not only potently antagonized the collagen-induced thrombocytopenia in the anesthetized guinea pig, but also antagonized collagen-induced platelet aggregation ex vivo. In the dog an oral dose of 1 mg/kg AH23848 produced a sustained inhibition over many hours. The effect was specific for collagen, indicating that the inhibition resulted from thromboxane-receptor blockade. Further evidence for long-lasting platelet thromboxane-receptor blockade in vivo was obtained in man.

Until the development of orally active specific thromboxane receptor–blocking drugs like AH23848 that can be administered to man,\(^{7}\) it was not possible to obtain good evidence for the involvement of TxA_{2} in human disease. Research workers have had to rely, to
a large extent, on observing whether or not a cyclo-
xygenase inhibitor, such as aspirin, or a thrombox-
ane-synthestase inhibitor, such as dazoxiben, ame-
liorated the condition. However, as already discussed
(see introduction), none of these tools will provide
definitive evidence for or against the involvement of
TxA2. Similarly, measurement of plasma levels of
TxB2 (the stable breakdown product of TxA2) may be
of little value because any increases could be second-
dary to some other pathologic event, TxA2 not being
the primary mediator, or artifactual as a consequence
of blood sampling problems. Alternatively, despite a
major involvement, thromboxane levels in peripheral
blood might not be raised since there is good reason to
believe that TxA2 (as well as prostacyclin) may be
released locally at the site of a pathologic lesion. Thus,
for example, after ligation of the left anterior
descending coronary artery in the anesthetized grey-
hound, marked increases in TxB2 production can be
shown in blood from a vein draining the ischemic area.

FIGURE 4. Rabbit isolated everted deendothelialized rabbit aorta superfused in a Baumgartner chamber with 111 In-labeled platelets in human whole blood. The effects of different concentrations of prostacyclin, AH23848, and aspirin on platelet deposition were compared with those on control (untreated) preparations. Each value is the mean of at least four determinations; vertical bars represent ± SEM.

FIGURE 5. Anesthetized guinea pig. The effect of AH23848 (μg/kg) on collagen (0.1 mg/kg iv)-induced thrombocytopenia (left), increases in diastolic blood pressure (center), and increases in tracheal inflation pressure (right). Each value is the mean from four to seven animals in each dosage group; vertical bars represent ± SEM.
but not in peripheral venous blood or even in blood from the coronary sinus.\textsuperscript{32}

If aspirin (or other cyclooxygenase inhibitors) is beneficial in an animal preparation or clinical disease state, this could be considered evidence for the possible involvement of TxA\textsubscript{2}. Thus, aspirin would prevent the conversion of arachidonic acid to the endoperoxide intermediate and thereby stop the production of TxA\textsubscript{2}. However, it would also prevent the formation of the antithrombotic prostanoids, PGD\textsubscript{2} and prostacyclin, which could be counterproductive and could even be potentially dangerous in the clinical situation. Indeed, aspirin has been shown to have a prothrombotic action in some animal preparations.\textsuperscript{34} Nevertheless, "low dose" aspirin has been shown to selectively inhibit thromboxane production in platelets\textsuperscript{35-37}; however, whether this can be achieved in the clinical setting in all patients on long-term treatment remains to be seen.\textsuperscript{38}

If a thromboxane-synthetase inhibitor were shown to ameliorate a condition clinically or in an animal preparation interpretation of the mechanism involved would be difficult. It could be effective because it reduces the levels of the deleterious primary mediator TxA\textsubscript{2} or alternatively because it increases the levels of circulating antithrombotic prostaglandins (e.g., prostacyclin or PGD\textsubscript{2}) by synthesis pathway diversion.\textsuperscript{39, 40} If, as has been suggested, thromboxane-synthetase inhibitors rely on conversion of prostaglandin endoperoxides to anti-aggregatory prostaglandins for their efficacy, such conversion may not occur when there is extensive vessel wall disease or a (local) congenital or pathologic enzyme deficiency. Under these conditions excess endoperoxides may accumulate that themselves mimic the biological actions of TxA\textsubscript{2}. Indeed, it has been shown that thromboxane-synthetase inhibitors do not have much effect on the platelet aggregation produced by arachidonic acid or PGH\textsubscript{2} in vitro because the endoperoxides cause platelet aggregation (and vasoconstriction) in their own right.\textsuperscript{10, 41, 42} Thus, even if a thromboxane-synthetase inhibitor did not work in a given clinical situation, it would not necessarily provide evidence for the lack of involvement of TxA\textsubscript{2}.

Definitive evidence for the involvement of TxA\textsubscript{2} can, however, come from clinical studies with a highly specific and potent thromboxane receptor--blocking drug such as AH23848. AH23848 would, if given in a sufficiently high dose, antagonize the effects of TxA\textsubscript{2}, even if the levels were as much as a 100 times or more greater than normal. As shown in this study, this could be achieved without inhibiting the normal synthesis of antithrombotic prostaglandins since the compound has no inhibitory effect on the enzymes involved. In addition, AH23848 antagonizes the actions of the endoperoxide PGH\textsubscript{2}, which acts directly via thromboxane receptors (table 2).\textsuperscript{43, 44} Importantly too, such a com-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6}
\caption{Human volunteer. U-46619--induced platelet aggregation was examined ex vivo in whole blood. Concentration--effect curves for U-46619 were constructed before (\textbullet) and at intervals (1 hr, \textgreater; 3 hr, \textbullet; 4 hr, \textangle; 6 hr, \textlt; 8 hr, \textbullet; and 24 hr, \texttriangle) after oral administration of 0.5 mg/kg AH23848. The ordinate represents the percentage decrease in platelet count and the abscissa is the log molar concentration of U-46619. The data are from a single study in one of two volunteers given this dose. Similar findings were observed in the other volunteer.}
\end{figure}
pound will not produce potentially undesirable hemodynamic effects such as vasodilation or hypotension that would be expected to compromise further an already ischemic vascular bed by "steal" or a reduction in driving pressure, respectively.

TxA₂ has been suggested to be involved in a variety of pathologic conditions, particularly those embraced by the title of occlusive vascular disease. Thus it has been reported a number of times in the literature that thromboxane levels are markedly raised in pacing-induced angina and that the rise appears to relate specifically to coronary disease since pacing does not induce such changes in healthy volunteers.⁵⁵, ⁵⁶ However, drugs that inhibit the synthesis of TxA₂, such as indomethacin and aspirin, appear to be without effect on the severity or incidence of symptomatic episodes of vasospastic angina.⁴⁷, ⁴⁸ One could argue, as indeed Chierchia et al.⁴⁸ have done, that the inhibition of prostacyclin production could explain the negative results in these studies.⁴⁹ In keeping with this view it has been shown that prostacyclin levels, as well as thromboxane levels, are markedly elevated in venous blood draining an ischemic myocardial region.³² The thromboxane-synthetase inhibitor dazoxiben has also been found to have no marked effect on the time to reach pacing- or exercise-induced angina.⁴⁹, ⁵⁰ However, in view of the interpretational problems discussed above, it can be argued that only the investigation of the effects of a thromboxane receptor-blocking drug will provide definitive evidence one way or the other for the involvement of TxA₂.

Importantly, AH23848 has now been examined clinically in pacing- and exercise-induced angina, but has been shown to have no obvious effect.⁵¹ We therefore conclude that TxA₂ is not important in the pathogenesis of pacing- or exercise-induced angina. Nevertheless, it may still be that TxA₂ is important in Prinzmetal’s angina or vasospasm and thrombosis, which lead to sudden death or myocardial infarction. In anesthetized greyhounds AH23848 (1 mg/kg iv) protects against death after ventricular fibrillation as a result of reperfusion of an ischemic area of myocardium.⁵² Clinically aspirin does appear to have some benefit in myocardial infarction, and it may be that its efficacy is reduced by failure to use the correct dosage.⁵³, ⁵⁴ The question of whether elevated thromboxane levels are only secondary to platelet aggregation or whether TxA₂ is the primary etiologic factor in these conditions remains to be determined.⁵⁵ Definitive evidence can only come from well-designed clinical trials with a potent effective thromboxane receptor-blocking drug.

Other clinical conditions in which TxA₂ might be important pathologically include postoperative complications resulting from platelet activation, cerebrovascular disease, and septic shock. Evidence has been presented that aspirin and AH23848 will inhibit platelet deposition onto isolated endothelialized vascular smooth muscle. The inhibitory action might be of value in maintaining the patency of vascular prostheses and in the prophylaxis of organ transplant rejection, particularly the kidney, which synthesizes substantial amounts of TxA₂ under ischemic conditions.⁶⁶ Interestingly, it has already been found clinically that AH23848 will inhibit platelet deposition onto aortofemoral prosthetic grafts and in this respect was perhaps surprisingly superior to a combination of aspirin and dipyridamole.⁵⁷ Whether the superior effect of AH23848 will be a consistent finding remains to be determined, but nevertheless a thromboxane receptor-blocking drug has a theoretical advantage over aspirin in that it would spare the production of the protective prostaglandins such as prostacyclin. Thus it may be preferable to use a thromboxane-receptor blocker and avoid the risk of compromising a sick patient’s own natural defenses, which may be at least partially functional at the site of the lesion and indeed intact elsewhere in the body.

Patients suffering from transient ischemic attacks commonly have advanced atherosclerosis of the intracranial circulation and this may provide the stimulus for TxA₂ release from aggregating platelets, thus producing a chain reaction for further aggregation and cerebral vasoconstriction.⁵⁸, ⁵⁹ Some evidence that such a pathologic sequence may indeed occur comes from the fact that aspirin is now considered to be an effective prophylactic in the treatment of transient ischemic attacks and cerebral infarction.⁶⁰, ⁶¹ The rationale for the use of a thromboxane-receptor blocker in patients with cerebrovascular disease is similar to that advanced for the use of such a drug in the prophylaxis of vascular graft occlusion.

There is evidence from animal experiments in preparations of endotoxin shock that plasma levels of thromboxane are raised by up to 10-fold or more.⁶², ⁶³ The early hemodynamic effects, which include hypotension, pulmonary vasoconstriction, and splanchnic infarction appear to be due to these high levels of TxA₂.⁶² However, there is some doubt about the importance of TxA₂ in the later stages of shock, which are characterized by progressive reductions in blood pressure and cardiac output associated with cellular damage, which leads to death.⁶⁴ Certainly studies with prostaglandin cyclooxygenase inhibitors should be in-
terpreted with caution since prostacyclin may have an important protective role in such conditions. Nevertheless, indomethacin has been shown to increase survival rates in dogs and rats. Inhibitors of thromboxane synthetase have also been shown to improve survival rates in rats in endotoxic shock and this seemed not to be dependent on the shunting of arachidonic acid metabolism to prostacyclin. Clinically, thromboxane levels have been shown to be very high in severe cases of septic shock and the condition is characterized by pulmonary complications, hypotension, thrombocytopenia, and hypoxia. Evidence has recently been provided that AH23848 protects against the early cardiopulmonary effects of endotoxic in cats. It may be that a thromboxane receptor–blocking drug like AH23848 would be of clinical value in this potentially fatal condition.

In conclusion, we have provided arguments to support the view that the controversy about the etiologic role of TxA2 in a variety of cardiovascular diseases can only be definitively resolved by a thorough clinical evaluation of a potent thromboxane receptor–blocking drug such as AH23848. Such studies may also reveal other, as yet unconsidered, pathophysiologic roles of TxA2.

We gratefully thank Dr. A. Wadsworth (Chemical Research Department, Glaxo Group Research Ltd., Ware) for synthesizing U-46619. We are also very grateful to Pfizer (U.K.), Sandwich, for the supply of UK 34787.

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Circulation. 1985;72:1208-1218
doi: 10.1161/01.CIR.72.6.1208

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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