Increased thromboxane biosynthesis in a human preparation of platelet activation: biochemical and functional consequences of selective inhibition of thromboxane synthase

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ABSTRACT Although thromboxane A\textsubscript{2} is a potent platelet agonist and vasoconstrictor in vitro, our knowledge of its pathophysiologic importance in human disease is limited. To facilitate the elucidation of its role in vivo, we sought to define a human syndrome in which pharmacologic interventions designed to inhibit the biosynthesis or biologic actions of thromboxane A\textsubscript{2} might be appropriately assessed. Patients with severe peripheral vascular disease were selected on the basis of elevated plasma \( \beta \)-thromboglobulin and circulating platelet aggregates and compared with healthy, age-matched control subjects. In addition to the platelet indexes, their bleeding time was shorter and excretion of 2,3-dinor-thromboxane B\textsubscript{2}, a noninvasive index of thromboxane formation in vivo, and 2,3-dinor-6-keto-prostaglandin F\textsubscript{1 \alpha}, the major urinary metabolite of prostacyclin, was markedly increased. A selective inhibitor of thromboxane synthase, imidazo (1,5-2) pyridine-5-hexanoic acid, was administered to these patients under randomized, double-blind, controlled conditions. Platelet aggregation ex vivo, the circulating platelet aggregate ratio, and the bleeding time were all unaltered, despite almost maximal inhibition of platelet thromboxane formation 1 hr after dosing. By contrast, pronounced inhibition of aggregation was observed when platelet cyclooxygenase was inhibited by aspirin. During long-term dosing with the synthase inhibitor, inhibition of thromboxane biosynthesis was incomplete, which would permit continued thromboxane-dependent platelet aggregation to occur. However, the failure of enzyme blockade to influence platelet function at the time of maximal drug action, despite efficient inhibition of serum thromboxane B\textsubscript{2}, suggests that accumulation of proaggregatory endoperoxides is also likely to have contributed to the persistence of platelet activation. We have characterized a human preparation in which platelet activation coexists with increased thromboxane biosynthesis. In this setting, platelet activation persists despite long-term administration of a thromboxane synthase inhibitor in a dosing regimen representative of that employed in clinical trials. Prolongation of drug action and combination with antagonists of the shared endoperoxide/thromboxane A\textsubscript{2} receptor may be necessary to assess the potential of selective inhibition of thromboxane synthase as a therapeutic strategy in man.

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IN VIEW OF the potential importance of the proaggregatory platelet eicosanoid thromboxane A\textsubscript{2} in the mediation of vascular occlusive events in vivo, there has been considerable interest in the effects of selective thromboxane synthase inhibitors in man.\textsuperscript{1} Despite apparent efficacy in animal preparations of acute thrombosis,\textsuperscript{2,4} endotoxic shock,\textsuperscript{5,6} ventricular fibrillation,\textsuperscript{7,8} and in an acute preparation of immune complex glomerulonephritis,\textsuperscript{9} clinical trials of these compounds have failed to demonstrate significant benefit.\textsuperscript{10-14}

Several factors may have contributed to this discrepancy. First, in the clinical conditions that have been studied, the pathogenic importance of thromboxane A\textsubscript{2} has not been clearly defined. In addition, while the animal studies reflect the short-term effects of thromboxane synthase inhibitors (those over a period during which maximal inhibition of thromboxane formation
can be maintained), the clinical investigations involved long-term drug administration. Thromboxane synthase inhibitors, unlike aspirin, are reversible enzyme inhibitors. Moreover, the brevity of their plasma drug half-lives suggests that recovery intervals commonly employed in clinical trials. Finally, in the presence of thromboxane synthase inhibitors, unlike aspirin, thromboxane biosynthesis may have occurred during the dosing intervals.

Methods

Clinical methods

Patient selection. All patients had severe, angiographically confirmed, obstructive arterial disease of the lower limb with pain at rest and/or ischemic ulcers (grades III or IV) and met preset criteria consistent with the presence of platelet activation in vivo: plasma β-thromboglobulin of greater than 20 ng/ml and a circulating platelet aggregate (CPA) ratio of greater than 1.3. Concomitant measurement of plasma platelet factor 4 allowed us to minimize artifacts caused by spurious platelet activation ex vivo by excluding samples with a platelet factor 4 of greater than 25 ng/ml. Five of the nine patients studied were insulin-dependent diabetics, seven had electrocardiographic evidence of chronic myocardial ischemia, although only one was taking antianginal medication (occasional sublingual nitroglycerine), and seven of nine were chronic cigarette smokers. Four had ischemic foot ulcers at the commencement of the study and one further patient developed an ulcer during the course of the study.

Study design. Nine patients (eight men and one woman) who were 49 to 75 years old participated in the study, which was performed in the Elliott V. Newmann Clinical Research Center of Vanderbilt University and which was approved by the Committee for the Protection of Human Subjects of Vanderbilt University.

THERAPY AND PREVENTION—PLATELET AGGREGATION

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Platelet aggregation was measured in response to increasing doses of arachidonic acid (0.33 to 1.33 mM; Sigma Chemical Co., St. Louis), epinephrine (1 to 10 μM; Sigma Chemical Co.), and collagen (9.5 to 95 μg/ml; Biodata Corporation, Horsham, PA). Platelet aggregation was quantitated with the lag time to 50% of maximal alteration in light transmission, expressed as the LT₅₀, and as the percentage of maximum light transmission achieved 6 min after addition of the agonist.

**Bleeding time.** The bleeding time was measured on the forearm in a blinded fashion by the template method (Simplate, General Diagnostics, Morris Plains, NJ). All determinations were performed by the same investigator.

**PGI-M.** Urinary excretion of PGI-M was measured with a stable isotope dilution assay with the use of gas chromatography/mass spectrometry modified from the original method as previously described. Briefly, 5 ng of a deuterated internal standard was added to 5 ml of urine, which was then subjected to extraction and backextraction under alkaline and acidic conditions. The sample was derivatized as the methoxime, pentafluorobenzyl ester, and purified by thin-layer chromatography and the derivatization was completed by formation of the trimethylsilyl ether derivative. Quantitation was accomplished with a Hewlett-Packard 5980 instrument operating in the negative ion mode, monitoring m/z 586.5 for endogenous PGI-M and m/z 590.5 for the deuterated internal standard.

**Tx-M.** Urinary excretion of Tx-M was measured by a newly developed stable isotope dilution assay in which gas chromatography/mass spectrometry is used, as described in detail elsewhere. Briefly, methoxymine hydrochloride (0.5 ng in 1.5 ml 6M acetate buffer) was added to a 5 ml aliquot of urine previously spiked with 12.5 ng of a deuterated internal standard. The urine was then applied to a phenylboronic acid column (Analytichem International, Harbour City, CA), rinsed with 0.05M HCl followed by methanol, and eluted from the column with 4 ml methanol/0.1N NaOH. The sample was then diluted to 15 ml with distilled water, acidified to pH 3.5 with 10% formic acid, and applied to a reverse-phase Sep-Pak (Waters Associates, Milford, MA). After washing with 5 ml distilled water followed by 5 ml methanol/water (1:3 by volume), the sample was eluted with ethyl acetate and evaporated to dryness under nitrogen. Further purification was carried out by thin-layer chromatography in a solvent system of ethyl acetate/acetic acid/hexane (54:12:25 vol/vol/vol). The sample was extracted into ethyl acetate and further derivatized as the pentafluorobenzyl ester by addition of 12.5% pentafluorobenzyl bromide and N,N-disopropylthiophosphonylamine and heating to 40°C for 30 min. This reaction mixture was subjected to thin-layer chromatography with a solvent system of ethyl acetate/isooctane (85:15 vol/vol) and extracted as above and final derivatization was completed by formation of the trimethylsilyl ether by treatment with bis(trimethylsilyl)tributylfluoracetamide and pyridine for a minimum of 15 min at 20°C. Quantitation was established with a Nermag 10-10C instrument operating in the negative ion mode monitoring m/z 586.5 for endogenous Tx-M and m/z 590.5 for the tetradeuterated internal standard.

**Thromboxane B₂.** Urinary excretion of thromboxane B₂ was measured by a stable isotope dilution assay reported in detail elsewhere. A 5 ml aliquot of urine was spiked with 12.5 ng of a deuterated internal standard of thromboxane B₂. Purification and derivatization of the sample was carried out as described above for Tx-M with the exception that, since the relative migration rates for thromboxane B₂ and Tx-M (approximately 0.33 and 0.21, respectively) are different, the appropriate areas of the plates were scraped at each step of the thin-layer chromatography procedure. Quantitation was established with the Nermag 10-10C instrument operating in the negative ion mode.
Although Tx-M excretion remained depressed during long-term dosing, significant biosynthesis of thromboxane persisted and no further decrement in Tx-M occurred with administration of the final dose. There was no significant difference between the 100 and 200 mg dose with respect to the effects observed on Tx-M excretion after short- and long-term dosing. In the four patients in whom we measured the urinary excretion of both thromboxane B₂ and Tx-M, a similar degree of depression of both metabolites was noted after administration of 200 mg CGS 13080 (table 1). In addition, basal levels of urinary thromboxane B₂ were markedly elevated compared with those observed in healthy subjects (247 ± 86 vs 46 ± 14 pg/mg creatinine).

Administration of placebo did not significantly alter serum thromboxane B₂ levels from predosing values. After short-term administration of CGS 13080, serum thromboxane B₂ generation ex vivo was reversibly inhibited, with peak inhibition measured 1 hr after dosing (figure 3). Recovery of serum thromboxane was linear and gave a calculated “biological” half-life (50% recovery to predosing values) of between 6 and 8 hr. The level of serum thromboxane B₂ immediately before the last dose (140 ± 54 ng/ml), which was measured 8 hr after the previous dose, was not significantly different from that measured 8 hr after the first dose (115 ± 38 ng/ml). Thus, no cumulative inhibition of serum thromboxane B₂ occurred during long-term dosing. As observed with Tx-M excretion, there was no difference in the effects of the two doses of CGS 13080 on serum thromboxane B₂ formation, suggesting their close proximity on the dose-response curve.

**TABLE 1**

Urinary excretion of Tx-M and thromboxane B₂ (TxB₂) in four atherosclerotic patients after long-term administration of 200 mg CGS 13080

<table>
<thead>
<tr>
<th>Time</th>
<th>Tx-M</th>
<th>TxB₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before last dose</td>
<td>84.7 ± 5</td>
<td>89.4 ± 1</td>
</tr>
<tr>
<td>After dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 hr</td>
<td>78 ± 6</td>
<td>88.2 ± 7</td>
</tr>
<tr>
<td>6–12 hr</td>
<td>71.6 ± 7</td>
<td>72.7 ± 4</td>
</tr>
<tr>
<td>12–24 hr</td>
<td>52.1 ± 12</td>
<td>52.3 ± 7</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of determinations in four patients. Depression of Tx-M and TxB₂ excretion was not significantly different by analysis of variance (p > .10).
Effect of drug on platelet function. Plasma β-thromboglobulin and platelet factor 4 were unchanged by placebo. There was no significant depression of β-thromboglobulin after short-term administration of either dose of CGS 13080 (figure 4). During long-term dosing, however, β-thromboglobulin levels fell significantly, although they remained more than three times normal: 61 ± 21 ng/ml (200 mg) and 45 ± 15 ng/ml (100 mg) vs 13 ± 3 ng/ml (age-matched control subjects). Platelet factor 4 levels were within the normal range and were unaltered by CGS 13080 (figure 4).

The CPA ratio and bleeding time (figure 5) were not significantly different before and after administration of CGS 13080 or placebo. Platelet aggregation in platelet-rich plasma in response to a wide range of doses of arachidonic acid, epinephrine, and collagen was also unchanged by CGS 13080 or placebo, whether assessed as the LT50 or as the maximum extent of increase in light transmission at 5 min after addition of the agonist (figure 5). By contrast, administration of aspirin significantly inhibited platelet function in these patients (table 2).

Effect of drug on prostacyclin formation. There was no change in PGI-M excretion with placebo. Coincident with the fall in Tx-M after 200 mg of CGS 13080, PGI-M excretion increased significantly (p < .05) from 291 ± 54 to 538 ± 150 and 442 ± 150 pg/mg creatinine in the 0 to 6 and 6 to 12 hr aliquots. Similar increments in PGI-M were obtained after the 100 mg dose. PGI-M remained significantly elevated during long-term dosing but no further increase occurred after the final dose of CGS 13080 (figure 2).

**TABLE 2**

<table>
<thead>
<tr>
<th>Platelet aggregation (%) after:</th>
<th>Before aspirin</th>
<th>After aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet agonist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA 0.3 mM</td>
<td>87 ± 0.9</td>
<td>No agg.</td>
</tr>
<tr>
<td>0.66 mM</td>
<td>87 ± 20</td>
<td>No agg.</td>
</tr>
<tr>
<td>1.33 mM</td>
<td>87 ± 1.5</td>
<td>No agg.</td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.5 µg/ml</td>
<td>61 ± 20</td>
<td>No agg.</td>
</tr>
<tr>
<td>95 µg/ml</td>
<td>92 ± 2</td>
<td>85 ± 4</td>
</tr>
<tr>
<td>Epinephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 µM</td>
<td>89 ± 0.8</td>
<td>No agg.</td>
</tr>
<tr>
<td>10 µM</td>
<td>88 ± 2.5</td>
<td>No agg.</td>
</tr>
<tr>
<td>Serum TxM2 (ng/ml)</td>
<td>332 ± 62</td>
<td>13 ± 7</td>
</tr>
<tr>
<td>Bleeding time (sec)</td>
<td>218 ± 39</td>
<td>308 ± 50</td>
</tr>
</tbody>
</table>

Aggregation was inhibited in all individuals at all doses of agonist except high-dose collagen. Serum TxB2 was depressed and bleeding time prolonged in all patients after aspirin.

AA = arachidonic acid; No agg. = no aggregation response 6 min after addition of agonist.
Plasma drug levels. Plasma concentrations of CGS 13080 are shown in table 3. These confirmed bioavailability of the compound and the relationship of plasma concentration to dose within each of the volunteers. Significant concentrations of CGS 13080 were not detectable in plasma 8 hr after short-term dosing and in eight of nine patients there was no evidence of accumulation during administration of multiple doses. One patient did, however, exhibit marked accumulation of the drug with long-term dosing, particularly at the higher dose, and this correlated with a greater degree of inhibition of serum thromboxane B\textsubscript{2}, Tx-M excretion, and plasma β-thromboglobulin (figure 6) than observed in the other patients.

Clinical evaluation. There was no significant alteration in the Doppler echocardiographic measurements of absolute ankle systolic pressure, the ankle/brachial systolic pressure index, or arterial waveform. In addition, supine systolic and diastolic blood pressure and heart rate were unaffected by short- or long-term administration of CGS 13080 or placebo. The scores measured on the visual analog scale used to assess pain at rest were not significantly different with placebo or either dose of CGS 13080. There were no changes in the complete blood counts or results of routine automated biochemistry screens.

Discussion

To explore the discrepancy between the apparent efficacy of thromboxane synthase inhibitors in diverse animal preparations of acute thrombosis and the disappointing results of their use in clinical trials, we defined a novel human preparation in which evidence of
Inhibition

TABLE 3
Plasma concentrations of CGS 13080 (ng/ml)

<table>
<thead>
<tr>
<th>Time after dosing (hr)</th>
<th>After 100 mg dose</th>
<th>After 200 mg dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 8</td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>53 ± 26</td>
</tr>
<tr>
<td>1</td>
<td>2333 ± 379</td>
<td>1704 ± 397</td>
</tr>
<tr>
<td>4</td>
<td>149 ± 36</td>
<td>128 ± 30</td>
</tr>
<tr>
<td>6</td>
<td>31 ± 8</td>
<td>49 ± 19</td>
</tr>
<tr>
<td>8</td>
<td>ND</td>
<td>21 ± 9</td>
</tr>
<tr>
<td>24</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of determinations in nine patients. ND = None detected (limit of sensitivity 10 ng/ml).

platelet activation in vivo coexists with enhanced thromboxane biosynthesis. We then addressed the hypothesis that inhibition of thromboxane biosynthesis and platelet function is incomplete during long-term administration of a selective inhibitor of thromboxane synthase.

The patients with severe atherosclerosis fulfilled predetermined criteria for the presence of platelet activation in vivo based on three commonly used indexes: plasma levels of the platelet-specific proteins β-thromboblobulin and platelet factor 4 and estimation of the CPA ratio. Plasma β-thromboblobulin and the platelet aggregate ratio were both elevated in the atherosclerotic patients. While the possibility of platelet activation ex vivo cannot be excluded, the absence of a coexistent increase of platelet factor 4 is highly suggestive of the presence of platelet activation in vivo.17 The limitations of these invasive methods27, 28 led us to employ an additional approach to the study of platelet activation in vivo: measurement of urinary Tx-M, a noninvasive index of thromboxane A2 formation in vivo. While under physiologic conditions the rate of secretion of thromboxane has been estimated to be very low — on the order of 0.1 ng/kg/min29 — this is likely to reflect the relative absence of provocative stimuli to its production. In the setting of severe atherosclerosis with evidence consistent with platelet activation in vivo, endogenous thromboxane biosynthesis is increased more than fourfold. Furthermore, we have shown an increased rate of in vivo prostacyclin production in these patients consistent with the hypothesis, previously proposed,30 that this is a consequence of enhanced interactions between activated platelets and the vessel wall, particularly at sites of vascular injury.

Short-term administration of CGS 13080, a selective inhibitor of thromboxane synthase31 resulted in greater than 95% inhibition of serum thromboxane B2 formation ex vivo 1 hr after dosing, with a biological half-life of 6 to 8 hr, which is comparable to that achieved with other thromboxane synthase inhibitors21, 32 and with this compound in healthy volunteers.33, 34 During long-term dosing, however, trough levels of serum thromboxane B2 remained at approximately 60% of predosing values. Furthermore, Tx-M excretion fell to a mean of 34% after a single dose, and, during long-term dosing, thromboxane biosynthesis persisted at a mean 25% of predosing levels. Although platelets are the predominant source of urinary Tx-M, residual metabolite formation may represent formation by minor, nonplatelet sources in addition to recovery of platelet thromboxane formation during the urine collection period.

The failure to induce complete, cumulative inhibition of thromboxane generation during long-term drug administration is important for several reasons. First, the dosing interval in this investigation, as in previous clinical studies with this class of compounds,10, 12, 13 was based, not on the plasma drug half-life of approximately 45 min,34 but on the much longer biological

FIGURE 6. Relationship between plasma concentrations of CGS 13080 and the effects on indexes of thromboxane biosynthesis and platelet function. The results are expressed as percentage inhibition from predosing control values and represent the data obtained immediately before administration of the final dose of 200 mg CGS 13080. There was a greater degree of inhibition of serum thromboxane B2, Tx-M, and plasma β-thromboblobulin in the single patient in whom substantial drug accumulation occurred (shaded bars) compared with that in the other patients (open bars, n = 8).
half-life. While cumulation to a steady-state effect would be expected in three to four half-lives when the dosing interval is based on the plasma drug half-life, this assumption cannot be made for the biological half-life. Indeed, 6 to 8 hr after dosing, plasma levels of CGS 13080 were barely detectable and it is thus not surprising that cumulative inhibition of thromboxane formation did not occur during this study. While such a discrepancy (between the biological and pharmacological half-lives) might be anticipated on the basis of established plasma drug kinetic-dynamic relationships,\textsuperscript{35} other factors, such as the presence of active metabolites or slow elimination of the drug from deep compartments, may have contributed to this observation. However, the same discrepancy has been reported with several structurally distinct synthase inhibitors.\textsuperscript{21, 32, 33} In no case have biologically active metabolites of these compounds been identified. This suggests that incomplete inhibition of thromboxane biosynthesis during the dosing intervals is likely to have occurred in clinical trials of synthase inhibitors other than CGS 13080.

In addition, using a variety of methods to characterize platelet function \textit{ex vivo} and \textit{in vivo}, we have shown that platelet activation persists in this population during long-term administration of a thromboxane synthase inhibitor. There was no change in the bleeding time, in platelet aggregation in response to three different agonists, or in the CPA ratio and, although \( \beta \)-thromboglobulin fell, it still remained more than three times normal. This is in contrast to our studies in healthy volunteers in whom CGS 13080 significantly prolonged the bleeding time and inhibited platelet aggregation \textit{ex vivo}.\textsuperscript{33} The reason for this discrepancy was not directly addressed in the present study. However, the relationship between inhibition of the capacity of platelets to form thromboxane \( \text{B}_2 \) and thromboxane-dependent platelet activation is nonlinear.\textsuperscript{36–38} There is also a marked discrepancy between the capacity to form eicosanoids and actual production rates in vivo. Thus, although capacity was inhibited by approximately 95% 1 hr after dosing in both groups, this would still theoretically permit a substantial augmentation of thromboxane synthesis \textit{in vivo} in the presence of stimuli to its production.\textsuperscript{29} In the patients in whom biosynthesis of the thromboxane \( \text{A}_2 \) was increased, this may have been sufficient to sustain thromboxane-dependent platelet function. The absence of any substantial changes in the measured indexes of platelet function corresponded to the lack of clinical benefit in these patients.

It is also possible that the lack of platelet-inhibitory effects cannot be completely accounted for by inadequate suppression of thromboxane synthesis alone. For example, accumulation of proaggregatory endoperoxides may also be a contributory factor in the observed continuing platelet activation during selective inhibition of thromboxane synthesis. Inhibition of formation of both thromboxane \( \text{A}_2 \) and prostaglandin endoperoxides by aspirin prolonged the bleeding time and inhibited aggregation in these patients. At present, conclusive evidence for the importance of endoperoxides during thromboxane synthase inhibition in man is lacking. However, in a canine preparation, the combination of a prostaglandin endoperoxide/thromboxane receptor antagonist with a synthase inhibitor has been shown to delay the time to coronary artery occlusion despite maximal inhibition of thromboxane synthesis by the synthase inhibitor alone,\textsuperscript{39} and preliminary evidence for the efficacy of this combination has also been obtained with human platelets \textit{in vitro}.\textsuperscript{40}

An alternative explanation is that despite the increase in biosynthesis of thromboxane \( \text{A}_2 \), platelet activation in these patients was largely reflective of non-eicosanoid-dependent mechanisms. Nevertheless, that incomplete suppression of thromboxane formation throughout the long-term dosing period is likely to have been the major factor in the continuing platelet activation that we observed is supported by data from the single patient in this study in whom significant drug accumulation occurred. In this patient, cumulative inhibition of thromboxane formation \textit{in vivo} and \textit{ex vivo} was virtually complete, coincident with a significantly greater fall in \( \beta \)-thromboglobulin.

It has previously been shown, in a rat glomerular preparation \textit{in vitro}, that the thromboxane synthase inhibitor dazoxiben has a higher concentration for 50% inhibition of glomerular thromboxane synthase than the enzyme in platelets, suggesting that there may be differential tissue sensitivity of thromboxane synthase to selective inhibitors.\textsuperscript{41} It has been proposed that urinary thromboxane \( \text{B}_2 \) primarily reflects renal formation under physiologic conditions.\textsuperscript{42} By contrast, dinor-thromboxane \( \text{B}_2 \) is the predominant urinary metabolite of systemically administered thromboxane \( \text{B}_2 \) in man.\textsuperscript{43} It is therefore interesting that the pattern of inhibition and recovery was similar for both metabolites. While this may be interpreted as evidence against the hypothesis of a differential effect on renal and platelet thromboxane synthase, it may also reflect species differences in the sensitivity of the enzyme to pharmacologic inhibition. The most likely explanation is that while urinary thromboxane \( \text{B}_2 \) is mainly of renal origin in healthy human subjects, in the setting of
platelet activation in vivo with increased platelet thromboxane formation, urinary thromboxane B2 may predominantly derive from platelets. Under such circumstances, a similar degree of inhibition of urinary thromboxane B2 and Tx-M might indeed have been expected. Further evidence in support of this hypothesis is provided by the observation that the basal levels of urinary thromboxane B2 as well as of dinor-thromboxane B2 were elevated compared with those measured in healthy subjects.

Endogenous prostacyclin biosynthesis increased approximately twofold in response to a single dose of the thromboxane synthase inhibitor, which is consistent with endoperoxide diversion to a source of prostacyclin synthase.44 While the percentage increment in prostacyclin biosynthesis is similar to that observed with the same drug in healthy volunteers,33 the absolute increment in metabolite excretion was at least twice as great in the patients. This raised the possibility that the transfer of prostaglandin endoperoxides may be enhanced in the presence of platelet activation in vivo. Despite elevated basal secretion of prostacyclin in these patients, the magnitude of the increase in prostacyclin after administration of the synthase inhibitor does not represent the maximum capacity of vascular tissue to generate prostacyclin45-47 and would not result in circulating levels sufficient to exert systemic effects.48 Nevertheless, endogenous prostacyclin may contribute to the efficacy of thromboxane synthase inhibitors by its actions at a local level. The initial significant increment in prostacyclin was not maintained during long-term dosing and there was no further increment after the final dose. These changes mirrored the fall in thromboxane formation and indicate that alterations in prostacyclin biosynthesis are dependent on increased provision of endoperoxide substrate after inhibition of thromboxane synthase.

In conclusion, we have characterized a human preparation of platelet activation in vivo. We have demonstrated enhanced biosynthesis of thromboxane in patients with severe atherosclerosis coincident with evidence of platelet activation in vivo as assessed by more conventional indexes. The use of pharmacologic probes in such a preparation is likely to elucidate the pathophysiologic role of thromboxane A2 in man. In the present study we have demonstrated that administration of a thromboxane synthase inhibitor on a dosage schedule representative of those used in clinical trials causes reversible inhibition of thromboxane formation in such patients. Cumulative, complete inhibition of thromboxane biosynthesis does not occur, however, and platelet activation persists during long-term drug administration. This may reflect incomplete suppression of thromboxane production during the dosing interval. Furthermore, the lack of effect on measured indexes of platelet function 1 hr after dosing, despite substantial inhibition of platelet thromboxane formation, and the response of these indexes to aspirin suggest that substitution for the proaggregatory effects of thromboxane A2 by accumulated prostaglandin endoperoxides may also play a role during inhibition of thromboxane synthase in vivo.

This study was designed to investigate the functional and biochemical effects of administration of a representative thromboxane synthase inhibitor in a manner typical of that used in clinical trials of these compounds. Although persistent platelet activation in atherosclerotic patients may indicate the importance of nonthromboxane-mediated platelet stimulation, incomplete suppression of thromboxane biosynthesis with or without the proaggregatory effects of prostaglandin endoperoxides would in itself be sufficient to account for the lack of clinical efficacy of thromboxane synthase inhibitors. Thus, realization of the therapeutic potential of this class of compounds is likely to depend on prolongation of drug action and combination with antagonists of the prostaglandin endoperoxide/thromboxane receptor.

References


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