Inhibition of Thromboxane Formation In Vivo and Ex Vivo: Implications for Therapy With Platelet Inhibitory Drugs

By Irene A.G. Reilly and Garret A. FitzGerald

The capacity of platelets to generate thromboxane A₂, reflected by measurement of serum thromboxane B₂ (TxB₂), greatly exceeds the systemic production of thromboxane in vivo. Thus, it is possible that substantial but incomplete inhibition of thromboxane formation ex vivo would still allow marked augmentation of thromboxane production in vivo. To address this hypothesis, we administered aspirin 120 mg, a selective inhibitor of thromboxane synthase (TxSI), 3-(1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid (UK-38,485) 200 mg, and a combination of both drugs to 12 healthy volunteers and measured the effects on serum TxB₂ and urinary 2,3-dinor-thromboxane B₂ (Tx-M), an index of endogenous thromboxane biosynthesis. Although serum TxB₂ was maximally inhibited by 94 ± 1% after aspirin and 96 ± 2% after the TxSI, maximal depression of Tx-M was only 28 ± 8% and 37 ± 9%, respectively. Combination of aspirin with the TxSI resulted in a small but significant increase in inhibition of thromboxane generation ex vivo (98 ± 1%) v 94 ± 1%; P < 0.05), but a disproportionately greater fall in thromboxane synthesis in vivo (58 ± 7%; P < 0.01). Consistent with further inhibition of platelet thromboxane synthesis, addition of the TxSI abolished the transient decline in prostacyclin formation after aspirin alone. Administration of a lower dose of aspirin (20 mg) to 6 healthy subjects caused a small reduction in Tx-M (12 ± 4%; P < 0.05) and inhibited serum TxB₂ by 48 ± 2%. The relationship between inhibition of platelet capacity to form thromboxane ex vivo (serum TxB₂) and synthesis in vivo (Tx-M) departed markedly from the line of identity. When total blockade of the capacity of platelets to generate thromboxane is approached, minor decrements in capacity result in a disproportionate depression of actual thromboxane biosynthesis. These results imply that pharmacologic inhibition of serum TxB₂ must be virtually complete before thromboxane-dependent platelet activation is influenced in vivo.

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MATERIALS AND METHODS

Study Design

Twelve healthy male volunteers (aged 21 to 46 years, weight 80.5 ± 5 kg) participated in the study which was approved by the Committee for the Protection of Human Subjects of Vanderbilt University. The main study, which was double blind, randomized, and placebo controlled, was performed on four study days, each separated by a washout period of ten days. On each study day, the volunteers received as a single dose of one of the following: (a) aspirin 120 mg; (b) a thromboxane synthase inhibitor, 3-(1H-imidazole-1-yl-methyl)-2-methyl-1H-indole-propanoic acid (UK-38,485) 200 mg; (c) aspirin 120 mg plus UK-38,485 200 mg, or (d) placebo, each administered as 5 identical unlabelled white capsules.
(kindly provided by Dr P. Urquilla of Pfizer Inc., Groton, Conn). All the subjects had fasted for a minimum of 10 hours prior to dosing and for four hours after dosing and none had taken aspirin or aspirin-like drugs in the 2-week period prior to or during the study.

Blood was drawn before and 1, 4, 6, 8, and 24 hours after dosing by fresh venipuncture from an antecubital vein for plasma drug levels and serum thromboxane determinations. Urine was collected for measurement of prostacyclin and thromboxane metabolites for the 24 hours before each study day and in 3 postdosing aliquots: 0 to 6, 6 to 12, and 12 to 24 hours. Physical examination, laboratory evaluation, and an EKG were carried out prior to inclusion in the study. Safety tests (full blood count, routine automated biochemistry screen, and urinalysis) were performed before dosing and on completion of each study day. Blood pressure, measured by mercury sphygmomanometer in the sitting position, and heart rate were monitored at each blood sampling time point prior to venipuncture.

On a separate occasion, aspirin was administered to 6 volunteers such as to result in partial inhibition of platelet thromboxane formation ex vivo. Four 5 mg aspirin capsules (prepared by the Vanderbilt Pharmacy) were administered two hours apart. Blood was drawn for estimation of serum thromboxane B2 formation before dosing and eight hours after the first dosage. Urine was collected for measurement of prostacyclin and thromboxane metabolites for the 24 hours before each study day and in 3 postdosing aliquots: 0 to 6, 6 to 12, and 12 to 24 hours. Physical examination, laboratory evaluation, and an EKG were carried out prior to inclusion in the study. Safety tests (full blood count, routine automated biochemistry screen, and urinalysis) were performed before dosing and on completion of each study day. Blood pressure, measured by mercury sphygmomanometer in the sitting position, and heart rate were monitored at each blood sampling time point prior to venipuncture.

Finally, blood and urine samples were obtained for measurement of serum thromboxane B2 and urinary 2,3-dinor-thromboxane B2, respectively, from 28 additional apparently healthy volunteers (14 males, 14 females; age 24 to 38 years; wt. 45 to 96 kg) and 4 patients attending the hematology outpatient clinic, two with secondary thrombocytosis, one with hypersplenism, and one with idiopathic thrombocytopenic purpura.

## Biochemical Analyses

### 2,3-dinor-thromboxane B2 (Tx-M)

Tx-M was measured employing a stable isotope dilution assay described in detail elsewhere. Briefly, 12.5 ng of a deuterated internal standard was added to a 5 mL aliquot of urine that was then converted to the methoxime derivative by the addition of methoxyamine hydrochloride in acetate buffer. The sample was then applied to a phenylboronic acid column, washed with acid salt followed by methanol, and eluted with 0.1 N sodium hydroxide in methanol. The eluate was acidified, applied to a silica Sep-Pak, washed, and eluted with ethyl acetate. The sample was purified by thin layer chromatography, derivatized to the methoxime, pentfluorobenzyl ester, and further purified by repeat thin-layer chromatography. Derivatization was completed by formation of the trimethylsilyl ether and quantititation accomplished on the Nermag Rb-bC operating in the negative ion mode, monitoring m/z 586 for endogenous Tx-M and 590 for the deuterated internal standard.

### 2,3-dinor-6-keto-PGF1α (PGI-M)

PGI-M was also measured by stable isotope dilution assay employing negative ion-chemical ionization gas chromatography-mass spectrometry as described elsewhere. Briefly, a 5 mL aliquot of urine was first spiked with 5 ng of a deuterated internal standard. The sample was then subjected to extraction and back extraction under alkaline and acidic conditions, and derivatized as the methoxime pentfluorobenzyl ester. Further purification was carried out by thin-layer chromatography and the derivatization completed by formation of the trimethylsilyl ether derivative. Quantification was accomplished by stable isotope dilution using a Hewlett Packard (Avondale, PA) 5980 instrument operating in the negative ion mode, monitoring m/z 586 for endogenous PGI-M and m/z 590 for the deuterated internal standard.

### Serum thromboxane B2

Serum thromboxane B2 generation ex vivo was measured as previously described. Venous blood was collected into a warmed glass tube, incubated at 37 °C for one hour, and then spun at 2,000 g for 15 minutes. Serum was separated and stored at −20 °C for later analysis by radioimmunoassay.

## Plasma Drug Levels

Plasma concentrations of UK-38,485 were analyzed by high-performance liquid chromatography (HPLC) using UK-37,418 as an internal standard. The drug was isolated from plasma by precipitation with acetonitrile, centrifuged at 2,000 g for five minutes, and evaporated to dryness under nitrogen. After being resuspended in tetramethylthelylenediame (TEMED)/citrate buffer, aliquots were injected onto a Spherisorb Phenyl column (Hichrom, Reading, UK) with a mobile phase of 75% methanol: 25% TEMED/citrate buffer 0.1 mol/L. UK-38,485 and the internal standard were detected by fluorescence at an excitation wavelength of 220 nm and emission of 370 nm. These analyses were kindly undertaken by Dr P. Gibson (Chapel Laboratories, Hynhe, Kent, UK) by arrangement with Dr J.F. Faulkner, Pfizer Central Research, Groton, CT. Plasma concentrations of salicylate were measured by a stable isotope dilution assay employing gas chromatography mass spectrometry.

## Statistical Analysis

Data were analyzed by nonparametric methods thereby avoiding assumptions as to the distribution of the variables involved. Two-way analysis of variance was by the method of Friedman and subsequent pair-wise analysis was by the Wilcoxon Rank Sum Test. Unless otherwise stated, our results are expressed as the mean ± the standard error of the mean (SEM).

## RESULTS

### Platelet Thromboxane Formation

Platelet thromboxane generation ex vivo (measured as serum thromboxane B2) was reduced by 94 ± 1% one hour after dosing with 120 mg aspirin from 349 ± 37 ng/mL to 18 ± 4 ng/mL. Maximal depression of serum thromboxane B2 after the thromboxane synthase inhibitor (200 mg UK-38,485) was not significantly different, falling by 96 ± 2% from 312 ± 33 ng/mL to 12 ± 3 ng/mL one hour after drug administration. While the inhibition induced by aspirin persisted throughout the study period, serum thromboxane B2 had returned to a mean of 70% of predosing values 24 hours after administration of the thromboxane synthase inhibitor (Fig 1). Combination of aspirin with the synthase inhibitor achieved a minor but significant increase in the degree of inhibition ex vivo over that observed with aspirin administration alone: serum thromboxane B2 fell by 98 ± 1% from 380 ± 19 to 8 ± 4 ng/mL one hour after dosing. The difference between the combination and aspirin alone, although small, remained statistically significant (P < 0.05) one, four, and six hours after drug administration (Fig 1). Serum thromboxane B2 formation did not change significantly following placebo and there was no significant difference between baseline values on each of the four study days. Administration of the lower dose of aspirin (4 × 5 mg) resulted in a concomitantly smaller reduction in serum thromboxane B2 by 48 ± 2% from 365 ± 21 to 190 ± 8 ng/mL.

### Thromboxane Synthesis In Vivo

Peak depression of Tx-M excretion following aspirin 120 mg, which was observed in the six- to 12-hour aliquot after
After administration, the inhibitory effect of aspirin 120 mg was significant only eight and 24 hours after dosing (P < 0.01; Table 1). Both of these subjects had higher levels of Tx-M excretion (353 and 363 pg/mg creatinine) during the second (5 mg x 4 aspirin) study in which smoking had not been restricted. However, the maximum degree of inhibition after aspirin administration was similar whether these subjects were included in the analysis or not: 218 ± 36 falling to 187 ± 52 at 12 hours after dosing (12 ± 4%) v 134 ± 21 falling to 112 ± 22 pg/mg creatinine (14 ± 4%).

Tx-M excretion was found to be proportional to platelet count over a wide range of platelet counts in apparently healthy subjects (Fig 2). In addition, in two patients with thrombocytopenia due to peripheral platelet destruction and a shortened platelet life span (one with ITP and the other with hypersplenism), Tx-M excretion was correspondingly elevated at 345 and 1600 pg/mg creatinine, respectively (Fig 2). Metabolite excretion was markedly increased in two patients with secondary thrombocytosis. The pattern of recovery of metabolite excretion after reversible (UK-38,485) and irreversible inhibition of platelet cyclooxygenase (Table 1) was also consistent with urinary 2,3-dinor-TxB2 principally derived from platelets.

### Table 1. Excretion of 2,3-Dinor-Thromboxane B2 (Tx-M) in 12 Healthy Male Subjects After Single-Dose Administration of Aspirin 120 mg, UK-38,485 200 mg, and Aspirin 120 mg in Combination With UK-38,485 200 mg

<table>
<thead>
<tr>
<th>Time After Dosing (hr)</th>
<th>Aspirin 120 mg</th>
<th>UK-38,485 200 mg</th>
<th>UK-38,485 200 mg + Aspirin 120 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>134 ± 21</td>
<td>97 ± 14</td>
<td>95 ± 12</td>
</tr>
<tr>
<td>0-6</td>
<td>108 ± 18*†</td>
<td>85 ± 10</td>
<td>65 ± 9*</td>
</tr>
<tr>
<td>6-12</td>
<td>92 ± 17**†</td>
<td>56 ± 6*</td>
<td>47 ± 11*</td>
</tr>
<tr>
<td>12-24</td>
<td>104 ± 17†</td>
<td>76 ± 9</td>
<td>37 ± 6*</td>
</tr>
</tbody>
</table>

Values are expressed in pg/mg creatinine (mean ± SEM). There were no significant differences between the predosing values.

*P < 0.01 compared to control values.

†P < 0.05 aspirin 120 mg v aspirin 120 mg + UK-38,485 200 mg.

‡P < 0.01 aspirin 120 mg v aspirin 120 mg + UK-38,485 200 mg.
THROMBOXANE FORMATION IN VIVO AND EX VIVO

2.3-Dinor-TxB₂ (Tx-M) pg/mg creatinine

Fig 2. The relationship between platelet count and urinary excretion of 2,3-dinor-TxB₂, in 32 subjects. Metabolite excretion was discordant with the platelet count in two patients with syndromes associated with an increase in platelet turnover (hypersplenism and idiopathic thrombocytopenic purpura: ITP). When these patients were excluded, the 2,3-dinor-TxB₂ excretion significantly correlated (r = .91; P < .001) with platelet count.

Prostacyclin Biosynthesis

Aspirin 120 mg caused a small transient (25 ± 11%) reduction in PGI-M excretion that was significant in the zero- to 6-hour urine aliquot after dosing (P < .01). This effect was not apparent when aspirin was administered in combination with the thromboxane synthase inhibitor (Table 3). PGI-M excretion was also unaltered by the lower dose of aspirin (4 × 5 mg; 120 ± 20 v 128 ± 20 pg/mg creatinine) but was significantly increased (P < 0.05) following administration of the thromboxane synthase inhibitor from 119 ± 18 to 143 ± 19 pg/mg creatinine six to 12 hours after dosing (Table 3). There was no significant difference in PGI-M excretion between either the control days that preceded each dosing period or in the three timed aliquots following placebo administration.

Comparison of Inhibition of Thromboxane Formation Ex Vivo and In Vivo

Mean data relating peak inhibition of thromboxane generation ex vivo to peak inhibition of metabolite excretion are illustrated in Fig 3. Maximum depression of metabolite excretion was measured in the aliquot collected six to 12 hours after dosing with aspirin 120 mg or with UK 38,485, but in the 12- to 24-hour aliquot with the other dosage regimens. The delay in depression of metabolite excretion is likely to reflect a delay both in transformation (as shown thromboxane B₂ infusion) and in renal clearance.

The relationship between the inhibition of platelet capacity to form thromboxane ex vivo and actual biosynthesis departed markedly from the line of identity. As maximal blockade of the capacity to generate thromboxane ex vivo was approached, minor increments in the degree of inhibition achieved resulted in a disproportionately greater depression of thromboxane synthesis in vivo.

Plasma Drug Levels

Plasma drug levels of UK-38,485 are shown in Table 4. There was no significant difference in the drug concentrations measured whether UK-38,485 was administered either as a single dose or together with 120 mg of aspirin, confirming the absence of any pharmacokinetic interaction between

Table 3. Excretion of 2,3-Dinor-6-Keto-PGF₁α (PGI-M) in 12 Healthy Male Subjects After Single-Dose Administration of UK-38,485 (200 mg), Aspirin 120 mg, or UK-38,485 Plus Aspirin 120 mg

<table>
<thead>
<tr>
<th>Time After Dosing (hr)</th>
<th>Aspirin 120 mg</th>
<th>UK-38,485 200 mg</th>
<th>UK-38,485 200 mg + Aspirin 120 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>-24</td>
<td>100 ± 10</td>
<td>119 ± 18</td>
<td>116 ± 20</td>
</tr>
<tr>
<td>0-6</td>
<td>70 ± 11†</td>
<td>122 ± 16</td>
<td>92 ± 14</td>
</tr>
<tr>
<td>6-12</td>
<td>104 ± 22</td>
<td>143 ± 19*</td>
<td>111 ± 23</td>
</tr>
<tr>
<td>12-24</td>
<td>110 ± 16</td>
<td>131 ± 19</td>
<td>108 ± 14</td>
</tr>
</tbody>
</table>

Values are expressed in pg/mg creatinine (mean ± SEM).
*P < 0.05 compared to placebo.
†P < 0.01 compared to placebo.

Table 4. Plasma Concentrations of UK-38,485 (ng/ml)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Time After Dosing (hr)</th>
<th>UK-38,485 200 mg</th>
<th>UK-38,485 200 mg plus aspirin 120 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>UK-38,485 200 mg</td>
<td>3207 ± 333</td>
<td>225 ± 46</td>
<td>66 ± 8</td>
</tr>
<tr>
<td>UK-38,485 200 mg</td>
<td>2992 ± 320</td>
<td>199 ± 22</td>
<td>66 ± 7</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM in 12 healthy male subjects. Limit of sensitivity 10 ng/ml.
these drugs. Peak drug levels were measured one hour after dosing and the decline in plasma levels following administration of UK-38,485 was rapid, less than 3% of the peak plasma drug levels being detectable after six hours. The temporal discrepancy between the offset in plasma drug levels and the inhibition of serum thromboxane B2 generation has previously been observed with thromboxane synthase inhibitors and appears to result from supramaximal concentrations of the parent compound at the time of peak drug levels, rather than the presence of active metabolites.18

DISCUSSION

We have previously established that there is a marked discrepancy between the capacity of platelets to generate thromboxane B2 and the actual production rate of thromboxane in vivo3 based on excretion of its urinary metabolite, 2,3-dinor-thromboxane B2. If platelets are the major source of this metabolite in urine, this observation suggests that activation of platelets ex vivo during blood sampling and processing, rather than endogenous biosynthesis, largely accounts for the plasma levels of thromboxane B2 widely reported in the literature. The calculated physiological secretion rate of thromboxane B2 is extremely low, approximately 0.1 ng/kg/min, which would result in a maximal plasma concentration of 1 to 2 pg/mL.2 Thus, in healthy individuals in whom minimal evidence of platelet activation in vivo would be expected, activation of platelets to less than 0.1% of their capacity would be sufficient to account for these plasma levels.

This concept has implications for therapy with platelet inhibitory drugs in conditions in which platelet activation is of importance. In several human syndromes in which platelet activation in vivo has been demonstrated and is thought to play a primary role, endogenous biosynthesis of thromboxane appears to be markedly enhanced.4,5,19 For example, urinary 2,3-dinor-TxB2 excretion is increased on average 4- to 5-fold during episodes of myocardial ischemia in patients with unstable angina.19 Such increments are of likely pathophysiologic importance in this disease as cardiovascular mortality is significantly reduced by aspirin.20 However, even in the presence of a 95% inhibition of serum thromboxane B2, thromboxane biosynthesis could, in theory, be increased 50-fold above basal levels in response to stimuli to its production in vivo. Thus, pathophysiologically important increments in thromboxane formation might still occur in patients receiving sufficient aspirin to cause substantial but incomplete inhibition of serum thromboxane B2 ex vivo.

To explore this possibility more directly, we designed the present study to examine the relationship between inhibition of platelet capacity to form thromboxane (serum thromboxane B2) and inhibition of thromboxane synthesis in vivo, as reflected by excretion of its urinary metabolite, 2,3-dinor-thromboxane B2. We postulated that as maximal blockade of the capacity to generate thromboxane ex vivo was approached, a disproportionately greater inhibition of actual thromboxane synthesis by platelets in vivo would be expected. On the basis of previously published work,6,21 we selected two doses of aspirin for these studies: the lower dose (5 mg x 4) was chosen to produce a minor but significant reduction in ex vivo thromboxane formation; and the higher dose (120 mg) as one predicted to cause substantial but incomplete inhibition of thromboxane generation ex vivo. In order to inhibit thromboxane formation ex vivo still further, we coadministered with aspirin an inhibitor acting as a site distinct from aspirin in the synthetic cascade rather than administering a higher dose of the cyclooxygenase inhibitor. We hypothesized that if such a combined approach resulted in further inhibition of platelet thromboxane synthesis in vivo, rediversion of platelet endoperoxides, which would accumulate after thromboxane synthase inhibition, might lead to an increase in vascular prostacyclin formation.22,23

We used the excretion of 2,3-dinor-thromboxane B2 as an index of endogenous thromboxane biosynthesis. This compound has been shown to be the major urinary metabolite of systemically administered thromboxane B2 in man.24 Several lines of evidence indicate that urinary 2,3-dinor-thromboxane B2 is largely derived from platelet thromboxane synthase. First, the pattern of inhibition and recovery of dinor thromboxane after administration of pharmacological inhibitors of thromboxane synthesis is consistent with a predominantly platelet origin of this metabolite. Thus, chronic administration of low-dose aspirin (20 mg daily6 and 20 mg twice a day7) has been shown in two studies in healthy volunteers to cause marked depression of 2,3-dinor-thromboxane excretion, by 85% and 70%, respectively. This occurred in the absence of any significant effect on prostacyclin biosynthesis that provides a marker of extraplatelet cyclooxygenase activity. Similarly, urinary thromboxane B2, which, in contrast to the dinor metabolite, is believed to be principally of renal origin under physiologic conditions in humans also remains unaltered following low-dose aspirin administration despite almost maximal inhibition of platelet thromboxane formation ex vivo.25 The importance of these observations is that they address the possibility that the platelet represents a source of cyclooxygenase that is extremely sensitive to inhibition by aspirin but that contributes little to the concentration of 2,3-dinor-thromboxane B2 in urine. Such a phenomenon would explain a discrepancy between the effects of single, low doses of aspirin (eg, 120 ng) on metabolite excretion and serum thromboxane B2, such as that illustrated in Fig 3. However, the marked depression of urinary 2,3-dinor-thromboxane B2 that can be achieved without evidence of inhibition of prostaglandin formation by tissues other than the platelet in vivo during chronic administration of low-dose aspirin, renders unlikely such an explanation of our data. Finally, the recovery of dinor thromboxane excretion after irreversible acetylation of platelet cyclooxygenase by aspirin corresponds to platelet life span,16,17 while recovery is much more rapid after reversible enzyme inhibition induced by thromboxane synthase inhibitors or indomethacin.16 The results reported in this study also show recovery of 2,3-dinor-thromboxane B2 occurring within 24 hours after the synthase inhibitor, but no recovery during the same observation period after aspirin administration.

A second line of evidence consistent with the platelet being the major source of urinary 2,3-dinor-thromboxane B2 derives from the measurement of this metabolite in a large number of healthy volunteers as well as in diverse human
syndromes in which platelet activation in vivo has been documented. Over a wide range of platelet counts we have demonstrated a significant correlation between platelet number and dinor metabolite excretion in this study. Of particular interest is the distinction between reduced metabolite excretion in those subjects with a low platelet count as a result of decreased platelet production, and the marked elevation in 2,3-dinor-thromboxane B₂ in the two patients with thrombocytopenia due to increased peripheral destruction of platelets. Enhanced thromboxane synthesis has also been described in association with evidence of platelet activation in vivo in patients with severe peripheral vascular disease, unstable angina, and systemic sclerosis with Raynaud’s phenomenon. Indeed, that it may be a highly sensitive index of platelet activation in vivo is suggested by the significant elevation is apparently healthy chronic smokers.

Finally, inhibition of platelet activation in vivo, using measures that do not directly inhibit the thromboxane biosynthetic pathway, significantly reduces dinor thromboxane excretion. For example, infusion of prostacyclin to patients with thrombotic thrombocytopenic purpura results in a striking reduction in metabolite excretion. Consistent with the hypothesis, cessation of smoking is followed by a corresponding reduction in dinor thromboxane. In addition, in patients with severe atherosclerosis and platelet activation in vivo, dietary supplementation with eicosapentaenoate markedly depressed excretion of the dinor metabolite.

Thus, if the assumption that the platelet is the predominant (although not only) source of the dinor metabolite in urine is valid, a progressive fall in excretion of this metabolite would be likely to reflect progressive inhibition of platelet thromboxane formation in vivo. The results of the present study would then indicate that inhibition of the capacity of platelets to generate thromboxane ex vivo may prove misleading as a guide to the inhibition of actual thromboxane biosynthesis by platelets and hence, thromboxane-dependent platelet activation in vivo. Inhibition of the final 6% of platelet capacity corresponded to inhibition of the final 72% of thromboxane formation in vivo (after aspirin 120 mg). The functional implications of this observation are illustrated by evidence that even a residual 10% capacity to generate thromboxane appears to be enough to fully sustain thromboxane-dependent platelet activation. Similarly, by increasing the inhibition of platelet thromboxane formation ex vivo from 95% to 99%, an apparently minimal increase, it is possible to achieve further measurable inhibition of platelet function in normal volunteers, as assessed both by platelet aggregation ex vivo and the bleeding time. While these observations are not likely to be of clinical importance in healthy individuals, they may be extremely important when stimuli to cause platelet activation are present. The results of the present study indicate that thromboxane biosynthesis may remain markedly enhanced, despite substantial, but incomplete (eg 90%) inhibition of serum thromboxane B₂.

The changes that we observed in prostacyclin biosynthesis provide further evidence that excretion of 2,3-dinor-thromboxane B₂ in the present study was derived predominantly from platelets. Aspirin 120 mg caused a transient depression in excretion of the prostacyclin metabolite, 2,3-dinor-6-keto-PGF₁α. This is compatible with rapid de novo synthesis of endothelial cyclooxygenase following aspirin exposure, as has previously been shown to occur in cultured endothelial cells. However, it contrasts with the more prolonged depression of prostacyclin metabolite excretion observed following chronic administration of much higher doses of aspirin than used in the present study. Interestingly, such a dose-dependent difference in recovery has also been shown to occur ex vivo in patients who have been receiving aspirin. By contrast, the thromboxane synthase inhibitor resulted in a significant increment in prostacyclin biosynthesis, consistent with utilization of accumulated platelet endoperoxides by vascular prostacyclin synthase. Addition of the thromboxane synthase inhibitor abolished the depression of prostacyclin biosynthesis seen when aspirin was administered alone. It is attractive to speculate that this may reflect reorientation of accumulated endoperoxides resulting from additional inhibition of platelet thromboxane formation in vivo, in this case occurring at the level of thromboxane synthase.

Measurements of the platelet capacity to generate thromboxane B₂ ex vivo are frequently employed as a guide to the adequacy of dosing with platelet-inhibitory drugs. The results of the present study suggest that biosynthesis of thromboxane is maintained to a substantial degree, unless >95% inhibition of thromboxane generation ex vivo is achieved. Lesser degrees of inhibition would not be expected to substantially influence thromboxane-dependent platelet activation in vivo.

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REFERENCES

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