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 tissues to generate eicosanoids in response to physical and chemical stimuli is known to greatly exceed the actual rates of biosynthesis observed in vivo. Formation by human platelets of the potent eicosanoid, thromboxane A₂, measured as its stable metabolite thromboxane B₂, is a good example of this phenomenon. Under physiological conditions, the maximal endogenous concentration of thromboxane B₂ in plasma has been estimated to be in the order of 1 to 2 pg/mL. However, the levels of thromboxane B₂ measured in serum, which reflect the capacity of platelets to form thromboxane in response to thrombin, are over a thousand-fold higher than 300 to 400 ng/mL. Thus, the normal rates of thromboxane synthesis observed in vivo can be accounted for by activation of platelets to less than 0.1% of their capacity for thromboxane generation.

The distinction between the capacity for thromboxane production ex vivo and endogenous thromboxane biosynthesis is an important one. In several human syndromes of platelet activation the capacity of platelets to form thromboxane is unchanged, but thromboxane biosynthesis in vivo is increased. Earlier work from our laboratory has suggested that the relationship between the inhibition of the capacity of platelets to generate thromboxane and the persistence of thromboxane-dependent platelet activation may not be linear. An increase in the degree of inhibition of serum thromboxane formation from a mean of 95% to 99% inhibition, for example, results in a further significant increase in the extent of inhibition of platelet aggregation and a further prolongation of the bleeding time. In addition, others have shown that even a residual 10% capacity to generate thromboxane is enough to fully sustain thromboxane-dependent platelet aggregation. These observations have important implications for the use of antiplatelet drugs and inhibitors of the cyclooxygenase and thromboxane synthase enzymes in human syndromes of platelet activation. Many of the clinical and pharmacological studies of platelet inhibitory therapy in such conditions have employed measurement of serum thromboxane B₂ as a guide to the adequacy of dosing. A discrepancy between inhibition of serum thromboxane B₂ and platelet thromboxane production in vivo raises the possibility that despite substantial, but incomplete inhibition of the capacity of platelets for thromboxane generation, actual thromboxane biosynthesis may remain significantly enhanced.

The aim of the present study was to investigate the relationship between inhibition of serum thromboxane generation ex vivo and endogenous thromboxane biosynthesis in order to explore the hypothesis that incomplete inhibition of the capacity of platelets to generate thromboxane allows significant synthesis to continue in vivo.

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-imidazol-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 comple-
tion 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
eicosanoid analysis for 24 hours prior to dosing and from 0 to 12 and
12 to 24 hours after initiation of dosing.

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
subsequently administering clopidogrel 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
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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 else-
where.13 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
dervative by the addition of methoxamine 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 forma-
tion of the trimethylsilyl ether and quantitation accomplished on the
Nermag R10-10C 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-PGF\textsubscript{1α} (PGI-M). PGI-M was also measured by
stable isotope dilution assay employing negative ion-chemical ioniza-
tion gas chromatography-mass spectrometry as described else-
where.13 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 pentafluorobenzyl ester. Further
purification was carried out by thin-layer chromatography and the
derivatization completed by formation of the trimethylsilyl ether
dervative. Quantitation 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.1 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 precipi-
tation with acetonitrile, centrifuged at 2,000 g for five minutes,
and evaporated to dryness under nitrogen. After being resuspended in
tetramethylmethylenediamine (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, Hythe, 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.13

Statistical Analysis

Data were analyzed by nonparametric methods14 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 significa-
cantly following placebo and there was no significant differ-
ence 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
the cyclooxygenase inhibitor resulted in a disproportionate fall in thromboxane metabolite excretion (58 ± 182 platelets to form thromboxane B2 (Table 2).

Despite the minor increase in inhibition of the capacity of the inhibitor was somewhat greater at 37°C throughout the study period (Table 1).

Inhibition of serum thromboxane was significantly greater after inhibition of serum thromboxane B2 was significant only eight and 24 hours after dosing (120 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 12 to 24 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 545 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-Tx B2 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.

Fig 1. The effect on platelet cyclooxygenase formation (serum thromboxane B2) of single dose administration of placebo, UK-38,485 (200 mg), low-dose aspirin (ASA 120 mg), and a combination of low-dose aspirin with UK-38,485 (200 mg) in 12 healthy male subjects. Serum thromboxane B2 was expressed as a percentage of the predosing control value for each treatment regimen. There was no significant difference in serum thromboxane B2 from control values following placebo administration. Inhibition of serum thromboxane was significantly greater after the aspirin/UK-38,485 combination compared to aspirin alone (P < 0.05; one, four, and six hours after dosing) and compared to UK-38,485 (P < 0.01; four, six, eight, and 24 hours after dosing). The inhibitory effect of aspirin 120 mg v UK-38,485 (200 mg) on serum thromboxane B2 was significant only eight and 24 hours after administration (P < 0.01).

dosing, was only 28 ± 8% from predosing values despite almost maximal inhibition of platelet cyclooxygenase throughout the study period (Table 1). The maximal depression of Tx-M excretion after the thromboxane synthase inhibitor was somewhat greater at 37 ± 9% (P = 0.06). Despite the minor increase in inhibition of the capacity of platelets to form thromboxane ex vivo (94 ± 1% v 98 ± 1%, P < 0.05), addition of the thromboxane synthase inhibitor to the cyclooxygenase inhibitor resulted in a disproportionate fall in thromboxane metabolite excretion (58 ± 7%) compared to that after aspirin alone (P < 0.01). To address the possibility that alterations in Tx-M excretion after aspirin resulted from altered renal tubular handling of the metabolite rather than inhibition of cyclooxygenase we administered an identical (120 mg) dose of sodium salicylate, which is a weak inhibitor of platelet cyclooxygenase compared with aspirin but is subject to the same renal tubular excretory mechanism. In contrast to aspirin 120 mg, sodium salicylate 120 mg did not alter Tx-M excretion or the capacity of platelets to form thromboxane B2 (Table 2).

Tx-M excretion did not differ significantly prior to drug administration on each of the study days (P > 0.2; Table 1). Although there was moderate intersubject variability, the ranges of Tx-M excretion on each of the study days (respectively, 73 to 241, 44 to 167, and 55 to 174 pg/mg creatinine after aspirin 120 mg alone, 200 mg UK-38,485 and the combination of aspirin with UK-38,485) was comparable to that of previous studies by ourselves and others.10,11,16 The intrasubject variability was small, with the exception of the two subjects who smoked, a factor that we have subsequently identified as being associated with increased Tx-M excretion.17 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 12 to 24 hours after dosing (12 ± 4%) v 134 ± 21 falling to 112 ± 22 pg/mg creatinine (14 ± 4%).

Table 2. Urinary Excretion of 2,3-Dinor-Thromboxane B2 (Tx-M), Serum Thromboxane (Tx)B2, and Plasma Concentrations of Salicylate in 3 Healthy Male Subjects After Single-Dose Administration of Salicylate 120 mg

<table>
<thead>
<tr>
<th>Time After Dosing (hr)</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to Dosing</td>
<td>Plasma Salicylate (µg/mL)</td>
</tr>
<tr>
<td>3</td>
<td>Serum Tx B2 (ng/mL)</td>
</tr>
<tr>
<td>6-12</td>
<td>3</td>
</tr>
<tr>
<td>12-24</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. ND, not detectable; assay sensitivity 10 pg/mL.13
THROMBOXANE FORMATION IN VIVO AND EX VIVO

2.3- Dinor-TxB2 (Tx-M) excretion was discordant with the platelet count in these patients were excluded. The 2,3-dinor-TxB2 excretion significantly correlated with platelet count. When syndromes associated with an increase in platelet turnover (hypersplenism and idiopathic thrombocytopenic purpura: ITP). When aspirin was administered in combination with the thromboxane synthase inhibitor (Table 2). There was no significant difference in the drug concentration 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 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 infusion2) 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 2 healthy male subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time After Dosing (hr)</th>
<th>UK-38,485 200 mg</th>
<th>UK-38,485 200 mg + Aspirin 120 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>3207 ± 333</td>
<td>225 ± 46</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>66 ± 8</td>
<td>26 ± 4</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>199 ± 22</td>
<td>66 ± 7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>28 ± 3</td>
<td></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 \( B_2 \) 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.

**DISCUSSION**

We have previously established that there is a marked discrepancy between the capacity of platelets to generate thromboxane \( B_2 \) and the actual production rate of thromboxane \( B_2 \) in vivo\(^2\) based on excretion of its urinary metabolite, 2,3-dinor-thromboxane \( B_2 \). 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 \( B_2 \), widely reported in the literature. The calculated physiological secretion rate of thromboxane \( B_2 \) 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-TxB\(_2\) 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 \( B_2 \), 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 \( B_2 \) 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 \( B_2 \)) and inhibition of thromboxane synthesis in vivo, as reflected by excretion of its urinary metabolite, 2,3-dinor-thromboxane \( B_2 \). 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 \( \times 4 \)) 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, redirection 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 \( B_2 \) as an index of endogenous thromboxane biosynthesis. This compound has been shown to be the major urinary metabolite of systemically administered thromboxane \( B_2 \) in man.\(^24\) Several lines of evidence indicate that urinary 2,3-dinor-thromboxane \( B_2 \) is largely derived from platelet thromboxane synthesis. 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 daily\(^4\) and 20 mg twice a day\(^7\)) 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 \( B_2 \), 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 \( B_2 \) 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 \( B_2 \), such as that illustrated in Fig 3. However, the marked depression of urinary 2,3-dinor-thromboxane \( B_2 \) 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\(^5\) or indomethacin.\(^16\) The results reported in this study also show recovery of 2,3-dinor-thromboxane \( B_2 \) 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 \( B_2 \) 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 B_{2} 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 B_{2}.

The changes that we observed in prostacyclin biosynthesis provide further evidence that excretion of 2,3-dinor-thromboxane B_{2} 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-PGF_{1a}. 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 B_{2} 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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Inhibition of thromboxane formation in vivo and ex vivo: implications for therapy with platelet inhibitory drugs

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