Differential Suppression of Thromboxane Biosynthesis by Indobufen and Aspirin in Patients With Unstable Angina

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Abstract

Background We have previously reported aspirin failure in suppressing enhanced thromboxane (TX) biosynthesis in a subset of episodes of platelet activation during the acute phase of unstable angina. The recent discovery of a second prostaglandin H synthase (PGHS-2), inducible in response to inflammatory or mitogenic stimuli, prompted us to reexamine TXA$_2$ biosynthesis in unstable angina as modified by two cyclooxygenase inhibitors differentially affecting PGHS-2 despite a comparable impact on platelet PGHS-1.

Methods and Results We randomized 20 patients (15 men and 5 women aged 59±10 years) with
unstable angina to short-term treatment with aspirin (320 mg/d) or indobufen (200 mg BID) and collected 6 to 18 consecutive urine samples. Urinary 11-dehydro-TXB\(_2\) was extracted and measured by a previously validated radioimmunoassay as a reflection of in vivo TXA\(_2\) biosynthesis. Metabolite excretion averaged 102 pg/mg creatinine (median value; n=76) in the aspirin group and 55 pg/mg creatinine (median value; n=99) in the indobufen group (\(P<.001\)). There were 16 samples (21%) with 11-dehydro-TXB\(_2\) excretion >200 pg/mg creatinine among patients treated with aspirin versus 6 such samples (6%) among those treated with indobufen (\(P<.001\)). In vitro and ex vivo studies in healthy subjects demonstrated the capacity of indobufen to largely suppress monocyte PGHS-2 activity at therapeutic plasma concentrations. In contrast, aspirin could only inhibit monocyte PGHS-2 transiently at very high concentrations.

**Conclusions** We conclude that in unstable angina, episodes of aspirin-insensitive TXA\(_2\) biosynthesis may reflect extraplatelet sources, possibly expressing the inducible PGHS in response to a local inflammatory milieu, and a selective PGHS-2 inhibitor would be an ideal tool to test the clinical relevance of this novel pathway of arachidonic acid metabolism in this setting.

**Key Words:** thromboxane • indobufen • aspirin • angina

### Introduction

Thromboxane A\(_2\) derives from arachidonic acid, is a potent agonist of platelet aggregation, and has vasoconstrictive properties.\(^1\)\(^2\) Several studies have suggested a lack of platelet activation in chronic stable angina.\(^3\)\(^4\)\(^5\)\(^6\)\(^7\) In contrast, episodic increases in thromboxane A\(_2\) biosynthesis, as reflected by plasma measurements of thromboxane B\(_2\) in the coronary sinus\(^8\) or urinary 11-dehydro-thromboxane B\(_2\) and 2,3-dinor-thromboxane B\(_2\) excretion, have been reported in patients with unstable angina.\(^3\)\(^9\)\(^10\) Enhanced thromboxane biosynthesis in this setting is likely to reflect episodes of platelet activation because it is largely suppressed by low-dose aspirin.\(^10\) However, despite >95% suppression of the cyclooxygenase activity of platelet PGHS-1 by aspirin, as monitored ex vivo, incomplete suppression of thromboxane metabolite excretion has been detected in some patients with unstable angina.\(^10\) Current knowledge indicates that the cyclooxygenase activity of platelet PGHS-1 can be inhibited by at least two distinct mechanisms. Aspirin irreversibly acetylates the serine residue at position 529 (Ser 529) in the polypeptide chain of PGHS-1.\(^11\) By virtue of this unique mechanism, the daily intake of low doses of aspirin selectively suppresses platelet thromboxane A\(_2\) synthesis for the life span of these anucleated cell fragments.\(^12\)\(^13\)\(^14\) The second mechanism is through reversible, competitive interaction between inhibitor and substrate for binding to the catalytic site of PGHS-1. Indobufen, a reversible inhibitor of platelet PGHS-1,\(^15\) has been reported to be at least as effective as a standard regimen
of aspirin plus dipyridamole in preventing early as well as late graft occlusion in patients undergoing artery bypass surgery.16 17

Whereas platelets contain only a constitutively expressed PGHS-1, in most other cells (such as vascular endothelial and smooth muscle cells and monocytes) an inducible isoform called PGHS-2 has been identified (reviewed in Reference 1818). Like other immediate-early genes, PGHS-2 is rapidly induced in response to cytokines, tumor promoters, or growth factors.18 In contrast to the constitutive isoenzyme, PGHS-2 has been shown to have a short half-life (on the order of 3 hours). It has been suggested that the induction of PGHS-2 may represent a mechanism to maintain prolonged states of increased PG production.18 Moreover, PGH₂ produced by the cyclooxygenase activity of PGHS-2 induced in human endothelial cells can restore the capacity of aspirin-treated platelets to generate thromboxane A₂, thus providing an aspirin-insensitive mechanism of thromboxane biosynthesis.19

In the present study, we tested the hypothesis that a component of enhanced thromboxane biosynthesis in unstable angina is dependent on the cyclooxygenase activity of nucleated cells, rapidly recovering after aspirin acetylation by virtue of new enzyme synthesis. Thus, we contrasted the effects of the short-lived aspirin (half-life in the human circulation of 15 to 20 minutes) with those of the long-lived indobufen (half-life of 8 hours) in a randomized, double-blind study of patients with unstable angina. Additional in vitro and ex vivo studies in healthy subjects were performed to assess the extent of PGHS-1 and PGHS-2 inhibition at therapeutic plasma concentrations of indobufen and aspirin.

Methods

Patients
Inclusion criteria for the study were a clinical presentation characteristic of primary unstable angina (class III B by Braunwald's classification)20 and angiographically proven coronary artery disease (see below). Informed consent was obtained from each subject. The study protocol was approved by the Institutional Ethical Committee.

Between December 1993 and November 1994, 20 consecutive patients with unstable angina from the same medical center were entered into the study. The mean age (±SD) was 59±10 years; 15 patients were male and 5 were female. The baseline clinical characteristics are detailed in Table 1.

View this table: Table 1. Baseline Characteristics of the Unstable Angina Patients
[in this window] Randomized to Aspirin or Indobufen
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Treatments
Patients with unstable angina were randomly assigned to one of the following treatments: (1) aspirin 320 mg/d for 2 days (n=10) or (2) indobufen 200 mg BID at 8 AM and 6 PM for 2 days (n=10). Treatment was allocated according to predefined randomization lists that were unknown to the investigators. Separate randomization lists were used for patients admitted while receiving aspirin treatment (n=16) and those who were not being treated with aspirin or other antiplatelet drugs (n=4). The aspirin regimen was the daily dose recommended for the treatment of patients with unstable coronary syndromes. The indobufen regimen was that approved for use in patients with coronary artery disease. Other drugs, such as β-adrenergic blocking agents, calcium channel blocking agents, nitrates, diuretics, and intravenous heparin, were also allowed as required by clinical judgment. There were no statistically significant differences between the two groups with respect to age, sex, body mass index, previous myocardial infarction, distribution of risk factors, or concurrent treatment (Table 1). The angiographically detected sites of coronary artery disease were triple-vessel disease in 4 patients assigned to aspirin and 3 assigned to indobufen, double-vessel disease in 5 patients in the aspirin group and 1 in the indobufen group, and single-vessel disease in 1 and 6 patients in the aspirin and indobufen groups, respectively. Intravenous heparin was given before coronary angiography (5000 IU); in addition, 1 patient received heparin and 1 received heparin (10 000 IU) plus tissue plasminogen activator during PTCA.

Urine Collection
Six to 18 consecutive urine collections were obtained from each patient during the first 48 hours after randomization for the analysis of 11-dehydro-thromboxane B$_2$, a major enzymatic derivative of thromboxane A$_2$. The timing and total volume of each urine sample were recorded, and a 50-mL aliquot was frozen and stored at -20°C until analysis.

Effects of Indobufen Ex Vivo on the Cyclooxygenase Activity of PGHS Isoenzymes in Healthy Subjects
Four healthy volunteers (2 women and 2 men aged 30 to 52 years) were studied on several occasions. Informed consent was obtained from each subject. None of the subjects had taken aspirin or aspirin-like drugs in the 2-week period before the study. Each subject received 200 mg of indobufen at 8 AM and 6 PM daily for 2 days. Whole-blood samples were drawn by venipuncture from an antecubital vein immediately before the first oral dosing and 2 hours after the fourth administration of indobufen. Duplicate 1-mL aliquots were immediately transferred into glass tubes and allowed to clot at 37°C for 60 minutes, and serum was separated by centrifugation (10 minutes at 3000 rpm) and kept at -70°C until assayed for thromboxane B$_2$ as a reflection of maximally stimulated cyclooxygenase activity of platelet PGHS-1 by endogenously formed thrombin. The effect of indobufen on the cyclooxygenase activity of monocyte PGHS-2 was evaluated by incubating 1-mL aliquots of heparinized whole blood (10 IU/mL) with LPS (10 µg/mL) for 24 hours at 37°C. Plasma was separated by centrifugation (10 minutes at 2000 rpm) and kept at -70°C until assayed for PGE$_2$. The platelet contribution to cyclooxygenase activity in whole blood was suppressed by adding aspirin in vitro (50 µg/mL), as previously described.
Effects of Aspirin on the Cyclooxygenase Activity of PGHS Isoenzymes In Vitro
Aspirin was dissolved in dimethyl sulfoxide (0.25 to 250 mg/mL), and 2 µL of the solutions was added to 1-mL aliquots of whole blood to give a final concentration of 0.5 to 500 µg/mL. The effect of aspirin on the cyclooxygenase activity of monocyte PGHS-2 was studied by incubating the drug at six different concentrations with multiple heparinized whole-blood samples in the presence of LPS (10 µg/mL) for 4 and 24 hours and then measuring plasma PGE$_2$ levels. The effect on platelet PGHS-1 activity was evaluated by incubating aspirin at six different concentrations with multiple blood samples that were allowed to clot at 37°C for 60 minutes and then measuring serum thromboxane B$_2$ levels.

Time Course of Aspirin Inactivation in Whole Blood
Because plasma esterases can deacetylate aspirin in a time-dependent fashion, which could influence its effects on PGHS-2 over extended incubations, we characterized the time course of deacetylation by transfer experiments assessing the capacity of aspirin recovered from anticoagulated blood at different time points to inactivate platelet cyclooxygenase activity in the whole-blood clotting system.

Five hundred micrograms of aspirin was incubated with 1-mL aliquots of heparinized blood samples for 0, 1, 2, and 3 hours at 37°C in the presence of LPS (10 µg/mL). At the end of each incubation, plasma was separated by centrifugation at 3000 rpm for 5 minutes at 4°C, and 20-µL aliquots (corresponding to 10 µg of aspirin) were immediately added to 1-mL samples of whole blood (of the same subject) that were allowed to clot at 37°C for 60 minutes; serum thromboxane B$_2$ levels were then measured.

Analyses of Urinary, Plasma, and Serum Eicosanoids
Immunoreactive 11-dehydro-thromboxane B$_2$ was extracted from 10-mL aliquots of each urine sample (the pH was adjusted to 4.0 to 4.5 with formic acid) on SEP-PAK C18 cartridges (Waters Associates) and eluted with ethyl acetate as previously described. The eluate was subjected to silica column chromatography and further eluted with benzene:ethyl acetate: methanol (60:40:30 vol/vol/vol). The overall recovery, as determined by the addition of [³H]thromboxane B$_2$, averaged 64±11%. Immunoreactive 11-dehydro-thromboxane B$_2$ eluted from silica columns was assayed at a final dilution ranging from 1:10 to 1:30 (vol/vol). The detection limit of the radioimmunoassay was 10 pg/mL. The assays were performed blindly as to clinical data and allocated treatment.

Plasma and serum concentrations of PGE$_2$ and thromboxane B$_2$ were measured by previously described and validated radioimmunoassays. Unextracted serum and plasma samples were diluted in the standard diluent of the assay (0.02 mol/L phosphate buffer, pH 7.4) and assayed in a volume of 1.5 mL at a final dilution of 1:50 to 1:20 000. The least-detectable concentration was 1 pg/mL for both assays. Thus, the detection limit of the assays was 0.05 ng/mL of sample.

Statistical Analysis
For the clinical data, variables were compared by use of the $\chi^2$ test. Having established that metabolite excretion rates were not normally distributed, we used a nonparametric approach to statistical analysis of the biochemical measurements, ie, the Mann-Whitney $U$ test.\textsuperscript{27} Statistical comparisons of plasma and serum eicosanoid measurements were performed by Student's unpaired $t$ test and by ANOVA. Statistically significant differences were determined by Student-Newman-Keuls test. Statistical significance was defined as $P<.05$. The values are expressed as median and range except for data presented in Figs 3\textsuperscript{8} and 4\textsuperscript{8}, which are expressed as mean±SE.

**Results**

**Clinical Course**
Two patients, one in each treatment group, underwent PTCA during the study. Moreover, one patient in the indobufen group was subjected to coronary angiography. One patient (in the aspirin group) undergoing PTCA developed total acute reocclusion at the angioplasty site immediately after the procedure. No patient in either group developed myocardial infarction or sudden death during the study; moreover, there were no bleeding complications that required blood transfusion. Ischemic events (ST-T segment changes) were detected during 18 (10.3%) of 175 urine collection periods by

Figure 3. Time course of 11-dehydro-thromboxane B$_2$ (11-dehydro-TxB$_2$) excretion during the first 24 hours after randomization of unstable angina patients to aspirin or indobufen. The numbers in parentheses represent the number of urine samples contributing to the mean value of each time point. Because of the blinded nature of the study, patients randomized to aspirin treatment received aspirin in the morning and placebo in the afternoon to match the BID dosing regimen of indobufen.

Figure 4. Mean rates of excretion of 11-dehydro-thromboxane B$_2$ (11-dehydro-TXB$_2$) measured during the first and second day of treatment in patients with unstable angina randomized to aspirin or indobufen. The numbers in parentheses represent the number of urine samples contributing to the mean value.
Holter monitoring.

**11-Dehydro-thromboxane B\(_2\) Excretion in Patients Randomized to Aspirin**

Six to 12 urine samples were collected from each patient, and a total of 76 urine samples were analyzed for 11-dehydro-thromboxane B\(_2\). The mean duration of each collection was 4.6±3 hours. There was no statistically significant difference in 11-dehydro-thromboxane B\(_2\) excretion between the 8 patients who were admitted to the study while taking aspirin and the 2 who were not being treated with antiplatelet drugs before randomization (Table 2). Thus, all data from the 10 patients were pooled for further analysis. The measurements obtained in aspirin-treated patients are depicted in Fig 1. Metabolite excretion averaged 102 pg/mg creatinine (median value; range, <10 to 897 pg/mg creatinine). There were 16 samples (21%) with 11-dehydro-thromboxane B\(_2\) >200 pg/mg creatinine among patients treated with aspirin. These were evenly distributed throughout the 48-hour sampling period: 6 of 20, 4 of 16, 3 of 20, and 3 of 20 samples during the 0- to 12-, 12- to 24-, 24- to 36-, and 36- to 48-hour collection periods, respectively. A total of 11 ST-T segment changes were recorded during the study by Holter monitoring in 4 patients. Myocardial ischemia was detected during 8 of 76 urine collections; all cases were of symptomatic ischemia, with chest pain plus ECG changes. In the patients with myocardial ischemia, metabolite excretion averaged 63 pg/mg creatinine (median value) in the samples collected during ischemia and 87 pg/mg creatinine in those collected during the ischemia-free periods. The range of 11-dehydro-thromboxane B\(_2\) excretion rates was 54 to 253 and 74 to 184 pg/mg creatinine during ischemia and ischemia-free periods, respectively. Moreover, in the patient who developed acute reocclusion after PTCA, the rate of 11-dehydro-thromboxane B\(_2\) excretion was not higher than in the other patients (96 versus 100 pg/mg creatinine, respectively [median value]). Moreover, there were no differences in metabolite excretion in the samples collected immediately before and after the procedure (102 versus 81 pg/mg creatinine, respectively).

**Table 2.** Urinary 11-Dehydro-thromboxane B\(_2\) Excretion in Unstable Angina Patients Treated With Aspirin or Indobufen, as a Function of Previous Aspirin Use

**Figure 1.** Rates of urinary 11-dehydro-thromboxane B\(_2\) (11-dehydro-TxB\(_2\)) excretion in unstable angina patients randomized to aspirin or indobufen as antiplatelet treatment. Dots represent individual measurements. The broken line represents the value of 200 pg/mg creatinine. The solid lines represent the median value of each treatment group.
11-Dehydro-thromboxane B₂ Excretion in Patients Randomized to Indobufen

Seven to 18 urine samples were collected from each patient in the indobufen group, and a total of 99 urine samples were analyzed for 11-dehydro-thromboxane B₂. The mean duration of each collection was 4.9±2.9 hours. There was no statistically significant difference in 11-dehydro-thromboxane B₂ excretion between the 8 patients who were admitted to the study while receiving aspirin therapy and the 2 who were not being treated with antiplatelet drugs before randomization (Table 2 ). Thus, all data from the 10 patients were pooled for further analysis. The measurements obtained in indobufen-treated patients are depicted in Fig 1. Metabolite excretion averaged 55 pg/mg creatinine (median value; range, <10 to 299 pg/mg creatinine).

There were only 6 samples (6%) with 11-dehydro-thromboxane B₂ >200 pg/mg creatinine among patients taking indobufen. Five of these were obtained during the first 12 hours of urine sampling and the sixth during the 12- to 24-hour collection period. A total of 12 ST-T segment changes were recorded during the study by Holter monitoring in 5 patients. Myocardial ischemia occurred during 10 of 99 urine collections. Chest pain and ST-T segment changes were recorded in 8 of these collection periods, whereas silent ischemia was present in 2. Metabolite excretion averaged 52 pg/mg creatinine (median value) in the samples collected during myocardial ischemia and 57 pg/mg creatinine in those collected during the ischemia-free periods. The range of metabolite excretion was 40 to 141 and 23 to 76 pg/mg creatinine during the ischemia and ischemia-free periods, respectively.

Comparison of Thromboxane Biosynthesis During Aspirin Versus Indobufen

There was a statistically significant difference in the urinary 11-dehydro-thromboxane B₂ excretion measured during aspirin versus indobufen (102 versus 55 pg/mg creatinine; \( P<.001 \)). Moreover, there were significantly \( (P<.001) \) more samples with abnormally high levels of 11-dehydro-thromboxane B₂ ( >200 pg/mg creatinine) in the aspirin group (21%) than in the indobufen group (6%) (Fig 1). The individual and median values of thromboxane metabolite excretion measured in each unstable angina patient during treatment with aspirin or indobufen are depicted in Fig 2. The unstable angina patient with the highest level of urinary 11-dehydro-thromboxane B₂ excretion in the aspirin group had 425 pg/mg creatinine as the median value, whereas the highest level was 92 pg/mg creatinine in the indobufen group ( \( P<.001 \)). In addition, 7 of the 10 patients randomized to receive aspirin had a median level of thromboxane metabolite excretion above the highest value in the group randomized to indobufen. The time course of 11-dehydro-thromboxane B₂ excretion during the first 24 hours is depicted in Fig 3. There were no statistically significant differences between the two groups in the level of urinary 11-dehydro-thromboxane B₂ measured during the first 9 hours after randomization. However, in the
successive samples collected after the second daily tablet, there was a relatively stable reduction in metabolite excretion in the indobufen-treated patients and less-consistent changes in the aspirin-treated patients. Overall, thromboxane biosynthesis was significantly lower in the indobufen group than in the aspirin group during the first ($P<.05$) and second ($P<.0001$) days of the study (Fig 4•).

**Figure 2.** Individual rates of 11-dehydro-thromboxane B$_2$ (11-dehydro-TxB$_2$) excretion and median values (solid lines) in the 10 patients randomized to aspirin (left) and the 10 patients randomized to indobufen (right). Six to 18 consecutive urine collections were obtained from each patient during the first 48 hours after randomization.

The number and duration of ischemic episodes did not differ between the two groups to any statistically significant extent, although there was a trend for a shorter duration of ischemia in the patients treated with indobufen (39 minutes for the entire group; 36 minutes in those recently exposed to aspirin) than in patients treated with aspirin (48 minutes).

### Effects of Indobufen and Aspirin on Platelet PGHS-1 and Monocyte PGHS-2

To evaluate to what extent indobufen (200 mg BID for 2 consecutive days) inhibits the cyclooxygenase activity of platelet PGHS-1 and monocyte PGHS-2, four healthy subjects were treated with the drug, and serum thromboxane B$_2$ levels and PGE$_2$ production by whole blood incubated with LPS for 24 hours were measured. Before indobufen administration, serum thromboxane B$_2$ and plasma PGE$_2$ averaged 657±150 and 11.1±5.3 ng/mL (mean±SD; n=4), respectively. Whole-blood thromboxane B$_2$ production (Fig 5A•) and LPS-induced PGE$_2$ production (Fig 5B•) were significantly ($P=.0001$ and $P=.015$, respectively) reduced by oral indobufen by 98±1% and 80±10%, respectively.

**Figure 5.** Inhibition of platelet PGHS-1 activity (A) and monocyte PGHS-2 activity (B) measured ex vivo after oral dosing with indobufen 200 mg BID for 2 days in healthy subjects. Whole-blood thromboxane (TX) B$_2$ and LPS-induced PGE$_2$ production were measured before the first dose (control) and 2 hours after the fourth dose of indobufen as a reflection of platelet-constitutive and monocyte-inducible
The instability of aspirin in blood at 37°C does not permit evaluation of its inhibitory effect ex vivo on the cyclooxygenase activity of monocyte PGHS-2 expressed in whole blood in response to LPS. In fact, LPS stimulated blood monocytes to produce PGE\(_2\) after a lag time of several hours that reflected de novo synthesis of PGHS-2. Therefore, we studied the inhibitory effects of aspirin added in vitro on the cyclooxygenase activity of platelet PGHS-1 and monocyte PGHS-2. Aspirin inhibited the cyclooxygenase activity of the constitutively expressed platelet PGHS-1, with an IC\(_{50}\) value of 3.5±0.9 µg/mL (mean±SD; n=6) (Fig 6). The addition of aspirin (0.5 to 500 µg/mL) to whole blood stimulated for 4 hours with LPS caused a dose-dependent inhibition of monocyte PGHS-2 activity that reached a plateau of 50% at 100 µg/mL (Fig 6). In contrast, after 24 hours of incubation, aspirin did not affect LPS-induced PGE\(_2\) production to any statistically significant extent (data not shown). The time-dependent loss of inhibition of the cyclooxygenase activity of PGHS-2 by aspirin is likely due to the rapid enzymatic hydrolysis of acetylsalicylic acid by plasma esterases and the rapid turnover of monocyte PGHS-2. Therefore, we studied the time course of aspirin deacetylation in blood by transfer experiments. Aspirin (10 µg/mL) inhibited the production of thromboxane B\(_2\) during whole-blood clotting by 81±9% (mean±SD; n=5). Preincubation of the same concentration of aspirin with LPS-stimulated whole blood for \(\leq3\) hours at 37°C caused a time-dependent loss of this capacity, with a half-life of \(\approx120\) minutes. These observations suggest that high concentrations of aspirin can indeed inhibit the cyclooxygenase activity of human monocyte PGHS-2. However, due to the instability of aspirin in blood, the degree of inhibition will depend on the amount of intact drug present at the time of induction of the functional PGHS-2.

**Figure 6.** Inhibition of platelet PGHS-1 activity (■) and monocyte PGHS-2 activity (▲) measured in vitro after the addition of aspirin to whole-blood samples drawn from healthy subjects. Thromboxane B\(_2\) production after 1 hour of whole-blood clotting and PGE\(_2\) production after 4-hours’ incubation of heparinized blood samples with LPS were measured in the absence and presence of increasing concentrations of aspirin added to blood samples at time 0. The graph depicts the dose-response relation for the inhibition of the cyclooxygenase activity of the two isoenzymes. For each of six experiments, serum thromboxane B\(_2\) (a measure of platelet PGHS-1) and plasma PGE\(_2\) (a measure of monocyte PGHS-2) are expressed as the percentage inhibition of the values obtained in the
Discussion

Previous studies have examined the rate of thromboxane biosynthesis in the setting of acute coronary syndromes through measurements of plasma levels of its hydration product, thromboxane B\(_2\), and of the urinary excretion of major enzymatic metabolites such as 11-dehydro-thromboxane B\(_2\) and 2,3-dinor-thromboxane B\(_2\). Episodic increases in metabolite excretion have been detected in the vast majority of unstable angina patients while they were receiving anti-ischemia drug therapy. The administration of low-dose aspirin was associated with statistically significant reductions in thromboxane biosynthesis by \(\geq 70\%\). However, abnormally high rates of thromboxane metabolite excretion have been detected in some 20% of 6- to 8-hour urine collections obtained from aspirin-treated unstable patients. The intravenous administration of aspirin as well as the ex vivo monitoring of platelet cyclooxygenase activity in that study allowed us to exclude noncompliance or inadequate bioavailability as a source of enhanced thromboxane biosynthesis under these circumstances. Because such instances of the failure of aspirin to suppress the enhanced formation of the vasoconstrictor and platelet-agonist thromboxane A\(_2\) might contribute to episodes of myocardial ischemia as well as to progression toward complete vascular occlusion, we set out to investigate the potential mechanism(s) underlying this phenomenon.

In the present study, we tested the hypothesis that a component of enhanced thromboxane biosynthesis in unstable angina is dependent on the cyclooxygenase activity of nucleated cells that, in contrast to platelets, would have the capacity to resynthesize the enzyme PGHS after aspirin clearance from the circulation. We reasoned that a reversible cyclooxygenase inhibitor with a longer half-life might affect both platelet and extraplatelet sources of thromboxane biosynthesis by virtue of its persistence in the bloodstream and therefore at the active site of the enzyme throughout the dosing interval. To this effect, we used the antiplatelet drug indobufen, which has been characterized as being a potent, reversible inhibitor of platelet cyclooxygenase activity. When administered at a dose of 200 mg BID to patients recovering after myocardial infarction, indobufen suppressed platelet thromboxane biosynthesis measured ex vivo by >95% throughout the dosing interval. Moreover, in patients with type II diabetes mellitus, who are characterized by persistently elevated levels of thromboxane biosynthesis, the same regimen of indobufen caused profound suppression of thromboxane metabolite excretion, comparable to that achieved by low-dose aspirin in the same setting. Additionally, two independent randomized studies have shown indobufen to be as effective as the combination of aspirin and dipyridamole in preventing coronary bypass graft occlusion.
Thus, in the present study we randomized patients with unstable angina to a short-term treatment with either indobufen or aspirin and collected consecutive urine samples up to 48 hours after randomization. The rationale for collecting as many as 18 urine samples from the same patient is related to the episodic nature of platelet activation and enhanced thromboxane metabolite excretion, as previously characterized in unstable angina.\textsuperscript{3,10} The main finding of the present study is that the rate of thromboxane biosynthesis was \textasciitilde 50\% lower in patients treated with indobufen than in patients treated with aspirin. Moreover, a significantly lower proportion of episodes of enhanced thromboxane metabolite excretion was noted in association with indobufen than with aspirin (6\% versus 21\%, respectively). Such a difference was not influenced by previous aspirin use, number of ischemic episodes, or invasive procedures during the study. Moreover, concurrent drug treatments are unlikely to have contributed to such different levels of thromboxane biosynthesis inasmuch as these were fairly well balanced in the two groups, with the possible exception of heparin. The latter was used in twice as many patients in the indobufen group as in the aspirin group (Table 1\textsuperscript{(a)}). Heparin usually results in inhibition of intracoronary thrombus formation in an animal model of coronary artery stenosis and endothelial injury characterized by periodic formation of platelet aggregates at the site of stenosis.\textsuperscript{31} However, heparin does not prevent procedure-related increases in thromboxane biosynthesis in patients undergoing coronary angiography\textsuperscript{32} and may in fact cause platelet activation in humans.\textsuperscript{33}

The difference in thromboxane metabolite excretion became apparent after the second daily dose of indobufen and persisted throughout the study (Figs 3\textsuperscript{(e)} and 4\textsuperscript{(d)}).

At least two alternative explanations might be considered for the mechanism(s) underlying the different effects of aspirin and indobufen in suppressing thromboxane biosynthesis. First, the difference may have reflected incomplete inhibition of platelet cyclooxygenase activity by aspirin during the first 2 days of oral dosing. However, this seems unlikely because (1) 320 mg causes a ceiling inhibitory effect on platelet cyclooxygenase activity,\textsuperscript{12,13} (2) the difference in 11-dehydro-thromboxane B\textsubscript{2} excretion was also apparent in patients who had been exposed to aspirin before randomization to aspirin or indobufen (Table 2\textsuperscript{(c)}), and (3) the episodes of enhanced metabolite excretion while patients were receiving aspirin therapy were uniformly distributed throughout the sampling period. Alternatively, the lower rate of thromboxane biosynthesis while patients were receiving indobufen therapy may have reflected the inhibition by this drug of extraplatelet sources of thromboxane biosynthesis, largely unaffected by aspirin because of rapid de novo synthesis of the enzyme PGHS in nucleated cells during the 24-hour dosing interval. Cells endowed with substantial amounts of thromboxane synthase include monocytes/macrophages and, to a lesser extent, vascular cells.\textsuperscript{34} In addition to expressing the same constitutive PGHS-1 as platelets, monocytes and vascular endothelial cells can respond to a variety of inflammatory or mitogenic stimuli by expressing an inducible isoform of the enzyme called PGHS-2.\textsuperscript{35,36,37,38,39} The cyclooxygenase activity of monocyte PGHS-2 can be inhibited by high concentrations of aspirin (Fig 6\textsuperscript{(e)}) that cannot be achieved after oral dosing with 320 mg and by low micromolar concentrations of indobufen\textsuperscript{25} compatible with the reported plasma levels of the drug after oral dosing with 200 mg BID.\textsuperscript{40} The catalytic activity of PGHS-2 might
contrivate to aspirin-insensitive thromboxane biosynthesis by two distinct mechanisms, ie, by generating the intermediate PGH$_2$ as a substrate for the thromboxane synthase of the same cell (eg, monocytes/macrophages) or through transcellular metabolism by providing exogenous PGH$_2$ to the thromboxane-synthase of aspirin-treated platelets.$^{19}$ The failure of indobufen to completely prevent episodes of increased thromboxane biosynthesis may have reflected the relatively limited potency of the drug in inhibiting human monocyte PGHS-2 versus platelet PGHS-1 (Fig 5* ) and/or less than maximal plasma levels of the drug during the first 24 hours, when all such episodes occurred. Thus, given the 8-hour half-life of the drug,$^{40}$ steady-state plasma levels would not be achieved until the second day of BID dosing.

We did not perform any measurements of PGHS-2 activity in the circulating monocytes of our patients, and therefore the interpretation of our findings remains entirely speculative at this stage. However, measurements of PGHS-2 activity after oral dosing with the same regimen of indobufen in healthy subjects demonstrate the capacity of the drug to largely suppress inducible cyclooxygenase activity at therapeutic plasma concentrations (Fig 5* ). Another limitation of our study is that we could not include a placebo arm for obvious ethical reasons. Moreover, the present study did not have sufficient size to probe any realistic difference in clinical end points between the two treatments. Also, it has been pointed out that thromboxane A$_2$ is only one of several mediators that accumulate at sites of vascular injury and arterial narrowing and lead to thrombosis and vasoconstriction.$^{41}$

Despite these limitations, we believe that our findings might have some clinical as well as research implications. Aspirin-insensitive thromboxane biosynthesis might provide a mechanism for the episodic formation of a potent agonist of the platelet and vascular thromboxane receptors, possibly contributing to a number of clinical aspirin failures. The availability of potent and long-lasting thromboxane receptor antagonists and selective PGHS-2 inhibitors offers the opportunity to test this hypothesis.

#### Selected Abbreviations and Acronyms

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<tr>
<th>Abbreviation</th>
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<tr>
<td>LPS</td>
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<td>PGHS</td>
<td>prostaglandin H synthase</td>
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<tr>
<td>PTCA</td>
<td>percutaneous transluminal coronary angioplasty</td>
</tr>
</tbody>
</table>

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Footnotes


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