

CYCLOOXYGENASE INHIBITORS AND THE ANTIPLATELET EFFECTS OF ASPIRIN

FRANCESCA CATELLA-LAWSON, M.D., MUREDACH P. REILLY, M.D., SHIV C. KAPOOR, PH.D., ANDREW J. CUCCHIARA, PH.D., SUSAN DEMARCO, R.N., BARBARA TOURNIER, R.N., SACHIN N. VYAS, PH.D., AND GARRET A. FITZGERALD, M.D.

ABSTRACT

Background Patients with arthritis and vascular disease may receive both low-dose aspirin and other nonsteroidal antiinflammatory drugs. We therefore investigated potential interactions between aspirin and commonly prescribed arthritis therapies.

Methods We administered the following combinations of drugs for six days: aspirin (81 mg every morning) two hours before ibuprofen (400 mg every morning) and the same medications in the reverse order; aspirin two hours before acetaminophen (1000 mg every morning) and the same medications in the reverse order; aspirin two hours before the cyclooxygenase-2 inhibitor rofecoxib (25 mg every morning) and the same medications in the reverse order; enteric-coated aspirin two hours before ibuprofen (400 mg three times a day); and enteric-coated aspirin two hours before delayed-release diclofenac (75 mg twice daily).

Results Serum thromboxane B₂ levels (an index of cyclooxygenase-1 activity in platelets) and platelet aggregation were maximally inhibited 24 hours after the administration of aspirin on day 6 in the subjects who took aspirin before a single daily dose of any other drug, as well as in those who took rofecoxib or acetaminophen before taking aspirin. In contrast, inhibition of serum thromboxane B₂ formation and platelet aggregation by aspirin was blocked when a single daily dose of ibuprofen was given before aspirin, as well as when multiple daily doses of ibuprofen were given. The concomitant administration of rofecoxib, acetaminophen, or diclofenac did not affect the pharmacodynamics of aspirin.

Conclusions The concomitant administration of ibuprofen but not rofecoxib, acetaminophen, or diclofenac antagonizes the irreversible platelet inhibition induced by aspirin. Treatment with ibuprofen in patients with increased cardiovascular risk may limit the cardioprotective effects of aspirin. (N Engl J Med 2001;345:1809-17.)

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NONSTEROIDAL antiinflammatory drugs (NSAIDs) are commonly prescribed,¹ and the use of aspirin has increased since it was shown to reduce the risk of myocardial infarction and stroke.^{2,3} Aspirin acts by irreversibly acetylating a serine residue at position 529 in platelet prostaglandin G/H synthase,⁴ an enzyme colloquially known as cyclooxygenase. The predominant product of cyclooxygenase in platelets is thromboxane A₂.⁵ The anucleate platelet affords a unique target for aspirin, since once cyclooxygenase has been

acetylated by aspirin, the substrate's access to its active site is impeded for the lifetime of the platelet. Thus, the formation of thromboxane A₂ requires the synthesis of new platelets, which are regenerated at a daily rate of approximately 10 percent.^{6,7}

Although aspirin is effective in the secondary prevention of important vascular events,^{2,3} the effectiveness of traditional NSAIDs in this respect is unknown. Prospective, controlled trials have been limited,^{8,9} and initial case-control analysis suggests that NSAIDs do not reduce the risk of a first myocardial infarction.¹⁰ NSAIDs, unlike aspirin, bind reversibly at the active site of the enzyme, usually depressing platelet thromboxane formation to the degree that platelet function is impaired for only a portion of the dosing interval.¹¹

The form of cyclooxygenase that is produced in human platelets, cyclooxygenase-1,^{4,12} has been crystallized,¹³ and the structural basis of inhibition by both aspirin¹⁴ and NSAIDs¹⁵ has been elucidated. Both the aspirin- and the NSAID-binding sites lie within a narrow hydrophobic channel within the core of the enzyme. The potential for a competitive interaction between aspirin and NSAIDs afforded by these structural relations is supported by evidence from previous studies.^{16,17}

Because many patients take both NSAIDs and aspirin,¹⁸ we undertook a study to address in a more detailed fashion the possibility of a pharmacodynamic interaction between the two. First, we performed a controlled study to determine whether such an interaction does indeed exist. We then determined whether the effects of an aspirin regimen of the type commonly used for cardioprotection would be influenced by a clinically relevant NSAID regimen and whether the effects would extend to other compounds of the NSAID class. We also examined the interaction of aspirin with a specific inhibitor of cyclooxygenase-2, a new subclass of NSAID that selectively targets a second cyclooxygenase isozyme thought to be of most relevance to prostanoid formation in inflammation and cancer.¹⁹ Finally, we investigated the interaction of aspirin with acetaminophen, since the effects of acetaminophen on cyclooxygenase activity remain controversial.^{20,21}

From the EUPenn Group of Investigators at the Center for Experimental Therapeutics (F.C.-L., S.D., B.T., G.A.F.), the Division of Cardiology (M.P.R.), and the General Clinical Research Center (F.C.-L., S.C.K., A.J.C., S.N.V., G.A.F.), University of Pennsylvania School of Medicine, Philadelphia. Address reprint requests to Dr. FitzGerald at the University of Pennsylvania School of Medicine, 153 Johnson Pavilion, 3620 Hamilton Walk, Philadelphia, PA 19104-6084, or at garret@spirit.grc.upenn.edu.

METHODS

Study Subjects

The study protocols were approved by the institutional review board and the General Clinical Research Center advisory committee of the University of Pennsylvania School of Medicine. Written informed consent was obtained from all study subjects. The subjects were between 18 and 65 years of age and within 30 percent of ideal body weight and had an unremarkable medical history, physical examination, and routine hematologic and biochemical studies. Smokers and subjects with a bleeding disorder, an allergy to aspirin or any other NSAID, or a history of any gastrointestinal or cerebrovascular disease were excluded. Subjects abstained from the use of aspirin and other NSAIDs for at least two weeks before enrollment.

Crossover Study with Single Daily Doses

Study Design, Treatments, and Assessment

The first study was a randomized, crossover study of combinations of single daily doses of two treatments for 6 days, with a washout period of at least 14 days (Fig. 1). One group received aspirin (81 mg) two hours before ibuprofen (400 mg) for six days and, after the washout period, the same medications in the reverse order. Another group received aspirin two hours before acetaminophen (1000 mg) for six days and then the same medications in the reverse order. And a third group received aspirin two hours before the cyclooxygenase-2 inhibitor rofecoxib (25 mg) and then the same medications in the reverse order.

The inhibition of platelet cyclooxygenase-1 was assessed by measurements of serum thromboxane B₂.²² Platelet aggregation induced by arachidonic acid was measured in platelet-rich plasma *ex vivo*.¹¹ Cyclooxygenase-2 activity was assessed through the measurement of the formation of lipopolysaccharide-stimulated prostaglandin E₂ in whole blood.²³ Measurements were performed immediately before the administration of the first drug and immediately before the administration of the second drug on day 1, and 2, 6, 12, and 24 hours after the administration of the first drug on day 1 and day 6. Urinary 2,3-dinor-6-keto prostaglandin F_{1α}, an index of prostaglandin I₂ biosynthesis,²⁴ was assessed during the 12 hours before the administration of the first dose of study medication and in three sequential collections after the first study drug was administered (from hour 0 to hour 6, from hour 6 to hour 12, and from hour 12 to hour 24) on days 1 and 6. Biochemical studies were performed by staff members who were blinded to the treatment-group assignments.

Statistical Analysis

The primary hypothesis was that administering ibuprofen before aspirin would antagonize the irreversible effects of aspirin, as assessed by the measurement of serum thromboxane B₂ (primary end point) and platelet aggregation (secondary end point) 24 hours after the administration of the first study drug on day 6 of combination therapy.

This study had a six-factor, balanced, incomplete block design with two nonrepeated factors (treatment group and sequence) and four repeated factors (period, order, day, and hour). A two-by-two crossover design with factor sequence, period, and order was considered for each stratum defined in terms of the treatment group, the day, and the hour. An analysis of variance, appropriate for a two-by-two crossover design, was produced with the use of the mixed and general linear model procedures. The estimate of sample size was based on the analysis of the effects of aspirin in subjects in the ibuprofen group. It was anticipated on the basis of previous studies that a sample size of 18 (6 per group) would afford a power in excess of 90 percent to detect a difference of 20 percent or greater in serum thromboxane measurements and platelet aggregation, with two-tailed tests of the hypothesis associated with a type I error rate of less than 0.05 for all the main effects.

Parallel-Group Study with Multiple Daily Doses

Study Design, Treatments, and Assessment

We also performed a parallel-group, randomized, open-label, six-day study in which one group was given enteric-coated aspirin (81 mg) at 8 a.m. and ibuprofen (400 mg) at 10 a.m., 3 p.m., and 8 p.m. and another group was given enteric-coated aspirin at 8 a.m. and delayed-release diclofenac (75 mg) at 10 a.m. and 6 p.m. (Fig. 1). The inhibition of platelet cyclooxygenase-1 activity was assessed by measurement of serum thromboxane B₂ and platelet aggregation.^{11,22}

Statistical Analysis

The primary hypothesis was that ibuprofen or diclofenac would antagonize the irreversible effects of enteric-coated aspirin 24 hours after the administration of the first study drug on day 6 of the combination therapy. The estimate of sample size was based on the difference we found in the first study between the serum thromboxane B₂ level after the administration of aspirin before ibuprofen as compared with the administration of these drugs in reverse order. It was anticipated that a sample of five subjects per group would afford a power in excess of 90 percent to detect the main effect, with two-tailed tests of the hypothesis and a type I error rate of 0.05.

RESULTS

Crossover Study with Single Daily Doses

Three subjects in the first study who withdrew before receiving the study drug were replaced; all subjects who began receiving drugs completed the study according to the protocol. One subject reported an episode of epistaxis, and a second had a drop in hemoglobin of 1 g per liter. Analysis of variance revealed that the randomized order of administration did not affect the study end points. This indicates that there was no carryover effect during the washout period. Therefore, the results were pooled according to the order of dosing.

In the single-dose-ibuprofen group, subjects who took aspirin first had at least 98 percent inhibition of serum thromboxane B₂ up to 24 hours after dosing on day 6 (Fig. 2A). When the same subjects took ibuprofen before aspirin, serum thromboxane B₂ was more than 97 percent inhibited two hours after the administration of ibuprofen on day 6, but it later recovered, as would be expected after the administration of a reversible cyclooxygenase-1 inhibitor¹¹ such as ibuprofen (Fig. 2A). Twenty-four hours after the administration of the first study drug on day 6, the mean (\pm SD) degree of inhibition of serum thromboxane B₂ was 99 \pm 0.3 percent when the subjects had taken aspirin before ibuprofen and 53 \pm 7 percent when the subjects had taken ibuprofen before aspirin ($P < 0.001$).

Platelet aggregation was also irreversibly inhibited when subjects took aspirin before ibuprofen and reversibly inhibited when subjects took ibuprofen before aspirin (Fig. 2B). Twenty-four hours after the administration of the first study drug on day 6, the mean degree of inhibition of platelet aggregation was 98 \pm 1 percent in subjects who had taken aspirin before ibuprofen.

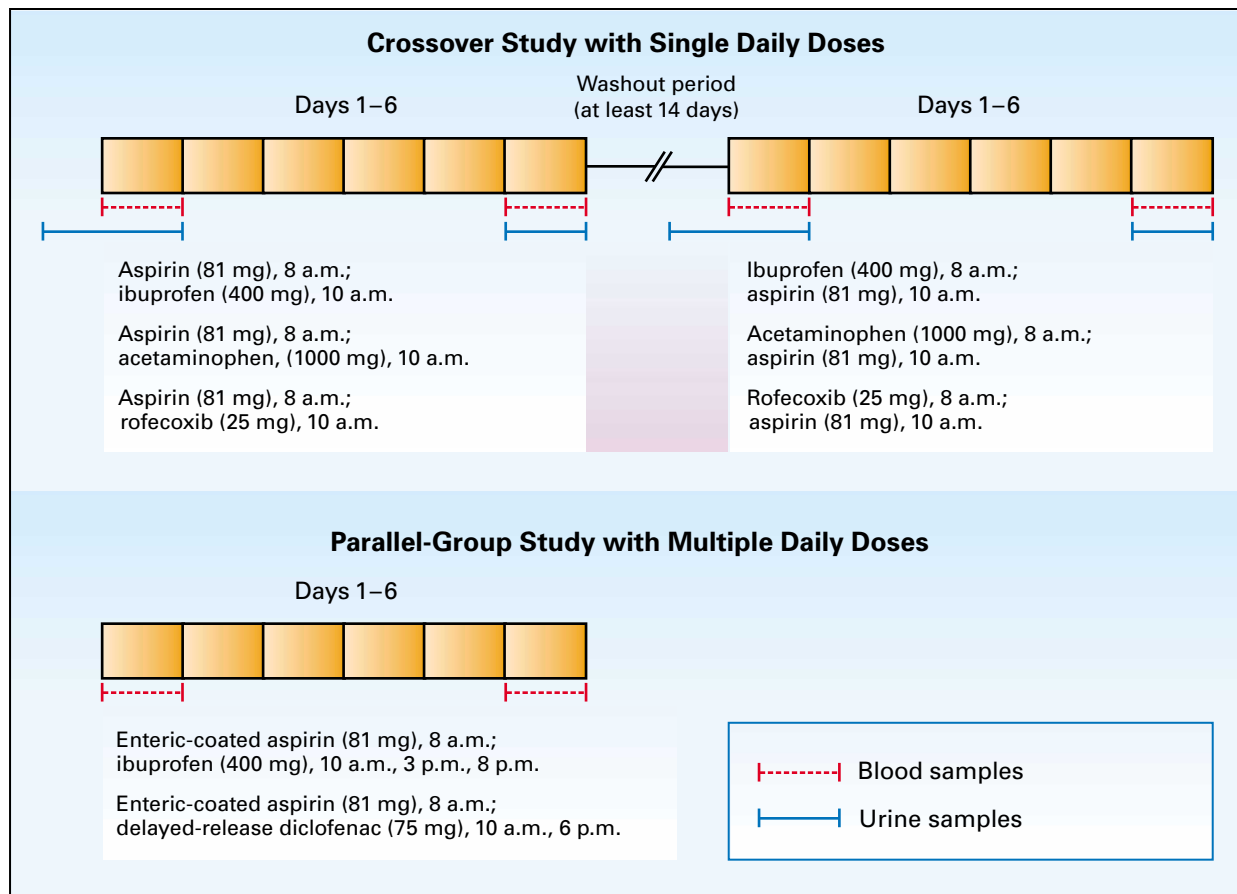


Figure 1. Flow Chart of Study Protocols.

Blood samples were taken at multiple time points on day 1 and day 6 of each treatment, and urine was collected throughout day 1 and day 6 of study 1.

profen and 2 ± 1 percent in subjects who had taken ibuprofen before aspirin ($P < 0.001$).

In the acetaminophen group, the inhibition of serum thromboxane B_2 in subjects who took aspirin before acetaminophen was similar to that observed in subjects in the single-dose-ibuprofen group who took aspirin before ibuprofen, with at least 96 percent inhibition up to 24 hours after treatment on day 6. Pretreatment with acetaminophen did not alter the antiplatelet effect of aspirin. The inhibition of serum thromboxane B_2 was similar on day 6 regardless of whether subjects had taken aspirin or acetaminophen first.

The inhibition of platelet aggregation on day 6 in subjects who had taken aspirin before acetaminophen was also similar to the inhibition in those who had taken acetaminophen before aspirin. Platelet aggregation was unaltered two hours after the administration of acetaminophen and before the administration of aspirin on day 1, when serum thromboxane B_2

was inhibited by 44 ± 14 percent. The failure of acetaminophen to inhibit platelet aggregation²⁵ — given its limited degree of inhibition of platelet cyclooxygenase-1 activity — was expected.^{7,26}

In the rofecoxib group, the patterns of serum thromboxane B_2 production (Fig. 3A) and platelet aggregation (Fig. 3B) when the subjects took aspirin first were similar to the patterns found when subjects took rofecoxib first. There was no inhibition of platelet cyclooxygenase-1 activity or platelet aggregation two hours after the administration of rofecoxib on day 1.^{27,28}

In all three groups, subjects who took aspirin before the other drug showed no inhibition of cyclooxygenase-2 activity in ex vivo studies, as reflected by the level of lipopolysaccharide-stimulated prostaglandin E_2 two hours after the administration of low-dose aspirin (and before the administration of the other drug) on day 1 (Table 1). Thus, the pattern of inhibition of cyclooxygenase-2 activity reflects inhibition

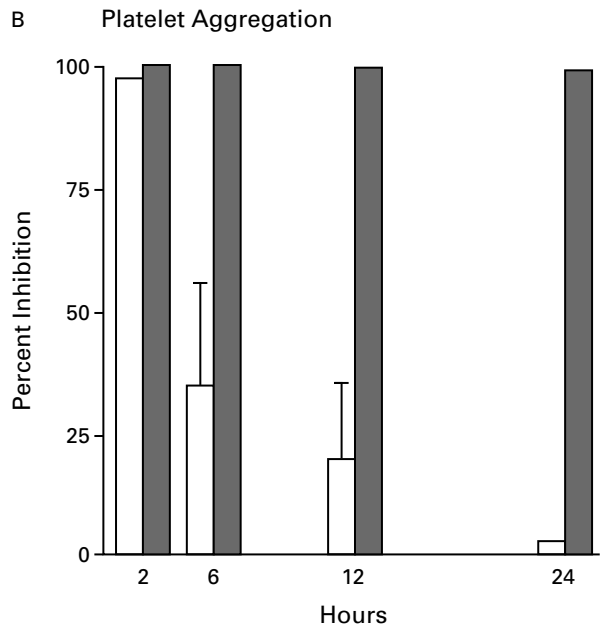
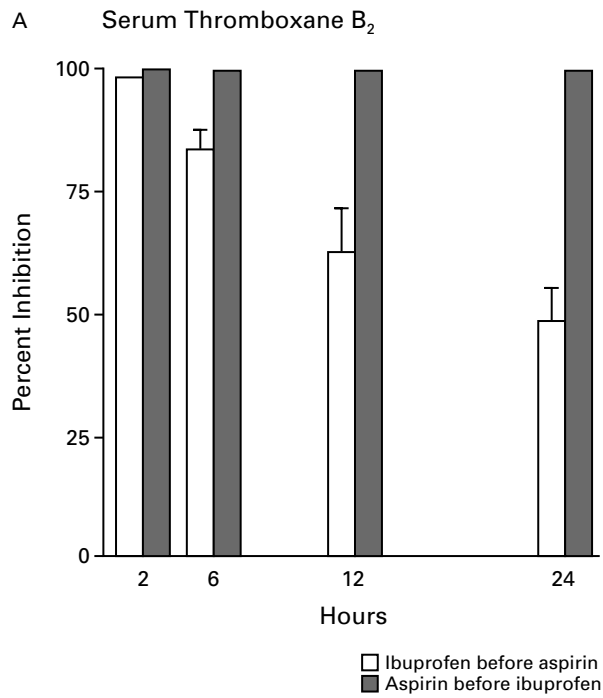


Figure 2. Mean Inhibition of Platelet Cyclooxygenase-1 Activity, as Assessed by Measurement of Serum Thromboxane B₂ (Panel A) and Inhibition of Platelet Aggregation (Panel B) in Subjects Taking Ibuprofen before Aspirin or Aspirin before Ibuprofen on Day 6 of Prolonged Dosing.

The base-line level of serum thromboxane B₂ was 473±92 ng per milliliter when ibuprofen was administered before aspirin and 503±57 ng per milliliter when aspirin was administered before ibuprofen. The I bars represent SEs. At 24 hours, P<0.001 for both comparisons between ibuprofen-before-aspirin and aspirin-before-ibuprofen. All times are hours after the administration of the first study drug.

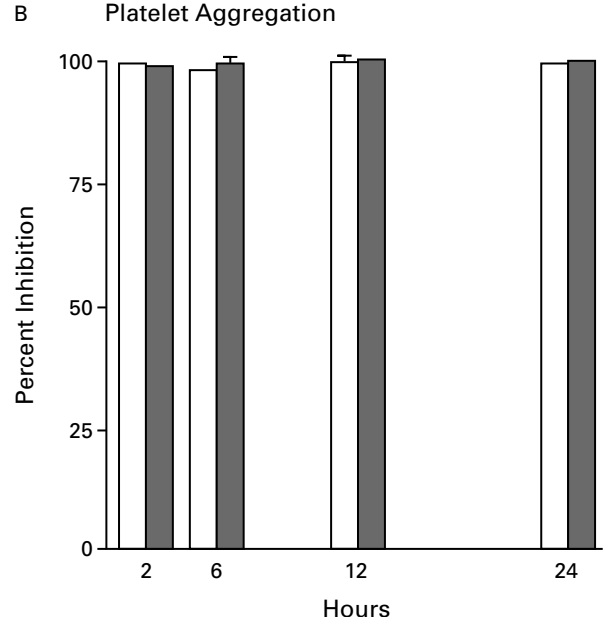
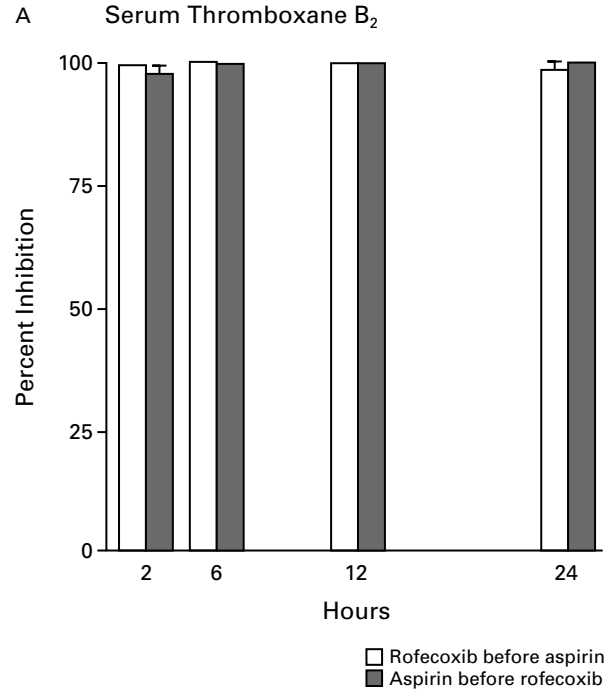


Figure 3. Mean Inhibition of Platelet Cyclooxygenase-1 Activity, as Assessed by Measurement of Serum Thromboxane B₂ (Panel A) and Inhibition of Platelet Aggregation (Panel B) in Subjects Taking Rofecoxib before Aspirin or Aspirin before Rofecoxib on Day 6 of Prolonged Dosing.

The base-line level of serum thromboxane B₂ was 411±50 ng per milliliter when rofecoxib was administered before aspirin and 416±60 ng per milliliter when aspirin was administered before rofecoxib. The I bars represent SEs. All times are hours after the administration of the first study drug.

TABLE 1. INHIBITION OF WHOLE-BLOOD LIPOPOLYSACCHARIDE-STIMULATED PROSTAGLANDIN E₂.*

| VARIABLE | DAY 1 | | | | DAY 6 | | | |
|------------------------------|--------------------|-------|-------|-------|--------|-------|-------|-------|
| | HR 2 | HR 6 | HR 12 | HR 24 | HR 2 | HR 6 | HR 12 | HR 24 |
| | percent inhibition | | | | | | | |
| Single-dose-ibuprofen group | | | | | | | | |
| Aspirin before ibuprofen | 8±4† | 63±6 | 61±8 | 21±10 | 22±10‡ | 67±9 | 64±6 | 19±13 |
| Ibuprofen before aspirin | 77±3 | 43±10 | 50±6 | 28±10 | 80±2 | 55±13 | 49±11 | 8±4 |
| Acetaminophen group | | | | | | | | |
| Aspirin before acetaminophen | 7±3† | 60±7 | 67±5 | 21±5 | 15±6‡ | 61±5 | 55±10 | 35±13 |
| Acetaminophen before aspirin | 44±5 | 46±6 | 60±9 | 29±10 | 53±8 | 32±11 | 41±12 | 14±10 |
| Rofecoxib group | | | | | | | | |
| Aspirin before rofecoxib | 4±2† | 54±5 | 82±3 | 37±6 | 68±3 | 76±3 | 85±3 | 55±9 |
| Rofecoxib before aspirin | 64±3 | 67±3 | 74±5 | 40±6 | 69±6 | 74±5 | 83±3 | 55±7 |

*Plus-minus values are means ±SE. The base-line level of lipopolysaccharide-stimulated prostaglandin E₂ was 40.4±8.9 ng per milliliter in the single-dose-ibuprofen group when aspirin was administered before ibuprofen and 47±4.3 ng per milliliter when ibuprofen was administered before aspirin, 27.9±3.4 ng per milliliter in the acetaminophen group when aspirin was administered before acetaminophen and 34.7±7.2 ng per milliliter when acetaminophen was administered before aspirin, and 47.2±8.1 ng per milliliter in the rofecoxib group when aspirin was administered before rofecoxib and 40.3±5.8 ng per milliliter when rofecoxib was administered before aspirin. All times are hours after the administration of the first study drug.

†P<0.001 for the comparison with the same drugs in the reverse order at the specific time point.

‡P=0.008 for the comparison with the same drugs in the reverse order at the specific time point.

by the additional therapy. Inhibition of cyclooxygenase-2 activity by ibuprofen, an isoform-nonspecific cyclooxygenase inhibitor, approached 80 percent two hours after the administration of the first study drug on day 6 and was rapid in onset and transient, as anticipated on the basis of the pharmacokinetic profile of the drug²⁹ (Table 1). Acetaminophen was also an inhibitor of cyclooxygenase-2 activity *ex vivo*, attaining 53±8 percent inhibition two hours after the administration of the first study drug on day 6 (Table 1). Rofecoxib caused prolonged and substantial inhibition of cyclooxygenase-2 activity *ex vivo* (more than 80 percent 12 hours after the administration of the first study drug on day 6) — an effect that is consistent with the drug's long half-life^{19,30} (Table 1).

The effects of the drugs on urinary 2,3-dinor-6-keto prostaglandin F_{1α} resembled their effects on the lipopolysaccharide-induced formation of prostaglandin E₂ *ex vivo* (Table 2). This finding was expected, given that cyclooxygenase-2 is the predominant source of 2,3-dinor-6-keto prostaglandin F_{1α} in healthy persons.^{27,28}

Parallel-Group Study with Multiple Daily Doses

One subject in the parallel-group study was replaced because of viral illness and the use of NSAIDs that were not part of the study regimen. When subjects received enteric-coated aspirin before the morning dose of ibuprofen, the serum thromboxane B₂ levels (Fig. 4A) and platelet aggregation (Fig. 4B)

recovered, as is consistent with the reversible inhibition of cyclooxygenase-1 activity and antagonism of aspirin's irreversible effect by the multiple-dose NSAID regimen. By contrast, the sustained inhibitory effect of enteric-coated aspirin on serum thromboxane B₂ and platelet aggregation was not altered by the administration of diclofenac (Fig. 4). Thus, 24 hours after the administration of the first study drug on day 6, the degree of inhibition of serum thromboxane B₂ was 92±3.8 percent when diclofenac was given after enteric-coated aspirin, as compared with 67±9.5 percent when multiple doses of ibuprofen were given after aspirin (P<0.05) (Fig. 4). Single doses of delayed-release diclofenac (75 mg), given two hours before aspirin (81 mg), for six days also produced no antagonism of the irreversible antiplatelet effect of aspirin (data not shown).

DISCUSSION

Aspirin reduces the incidence of recurrent myocardial infarction and stroke.^{2,3} Although it also reduces significantly the incidence of a first nonfatal myocardial infarction,^{31,32} this benefit is balanced by its propensity to induce gastrointestinal hemorrhage.³ NSAIDs are available over the counter for various indications and are prescribed widely for symptomatic relief for patients with arthritis.¹ More recently, selective inhibitors of the cyclooxygenase-2 isozyme have been shown to cause fewer gastrointestinal complications than traditional NSAIDs.³³

TABLE 2. INHIBITION OF URINARY 2,3-DINOR-6-KETO PROSTAGLANDIN F_{1α}*

| VARIABLE | DAY 1 | | | DAY 2 | | |
|------------------------------|--------------------|---------|----------|--------|---------|----------|
| | HR 0-6 | HR 6-12 | HR 12-24 | HR 0-6 | HR 6-12 | HR 12-24 |
| | percent inhibition | | | | | |
| Single-dose-ibuprofen group | | | | | | |
| Aspirin before ibuprofen | 34±8 | 67±10 | 32±10 | 74±6 | 54±10 | 33±7 |
| Ibuprofen before aspirin | 51±10 | 57±13 | 29±9 | 67±4 | 61±13 | 30±13 |
| Acetaminophen group | | | | | | |
| Aspirin before acetaminophen | 33±10 | 33±12 | 11±6 | 54±13 | 54±11 | 16±8 |
| Acetaminophen before aspirin | 36±12 | 37±13 | 17±9 | 59±7 | 40±11 | 40±5 |
| Rofecoxib group | | | | | | |
| Aspirin before rofecoxib | 36±8 | 81±5 | 66±9 | 80±4 | 84±3 | 82±6 |
| Rofecoxib before aspirin | 36±11 | 63±9 | 44±12 | 80±5 | 84±3 | 69±6 |

*Plus-minus values are means ±SE. The base-line level of urinary 2,3-dinor-6-keto prostaglandin F_{1α} was 94±23 pg per milligram of creatinine in the single-dose-ibuprofen group when aspirin was administered before ibuprofen and 89±18 pg per milligram of creatinine when ibuprofen was administered before aspirin, 96±29 pg per milligram of creatinine in the acetaminophen group when aspirin was administered before acetaminophen and 116±37 pg per milligram of creatinine when acetaminophen was administered before aspirin, and 113±18 pg per milligram of creatinine in the rofecoxib group when aspirin was administered before rofecoxib and 99±9 pg per milligram of creatinine when rofecoxib was administered before aspirin. There were no significant differences between a drug combination and the same drugs in the reverse order. All times are hours after the administration of the first study drug.

Neither traditional NSAIDs nor selective cyclooxygenase-2 inhibitors would be expected to afford substantial cardioprotection. NSAIDs inhibit the activity of the cyclooxygenase-1 isoform in the platelet, but most have actions that do not persist throughout the dosing interval rather than providing an irreversible effect like that of aspirin.^{5,15} The relation between the inhibition of platelet cyclooxygenase-1-dependent thromboxane A₂ generation and the inhibition of thromboxane-dependent platelet function is nonlinear,^{7,26} so the inhibition of platelet aggregability commonly does not persist during dosing with NSAIDs. Recently, it has been suggested that naproxen may differ from other NSAIDs in sustaining functionally important degrees of inhibition of platelet cyclooxygenase-1 activity throughout the dosing interval.³⁴ However, whether naproxen has a cardioprotective role remains controversial.¹⁰ Thus, many patients taking NSAIDs also take aspirin, usually in low doses, for cardioprotection. Platelet cyclooxygenase-1 activity is unaffected by the use of cyclooxygenase-2 inhibitors.²⁸ Concern about the depression of vascular prostaglandin I₂ production in the absence of concomitant platelet inhibition^{19,27,35} has enhanced awareness of the need for adjuvant antiplatelet therapy in appropriate patients who are receiving cyclooxygenase-2 inhibitors.

We wished to address the hypothesis that an NSAID, but not a cyclooxygenase-2 inhibitor, might competitively inhibit the ability of low-dose aspirin

to cause an irreversible inhibition of platelet function. Low-dose aspirin cumulatively inactivates cyclooxygenase in the anucleate platelet during prolonged dosing.^{36,37} Pretreatment with ibuprofen did, indeed, block the inhibition of platelet cyclooxygenase-1 activity and the impairment of platelet aggregation achieved by aspirin with prolonged dosing. These results are consistent with competitive inhibition by NSAIDs of the access of aspirin to the acetylation site in platelet cyclooxygenase-1 (Fig. 5).¹⁴ This interaction may be clinically relevant, because platelet aggregation may be sustained through the thromboxane pathway even if only 10 to 15 percent of the platelets remain functional. In our first study, this effect of ibuprofen could be bypassed by giving subjects aspirin two hours before a single daily dose of ibuprofen. However, in our second study, we simulated a more clinically relevant ibuprofen dosing regimen. Ibuprofen was administered three times per day, and an enteric-coated preparation of aspirin was administered once daily, as it is commonly used for cardioprotection in patients taking NSAIDs. Under these circumstances, the administration of aspirin before the morning dose of ibuprofen failed to circumvent the interaction. Thus, the inhibitory effects of daily low-dose aspirin on platelets are competitively inhibited by the prolonged use of multiple daily doses of ibuprofen, even when aspirin is administered before the first dose of the NSAID.

By contrast, the prolonged administration of a typ-

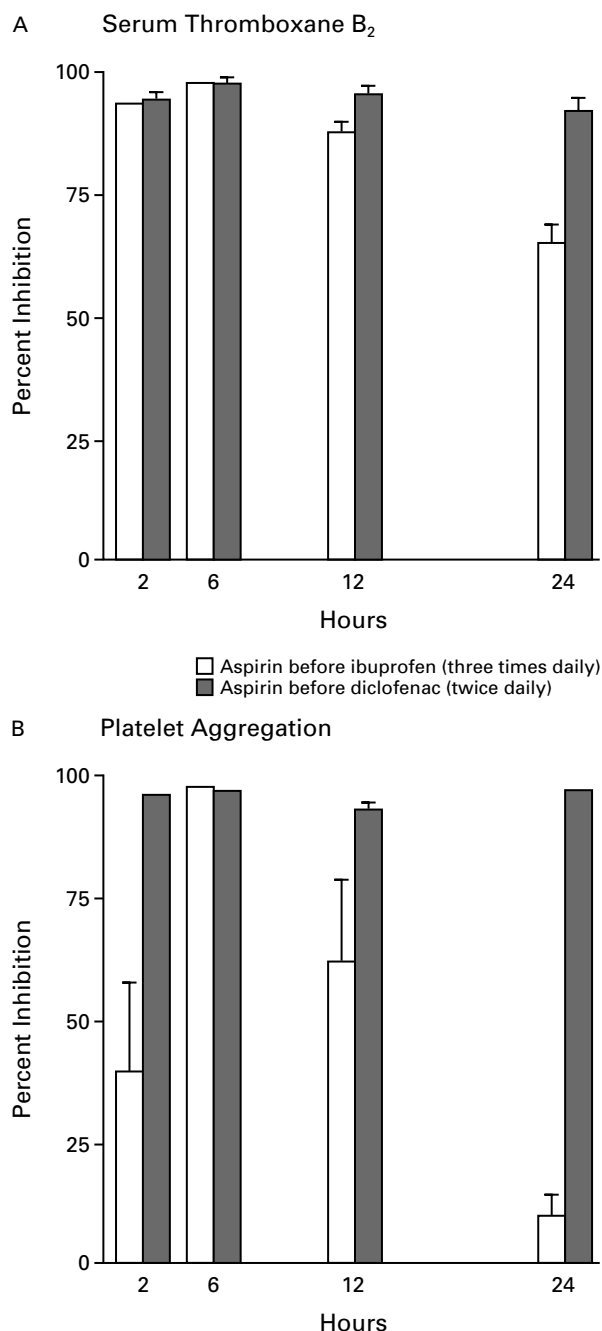


Figure 4. Mean Inhibition of Platelet Cyclooxygenase-1 Activity, as Assessed by Measurement of Serum Thromboxane B₂ (Panel A) and Inhibition of Platelet Aggregation (Panel B) in Subjects Taking Enteric-Coated Aspirin (81 mg) Two Hours before Either Ibuprofen (400 mg Three Times Daily) or Delayed-Release Diclofenac (75 mg Twice Daily) on Day 6 of Prolonged Dosing.

The base-line level of serum thromboxane B₂ was 423±54 ng per milliliter in the multiple-dose-ibuprofen group and 400±60 ng per milliliter in the diclofenac group. The I bars represent SEs. P=0.04 for the comparison of the serum thromboxane B₂ level between the groups at 24 hours (Panel A), and P=0.006 for the comparison of platelet aggregation between groups at 24 hours (Panel B). All times are hours after the administration of the first study drug.

ical regimen of delayed-release diclofenac (twice daily) did not inhibit the antiplatelet effect of enteric-coated aspirin. This lack of interaction may reflect the lower potency and shorter duration of action of the diclofenac regimen. In studies of short-term dosing with delayed-release diclofenac, we found that the degree of inhibition of serum thromboxane B₂ was 87±6.2 percent two hours after dosing and had returned to 54.6±7.4 percent inhibition within six hours. By contrast, inhibition was more pronounced two hours after short-term dosing with ibuprofen (94.6±2.3 percent, P<0.05) and was more sustained six hours after dosing with ibuprofen (81.4±4.1 percent, P<0.05) than after the administration of delayed-release diclofenac. An alternative hypothesis is that the diclofenac and ibuprofen afford differential impedance of the access of aspirin to the serine residue at position 529 in prostaglandin G/H synthase. Although ibuprofen, flurbiprofen, indomethacin, and suprofen all bind cyclooxygenase-1 in superimposable configurations,¹⁵ the binding of diclofenac may be spatially segregated from such inhibitors within the hydrophobic channel.^{38,39} However, the relevance of this observation and of the kinetic distinctions between the two drugs to our findings regarding platelet cyclooxygenase-1 remain to be addressed directly.

As expected,⁴⁰ rofecoxib did not influence the effects of aspirin. Cyclooxygenase-2 inhibitors bind to a side pocket that is present in the hydrophobic channel of cyclooxygenase-2 but not in that of cyclooxygenase-1.⁴¹ Consideration of the cost of treatment with traditional NSAIDs and recognition of the failure of selective cyclooxygenase-2 inhibitors to afford cardioprotection have raised interest in their combination with aspirin.¹⁹

Finally, we obtained detailed information on the effect of acetaminophen on cyclooxygenase function. Although acetaminophen is recommended for osteoarthritis of the hip and knee⁴² and is widely used as an antipyretic, it is less effective than traditional NSAIDs as an antiinflammatory agent.^{43,44} We found that, at a dose of 1000 mg, acetaminophen is a weak, reversible, isoform-nonspecific cyclooxygenase inhibitor. Such limited inhibition of cyclooxygenase-1 does not inhibit platelet function. Moreover, since competitive inhibition at the active site of cyclooxygenase would be expected to be concentration-dependent and saturable,¹⁵ it is perhaps not surprising that acetaminophen also fails to modify the antiplatelet action of aspirin. Higher doses of acetaminophen or regimens entailing multiple daily doses have been reported to have a gastrointestinal adverse-effect profile that resembles that of traditional NSAIDs.⁴⁵ It remains to be determined whether such regimens might afford levels of cyclooxygenase inhibition similar to those attained with 400 mg of ibuprofen and have similar effects on the action of aspirin.

In summary, our findings suggest that commonly

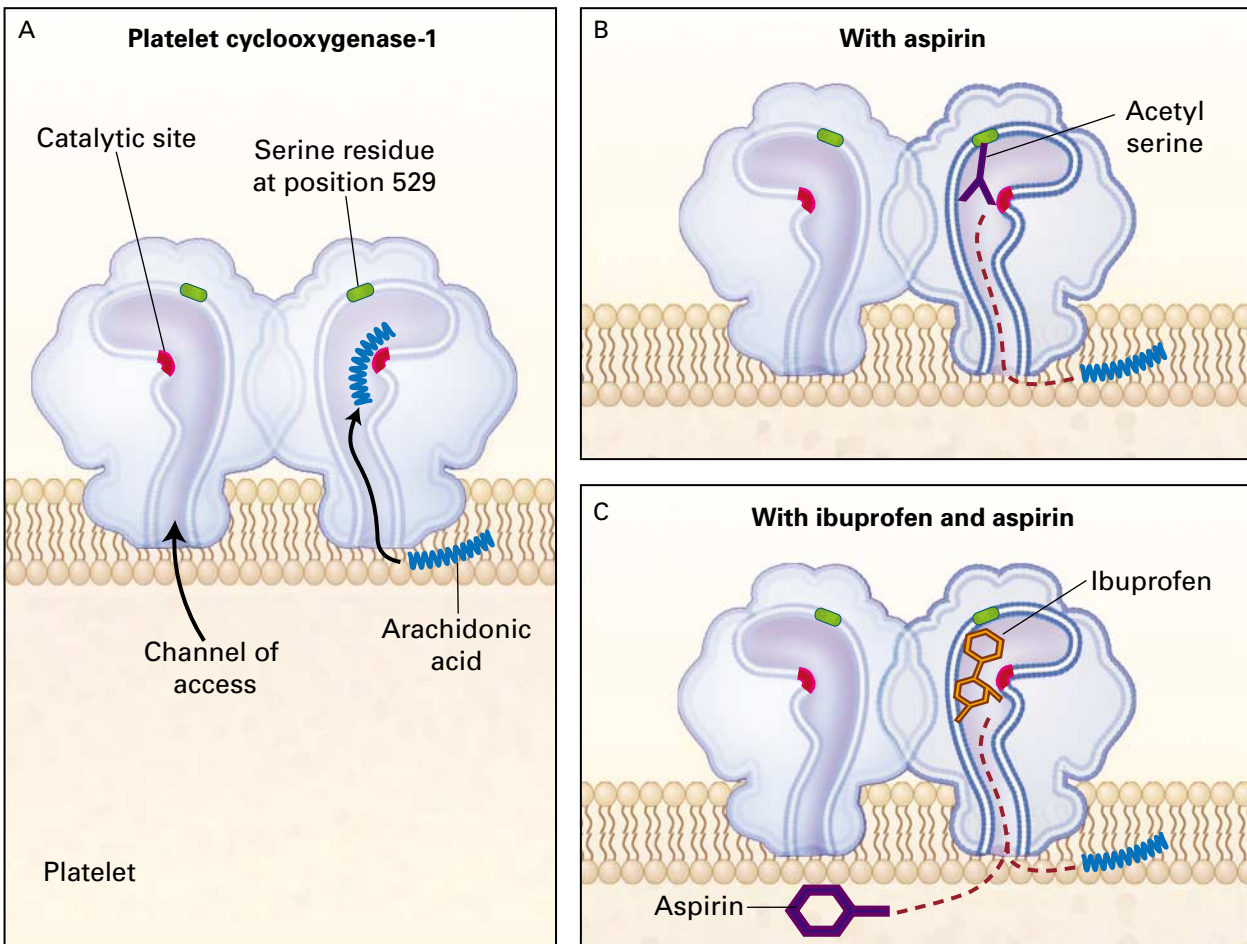


Figure 5. The Effect of Aspirin Alone and of Ibuprofen plus Aspirin on Platelet Cyclooxygenase-1.

The platelet prostaglandin G/H synthase-1 (cyclooxygenase-1) is depicted as a dimer. The arachidonic acid substrate gains access to the catalytic site (red area) through a hydrophobic channel that leads into the core of the enzyme (Panel A). Aspirin blocks the access of arachidonic acid to the catalytic site by irreversibly acetylating a serine residue at position 529 in platelet cyclooxygenase-1, near but not within the catalytic site (Panel B). Interpolation of the bulky acetyl residue prevents metabolism of arachidonic acid into the cyclic endoperoxides PGG₂ and PGH₂ for the lifetime of the platelet. Because PGH₂ is metabolized by thromboxane synthase into thromboxane A₂, aspirin prevents the formation of thromboxane A₂ by the platelets until new platelets are generated. Nonsteroidal antiinflammatory drugs, such as ibuprofen, are reversible, competitive inhibitors of the catalytic site (Panel C) whose use results in the reversible inhibition of thromboxane A₂ formation during the dosing interval. Prior occupancy of the catalytic site by ibuprofen prevents aspirin from gaining access to its target serine.

used analgesics may modulate the cardioprotective effects of low-dose aspirin to differing degrees. A clinical dosing regimen of ibuprofen may competitively inhibit the sustained inhibitory effect on platelets that underlies the cardioprotective property of aspirin. This observation is likely to extend to a second NSAID, indomethacin,^{15,16} but not to diclofenac or the cyclooxygenase-2 inhibitor rofecoxib. Finally, acetaminophen is a weak, reversible, isoform-nonspecific cyclooxygenase inhibitor.

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Dr. Catella-Lawson is an employee of Merck.

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