Clinical pharmacology of platelet cyclooxygenase inhibition

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ABSTRACT Nonsteroidal anti-inflammatory drugs and sulfinpyrazone compete dose-dependently with arachidonate for binding to platelet cyclooxygenase. Such a process closely follows systemic plasma drug concentrations and is reversible as a function of drug elimination. Peak inhibition and extent of its reversibility at 24 hr varies consistently with individual pharmacokinetic profile. Inhibition of platelet cyclooxygenase activity by these agents is associated with variable effects on prostaglandin (PG) synthesis in the gastric mucosa and the kidney. Aspirin acetylates platelet cyclooxygenase and permanently inhibits thromboxane (TX) A₂ production in a dose-dependent fashion when single doses of 0.1 to 2.0 mg/kg are given. Acetylation of the enzyme by low-dose aspirin is cumulative on repeated dosing. The fractional dose of aspirin necessary to achieve a given level of acetylation by virtue of cumulative effects approximately equals the fractional daily platelet turnover. Serum TXB₂ measurements obtained during long-term dosing with 0.11, 0.22, and 0.44 mg/kg aspirin in four healthy subjects could be fitted by a theoretical model assuming identical acetylation of platelet (irreversible) and megakaryocyte (reversible) cyclooxygenase. For a given dose within this range, both the rate at which cumulative acetylation occurs and its maximal extent largely depend upon the rate of platelet turnover. Continuous administration of low-dose aspirin (20 to 40 mg/day) has no statistically significant effect on urinary excretion of either 6-keto-PGF₁₀ or 2,3-dinor-6-keto-PGF₁₀, i.e., indexes of renal and extrarenal PGI₂ biosynthesis in vivo. Whether a selective sparing of extraplatelet cyclooxygenase activity by low-dose aspirin will result in increased antithrombotic efficacy, fewer toxic reactions, or both remains to be established in prospective clinical trials.

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PLATELET CYCLOOXYGENASE or prostaglandin (PG) H synthase (i.e., the enzyme that converts arachidonate released from membrane phospholipids into PG endoperoxides) is the target of several antiplatelet agents that can reversibly or irreversibly block the activity of the enzyme by competing with the substrate or permanently altering the active site, respectively. Such drugs belong to the class of so-called nonsteroidal anti-inflammatory agents (e.g., indomethacin, aspirin), but also include a uricosuric agent, i.e., sulfinpyrazone. This article will review the available information on platelet cyclooxygenase inhibition, as derived from human studies in health and disease, and present novel findings related to the mechanism of and variables affecting cumulative inhibition of the enzyme by low-dose aspirin.

Assessment of human platelet cyclooxygenase activity. Platelet PGH synthase levels can be determined with an immunoradiometric assay. However, PGH synthase immunoreactivity is not influenced by inactivation of the enzyme with aspirin. Similarly, a dissociation between platelet PGH synthase concentration and activity has been shown in uremic patients. Also, the enzyme can be measured by its ability to interact selectively with (acetyl-³H) aspirin and undergo a site-specific acetylation reaction, giving rise to (acetyl-³H) PGH synthase. Inactivation of platelet cyclooxygenase by aspirin in vivo can be measured as a reduction in the ability of (acetyl-³H) aspirin to acetylate the enzyme in washed platelets in vitro.

In the vast majority of studies assessing pharmacologic inhibition of platelet cyclooxygenase enzyme activity was measured ex vivo by quantitative analysis of the main product(s). These analyses may include measurement of (1) thromboxane (TX) A₂-like biological activity released by exogenous arachidonate in platelet-rich plasma (PRP), (2) malondialdehyde (MDA) production in PRP stimulated with N-ethyl-malei-
mide,7 and (3) TXB2 production in whole blood,8 agonist-stimulated PRP,9,10 or bleeding-time blood.11 The latter have gained wider acceptance as indicators of platelet inhibition because of the more accurate and specific determination of TXB2 by radioimmunoassay (RIA) or gas chromatography/mass spectrometry (GC/MS). These methods assess the capacity of platelets to synthesize TXB2 in response to native or exogenously added stimuli and by no means reflect the actual production rate of TXB2 in vivo.12 Such platelet-specific indexes can be used appropriately in pharmacologic studies in which drug-induced changes in cyclooxygenase or TX synthase are being investigated.

In our studies, we have used serum TXB2 determinations as a reflection of thrombin-induced platelet TXA2 production during whole blood clotting.5 Serum levels of this eicosanoid averaged 300 ± 108 ng/ml (mean ± SD) in a group of 177 healthy subjects, with no statistically significant differences between men and women.13 In this study, no statistically significant correlation was found between serum TXB2 and any of the continuous variables examined (age, body mass index, blood pressure, cigarette smoking, and serum cholesterol, triglyceride, and glucose) except for the number of platelets. Upon repeated blood sampling on successive days in healthy subjects, the intrasubject variability in serum TXB2 determinations averaged 14 ± 5%,14 a value allowing accurate determination of marginal inhibition of enzyme activity. Consistent results have been reported from different laboratories, whether serum TXB2 was measured by RIA14-16 or GC/MS.17

The dinor metabolite of TXB2 is the most abundant urinary derivative of systemically administered radioactive TXB2 in man,18 and it largely reflects systemic (extrarenal) TXB2 synthesis in vivo.12 The relative contribution of platelet and extraplatelet sources to urinary 2,3-dinor-TXB2 remains to be determined under pathophysiologic conditions. The finding that daily dosing with 20 mg aspirin can reduce urinary 2,3-dinor-TXB2 excretion by 67% in healthy subjects8 suggests that it may represent primarily platelet TXB2 production under physiologic conditions.

Reversible inhibition of platelet cyclooxygenase. Nonsteroidal anti-inflammatory drugs (with the exception of aspirin) and sulfinpyrazone compete dose dependently with arachidonate for binding to cyclooxygenase. Such a process closely follows systemic plasma drug concentrations and is reversible as a function of drug elimination. The extent and duration of inhibition of human platelet cyclooxygenase activity after a single dose of each of several of the currently available nonsteroidal anti-inflammatory drugs is detailed in table 1. Peak inhibition and extent of its reversibility at 24 hr varied consistently with individual pharmacokinetic profile.

Upon repeated dosing, no evidence of cumulative inhibition of platelet cyclooxygenase activity was found with ibuprofen or sulindac in patients with chronic glomerular disease.19 Thus, 85% reduction of platelet TXB2 production was measured after 1 week of treatment with 200 mg bid sulindac.19

With the notable exception of indomethacin, it appears that none of these inhibitors with reversible effects can achieve and sustain greater than 95% inhibition of platelet enzyme activity. Inasmuch as virtually complete and long-lasting suppression of TXA2 production may be required for maximal inhibition of platelet function,5 it appears likely that these and similar agents have limited antiplatelet efficacy.

Similar considerations might apply to partial reversible inhibition of platelet cyclooxygenase activity by sulfinpyrazone. Inhibition of cyclooxygenase activity is dependent on reduction to an active sulfide metabolite.20 When added to human whole blood in vitro this metabolite inhibits TXB2 production with 50% inhibitory concentration of 11 μM as compared with the 247 μM of the parent sulfide.* When given to healthy volunteers, sulfinpyrazone (200 mg qid) reduced platelet TXB2 production by 65%, with no evidence of cumulative inhibition over 7 days.21 This finding is consistent with similar sulfide levels measured after 1 and 5 days of sulfinpyrazone dosing in healthy subjects.22 The contribution of such limited suppression of platelet cyclooxygenase activity to the reported clinical efficacy of therapy22 remains to be established.

Inhibition of platelet cyclooxygenase activity by


| TABLE 1 |
| Reversible inhibition of platelet cyclooxygenase activity (as reflected by serum TXB2 levels) by single oral doses of nonsteroidal anti-inflammatory drugs in humans (n = 5 for each drug) |
| Drug | Dose (mg) | Percentage of initial enzyme activity |
| --- | --- | --- | --- |
| Indomethacin | 75 | 2 ± 2 | 2 ± 1 | 50 ± 23 |
| Diflunisal | 750 | 46 ± 20 | 35 ± 17 | 65 ± 21 |
| Naproxen | 250 | 11 ± 3 | 12 ± 3 | 22 ± 12 |
| Diclofenac | 50 | 19 ± 7 | 34 ± 6 | 57 ± 3 |
| Sulindac | 200 | 38 ± 16 | 22 ± 2 | 62 ± 33 |
| Piroxicam | 20 | 68 ± 22 | 41 ± 16 | 27 ± 18 |
| Ibuprofen | 300 | 33 ± 14 | 12 ± 4 | 59 ± 39 |

Values are mean ± SD.
these agents is associated with variable effects on PG synthesis in the gastric mucosa and the kidney. Such a differential pattern of inhibition may account for the diverse incidence of gastric and renal side effects associated with long-term use of cyclooxygenase inhibitors. One clear example of tissue-selective inhibition of cyclooxygenase activity is provided by sulindac and sulfipyrazone. We have previously shown that sulindac does not inhibit renal PG synthesis in man. This property is probably related to reoxidation of the active sulfide metabolite to its prodrug form within the kidney. Sulindac and sulfipyrazone share the following pharmacokinetic and pharmacodynamic features: (1) the major biotransformations involve valence changes at the sulfur atom, (2) inhibition of cyclooxygenase activity is dependent on reduction to an active sulfide metabolite, and (3) the sulfide metabolite is not detected in urine after oral dosing of the prodrug. Sulfipyrazone, similar to sulindac, does not inhibit renal cyclooxygenase activity when given to healthy subjects at a dosage (200 mg qid for 7 days) that significantly reduces platelet TXB₂ production and urinary 2,3-dinor-6-keto-PGF₁α excretion, i.e., that reducing cyclooxygenase activity at platelet and vascular sites. The finding of a selective sparing of renal cyclooxygenase activity by sulfipyrazone is consistent with the absence of short-term effects on glomerular filtration rate both in healthy subjects and patients with myocardial infarction.

Irreversible inactivation of platelet cyclooxygenase. Aspirin selectively acetylates the hydroxyl group of a single serine residue within the polypeptide chain of PGH₂ synthase, thereby inactivating the enzyme. The serine side chain is probably located near the active site of the enzyme. As a consequence of this acetylation, the cyclooxygenase activity (arachidonate → PGG₂) of the enzyme is lost, while the peroxidase activity (PGG₂ → PGH₂) is unaffected. The stoichiometry of this reaction is 1:1, with one acetyl group transferred per enzyme monomer (Mᵣ = 70,000). At low concentrations, aspirin acetylates PGH₂ synthase rapidly (within minutes) and selectively. At high concentrations, over longer time periods, aspirin will also acetylate nonspecifically a variety of proteins and nucleic acids. When given orally to healthy subjects, aspirin acetylates circulating platelet cyclooxygenase and inhibits TXB₂ production in a dose- and time-dependent fashion. A linear inhibition of platelet cyclooxygenase activity was found after a single dose in the range of 0.1 to 2 mg/kg. Moreover, such a dose-response relationship is substantially identical in healthy subjects and in patients with atherosclerosis when assessed by the same technique of determination of TXB₂ production ex vivo in whole blood. No sex-related difference in the effect of aspirin was noted in these studies.

Evidence has been presented that the return of cyclooxygenase activity after a single dose (100 to 400 mg) of aspirin does not occur for approximately 48 hr. The 2 day lag in the return of unacetylated enzyme to the circulation has been interpreted as evidence that aspirin acetylates cyclooxygenase in the megakaryocyte. However, platelet aggregation and secretion have been reported to occur as early as 4 hr after ingestion of aspirin (650 mg) in response to a combination of arachidonate with epinephrine, collagen, or ADP. This finding may suggest the early entry into the circulation of platelets originating from megakaryocytes in which cyclooxygenase has not been completely acetylated. It should be pointed out that the functional significance of a small percentage of aspirin-free platelets may be greatly enhanced by the performance of platelet aggregation studies in vitro, under conditions in which both removal (by flow) and metabolism (by lung and liver enzymes) of TXA₂ are absent.

Because of irreversible enzyme inactivation and lack of enzyme synthesis in platelets de novo, acetylation of platelet cyclooxygenase and consequent inhibition of TXA₂ production by low-dose (20 to 40 mg) aspirin is cumulative on repeated dosing. To investigate the variables affecting the rate and maximal degree of cumulative inhibition, we studied seven healthy subjects (four men and three women 23 to 38 yr old) receiving short- and/or long-term therapy with oral aspirin, 5 to 40 mg. As shown in figure 1, a single oral 20 mg dose of aspirin caused a time-dependent reduction in serum TXB₂ levels. Serum TXB₂ was significantly reduced to 82% of the control value, as early as 5 min after dosing. Inasmuch as no acetylsalicylic acid can be detected in peripheral venous blood at this time, acetylation of cyclooxygenase during the first 5 min may occur by exposure of platelets to the drug in the presystemic circulation.

Serum TXB₂ reached a 52% nadir at between 30 and 60 min and remained stable thereafter up to 24 hr. Upon repeated daily dosing with 20 mg aspirin, each dose caused a time-dependent reduction in serum TXB₂ that reached a nadir at 60 min and persisted after 24 hr (data not shown). This pattern is consistent with acetylation of megakaryocyte cyclooxygenase by low-dose aspirin. Serum TXB₂ was reduced to 46% of the control value after the first dose, to 27% after the second dose, and to 11% after the third dose. Platelet
cyclooxygenase activity, as reflected by TXB₂ generation in serum measured after daily administration of 20 mg aspirin for 3 days, is illustrated in figure 2 as a percentage of basal activity before drug in the same subject. Serum TXB₂ was reduced to 46% of control 60 min after the first dose, to 53% after the second dose, and to 43% after the third dose, with a similar time course. Given a 14% intrasubject coefficient of variation in serum TXB₂ determinations in our laboratory,¹⁴ the data presented in figure 2 demonstrate induction of a virtually identical acetylation process of platelet cyclooxygenase by successive doses of aspirin, irrespective of the initial level of acetylation of the enzyme.

To study the time and dose dependence of cumulative inhibition, four healthy subjects received aspirin in daily doses of 0.44, 0.22, and 0.11 mg/kg on separate occasions. Each dose was given for 1 month and serum TXB₂ was measured before, during, and for 2 weeks after aspirin was given. Daily dosing with 5 to 10 mg aspirin (0.11 ± 0.02 mg/kg, mean ± SD; n = 4) caused a maximal inhibition of cyclooxygenase activity of 56% to 76% within 18 to 30 days. Daily dosing in the same subjects with 10 to 20 mg (0.22 ± 0.03 mg/kg) caused a maximal inhibition of 68% to 90% within 10 to 20 days. Daily dosing with 20 to 40 mg (0.44 ± 0.06 mg/kg) caused a maximal inhibition of 92% to 95% within 6 to 12 days. As shown in figure 3, a linear relationship exists between the oral aspirin dose and the percentage inhibition of platelet TXB₂ production, both after single and daily dosing. The 50% inhibitory dose for one-time dosing was 26 mg, while that for repeated dosing (measured at steady-state) was 3.2 mg, or only 12.5% of the former.

This finding suggests that the fractional dose of aspirin necessary for achieving a given level of acetylation by virtue of cumulative effects approximately equals the fractional daily platelet turnover (10% to 15%). Such an interpretation is further supported by an experiment in which virtually complete suppression of platelet TXB₂ production was achieved by a single dose of 4 mg/kg and maintained over 5 weeks by daily dosing with 11% of this dose (0.44 mg/kg; data not shown). The inverse linear relationship (r = −.92, n
achieve steady-state inhibition with long-term dosing, and percentage inactivation of platelet cyclooxygenase after first dosing with aspirin. Individual data from 12 experiments are plotted. Each of four healthy subjects underwent daily treatment for 1 month with three different doses in the range of 5 to 40 mg. Serum TXB$_2$ was measured as a reflection of platelet cyclooxygenase activity.

\[ \text{Max % initial acetylation: 15, 35, 55%} \]

\[ \text{Platelet life span: 15, 15, 15 days} \]

$\Delta$ 25 mg/d  
$\triangle 12.5$ mg/d 
$\downarrow 6$ mg/d

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<tr>
<th>Max inhib.</th>
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<td>95</td>
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<td>89</td>
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FIGURE 5. Time course of platelet cyclooxygenase inactivation and reappearance as a function of aspirin dosage. Results of three monthly experiments performed in the same healthy subject are depicted. Serum TXB$_2$ was measured as a reflection of platelet cyclooxygenase activity before, during, and after dosing with 6 (●), 12.5 (□), and 25 (▲) mg/day. The open symbols represent computer-simulated daily levels of enzyme activity. The best fit of experimental data was provided by a combination of 15%, 35%, and 55% initial acetylation (after first dosing) at 6, 12.5, and 25 mg, respectively, and a platelet life span of 15 days. Percentage maximal inactivation and time interval necessary to achieve steady-state inhibition of platelet cyclooxygenase, as obtained by computer simulation, is also indicated.

$\Rightarrow 12, p < .001$) between the time interval necessary to achieve steady inhibition with long-term dosing and percentage inactivation of platelet cyclooxygenase after the first dose is shown in figure 4. Each set of experimental data could be fitted by a theoretical model assuming that (1) aspirin acetylates platelet as well as megakaryocyte cyclooxygenase, (2) the two enzymes are equally sensitive to aspirin, (3) synthesis of the enzyme de novo occurs within 24 hr in megakaryocytes but not in platelets, and (4) platelets with acetylated and those with unacetylated cyclooxygenase have a similar life span. As shown in figure 5, which illustrates results of three monthly experiments performed in the same subject, the computer-simulated pattern of inhibition closely matched the experimental data obtained at three dose levels. As an average, the two sets of data (computer-generated vs actual measurements of serum TXB$_2$) did not differ from each other by a greater interval than the intrasubject coefficient of variation for the method.

In view of the above findings and of recent studies of Pedersen and FitzGerald demonstrating unchanged systemic bioavailability of aspirin in doses of 20 to 1300 mg and presystemic acetylation of platelet cyclooxygenase by low doses (20 to 40 mg) of the drug, it
appears likely that platelet life span represents a major variable affecting cumulative inhibition within this particular dose range. For a given dose, both the rate at which cumulative acetylation occurs and its maximal extent would essentially depend on the rate of platelet turnover and dosing interval.

Although the long-lasting suppression of TXA$_2$-related platelet function by aspirin has been disputed on the basis of studies using pairs of aggregating agents, the likely occurrence of this phenomenon in vivo is suggested by the positive results (>50% reduction in thrombotic events) of three clinical trials of once-daily dosing: a study of 160 mg in patients on hemodialysis, one of 324 mg in men with unstable angina, and one of 100 mg in patients with aortocoronary bypass. These results are consistent with the hypothesis that the antithrombotic effect of aspirin is largely or entirely related to irreversible inactivation of platelet cyclooxygenase and consequent suppression of TXA$_2$-related platelet function. Further support for this contention derives from recent studies of Steele et al. demonstrating that low-dose aspirin (1 mg/kg/day) is as effective as high-dose aspirin (20 mg/kg/day) combined with dipyridamole in preventing platelet deposition and mural thrombus formation after arterial balloon angioplasty in pigs. Although mechanisms other than acetylation of platelet cyclooxygenase may be operative after doses of aspirin of 10 to 20 mg/kg, the likelihood of this occurring at doses 10 to 20 times lower is limited by the stoichiometry of the acetylation reaction and its dose dependence in vivo.

**Is low-dose aspirin a selective inhibitor of platelet cyclooxygenase?** Dissociation of the vascular from the platelet inhibiting effects of aspirin has been attempted on the assumption that intact PGI$_2$ production might enhance the antithrombotic efficacy of the drug. Although probably not serving the role of a circulating antiplatelet hormone, as originally proposed, PGI$_2$ may still be important in the local modulation of platelet-endothelial interactions, particularly in patients with severe atherosclerosis and platelet activation. Complete separation of platelet-inhibiting from vascular effects of aspirin can not be demonstrated after single doses. However, continuous administration of aspirin in low doses (20 to 40 mg/day) has no statistically significant effects on urinary excretion of either 6-keto-PGF$_{1\alpha}$ or 2,3-dinor-6-keto-PGF$_{1\alpha}$, two indexes of renal and extrarenal PGI$_2$ biosynthesis in vivo. Inasmuch as the cumulative nature of aspirin-induced inhibition of cyclooxygenase activity is a function of the different rates of daily acetylation and turnover of the enzyme (cell turnover or synthesis de novo), careful consideration should be given to the possibility of variable effects of the drug when pathologic processes affect platelet and/or endothelial behavior. Thus, enhanced platelet turnover and/or endothelial damage might decrease the effectiveness and/or selectivity of low doses of aspirin.

De Caterina et al. have reported that in patients recovering from myocardial infarction, low-dose aspirin (0.4 mg/kg/day) persistently inhibits platelet cyclooxygenase activity and TXA$_2$-dependent platelet function over 1 month of therapy without significantly reducing urinary 6-keto-PGF$_{1\alpha}$ excretion. In contrast, Weksler et al. have reported evidence for a significant cumulative inhibition of vascular PGI$_2$ synthesis in atherosclerotic patients given 20 mg aspirin daily for 7 days. This study showed that there was a cumulative inhibition of maximal production of PGI$_2$ ex vivo in vascular tissue, but that synthesis was 50% preserved. This may indicate that sufficient reserve exists, that urinary 6-keto-PGF$_{1\alpha}$ or 2,3-dinor-6-keto-PGF$_{1\alpha}$ might not be depressed, and that sufficient PGI$_2$ for local modulation of platelet function might persist. The long-term effects of intermittent aspirin regimens have not been explored, although evidence exists that they may effectively abolish platelet TXA$_2$ production in healthy subjects. On theoretical grounds, it appears that the alleged benefit of giving a single fully effective dose (2 to 3 mg/kg) every 2 or 3 days might be offset by the profound and long-lasting inhibition of vascular PGI$_2$ production after each dose. An alternative regimen of 40 mg every 48 hr has been proposed recently. However, it does not appear that this regimen offers any appreciable advantage with respect to sparing venous PGI$_2$ production when compared with the daily administration of the same dose.

**Is selective inhibition of platelet cyclooxygenase activity by low-dose aspirin clinically important?** The observation that aspirin can exert an antithrombotic effect when given in a dose that largely suppresses vascular PGI$_2$ production and a critical appraisal of its role in platelet-vessel wall homeostasis have raised skepticism on the clinical relevance of sparing extraplatelet sites of cyclooxygenase activity. To the extent that PGI$_2$ biosynthesis is enhanced in patients with severe atherosclerosis, possibly as a consequence of platelet interactions with endothelium or other vascular insults, preservation of the capacity of the vessel wall to produce this natural platelet-inhibitory compound would be desirable in the setting of platelet activation in vivo. Moreover, aspirin-induced reduction of PGI$_2$ production in the gastric mucosa and in glomeruli has been
proposed to contribute importantly to gastric and renal side effects, respectively, that are associated with long-term use of the drug at high dosage.

Whether a selective sparing of extraplatelet cycloxygenase activity by low-dose aspirin will result in increased antithrombotic efficacy, fewer toxic reactions, or both remains to be established in prospective clinical trials.

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References

37. Weksler BB, Tack-Goldman K, Subramanian VA, Gay WA Jr: Cumulative inhibitory effect of low-dose aspirin on vascular pros-
Clinical pharmacology of platelet cyclooxygenase inhibition.
C Patrono, G Ciabattoni, P Patrignani, F Pugliese, P Filabozzi, F Catella, G Davì and L Forni

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