Transdermal Modification of Platelet Function

A Dermal Aspirin Preparation Selectively Inhibits Platelet Cyclooxygenase and Preserves Prostacyclin Biosynthesis

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Background. Even low doses of oral aspirin inhibit prostacyclin (prostaglandin [PG] I_2) formation and cause gastrointestinal toxicity. We examined the skin as a novel route for continuous low-dose aspirin administration and selective inhibition of platelet cyclooxygenase in humans.

Methods and Results. Aspirin 250 or 750 mg/d for 10 days induced a dose-dependent inhibition of serum thromboxane (TX) B_2. At the highest dose, five of six subjects responded, with a mean reduction in serum TXB_2 of 95±3% (P=.003). Urinary 2,3-dinor TXB_2, an index of in vivo TXA_2 formation, decreased by 68±7% and recovered slowly, consistent with inhibition of platelet cyclooxygenase in vivo. In contrast, PGI_2 biosynthesis, determined as excretion of 2,3-dinor-6-keto PGF_1alpha, was 81±5% of baseline at 10 days. Intravenous bradykinin increased PGI_2 biosynthesis 5.1±1.6-fold (n=4) before aspirin treatment. Oral aspirin 75 mg/d for 14 days abolished bradykinin-induced PGI_2 formation, whereas dermal aspirin 750 mg/d had no effect despite similar inhibition of TXA_2 biosynthesis. In five subjects, plasma aspirin and salicylate were determined after a single application of 750 mg. Aspirin was absorbed slowly, with peak levels of 0.24±0.11 µg/mL at 3 hours. Salicylate levels peaked at 6 to 12 hours, with plasma levels of 0.79±0.14 µg/mL.

Conclusions. Thus, it is possible to achieve selective inhibition of platelet cyclooxygenase by aspirin applied to the skin. This approach may be applicable to other antiplatelet agents and be useful in patients at risk for gastrointestinal bleeding or toxicity. (Circulation 1993;88:556-561)

Key Words • aspirin • platelets • thromboxanes

Aspirin is an effective antithrombotic agent, a response that has been attributed to inhibition of platelet cyclooxygenase and thromboxane (TX) A_2 formation. TXA_2 is formed by all known platelet agonists and is itself a potent platelet activator. Inhibition of platelet TXA_2 generation is maintained at doses of aspirin as low as 30 to 80 mg/d, which have also been effective clinically. Low-dose aspirin has been used increasingly in an effort to preserve prostacyclin (prostaglandin [PG] I_2) biosynthesis and reduce gastrointestinal (GI) toxicity. However, biosynthesis of PGI_2, the major cyclooxygenase product of vascular endothelium and a potent platelet inhibitor, is suppressed at these low doses. Moreover, the threshold dose of aspirin for increased gastric blood loss is estimated to be 30 mg/d, and major GI bleeds have been reported at 30 and 75 mg/d.

A greater degree of platelet selectivity can be achieved by administering aspirin as a continuous, low-dose infusion rather than as a bolus. Platelets are exposed to a relatively high concentration of aspirin at the site of drug administration. Since the enzyme is irreversibly inhibited and cannot be regenerated, cumulative inhibition of platelet cyclooxygenase occurs over time. On the other hand, the absorbed aspirin is metabolized to the largely inactive salicylate by esterases in blood and tissues before it reaches the systemic vasculature. A very high degree of platelet selectivity has been achieved with a controlled-release oral preparation of aspirin. Since the gut is still exposed to the drug, however, there may be a potential for bleeding. In this study, we examined the skin as a novel route for continuous, low-dose aspirin administration and selective inhibition of platelet cyclooxygenase. The skin acts as a reservoir for aspirin, with as much as 10% to 15% absorbed over 24 to 48 hours after a single application. Assuming that 40 mg/d is required for >90% inhibition of platelet cyclooxygenase, we examined the effect of aspirin 250 to 750 mg applied daily on thromboxane A_2 and PGI_2 biosynthesis in healthy subjects.

Methods

Prostaglandin Biosynthesis

The protocol was approved by the Human Subjects Committees of the Institutional Review Boards of the Gundersen Clinic and Vanderbilt University. All subjects gave written, informed consent before enrollment. Only healthy volunteers were studied. The subjects were asked to avoid aspirin and any other cyclooxygenase inhibitors.
inhibitors for 10 days before and throughout the period of investigation. Aspirin (acetyl salicylic acid, USP) powder was dissolved in isopropyl alcohol or ethanol and propylene glycol (1:7:1 vol/vol) to a final concentration of 94 mg/mL. Preliminary studies demonstrated that aspirin was stable in this vehicle, with <1% salicylate detected after 24 hours at room temperature. The aspirin preparation was made daily immediately before its application. Volunteers attended the pharmacy where the preparation was applied and were asked not to wash the area for at least 12 hours. The aspirin solution was applied to the forearm and upper arm over a 15-minute interval. Volunteers received aspirin 250 mg (n=4), aspirin 750 mg (n=8), or vehicle (n=6) for 10 days and were followed for a further 8 days after drug withdrawal. The volunteers were 31 to 56 years old, with equal numbers of men and women in each treatment group.

Blood without anticoagulant was obtained for serum TXB2, the stable metabolite of TXA2, before and at intervals during and after aspirin administration. The blood was allowed to clot in glass at 37°C for 60 minutes, and the serum was removed and stored at −20°C until analyzed. Urine was collected over a period of 24 hours at corresponding times for measurements of 2,3-dinor-TXB2 (TXM) and 2,3-dinor-6-keto-PGF1α (PGI-M), major enzymatic metabolites of TXA2 and PGI2, respectively.19 Excretion of these products is an index of the in vivo formation of their parent compounds.20,21 Serum TXB2 and urinary metabolites were determined by negative ion chemical ionization, gas chromatography/mass spectrometry (NICI-GCMS) using authentic deuterium-labeled analogues as internal standards as previously described.22,23 The results are expressed in respect to creatinine concentration to account for changes in urine volume.

Bradykinin Stimulation Studies

In an additional four subjects, we examined the increase in PGI2 formation in response to intravenous bradykinin (Calbiochem, La Jolla, Calif) before and after oral aspirin 75 mg or dermal aspirin 750 mg daily for 14 days. The protocol for bradykinin has been described previously.13,24 Volunteers were admitted after an overnight fast to the Clinical Research Center. Blood samples were obtained for serum TXB2, and the subjects were asked to void. Through a peripheral vein, 1 L of normal saline was infused over a period of 1 hour. After another hour, bradykinin was infused in incremental doses of 100 to 800 ng·kg−1·min−1, each over a period of 15 minutes. The infusion was continued at the maximum tolerated dose for a total period of 2 hours. Blood pressure and heart rate were monitored continuously. Urine was collected in separate 2-hour aliquots before, during, and after the bradykinin infusion.

Pharmacokinetic Studies

In five subjects demonstrating a marked (>90%) decrease in serum TXB2, plasma aspirin and salicylate were determined at timed intervals (0, 0.25, 0.5, 1, 2, 3, 6, 12, and 24 hours) after the application of aspirin. Aspirin was applied in a dose of 750 mg on one limb over a 15-minute interval, and venous blood samples were drawn from the opposite arm. Aspirin was then administered for 14 days, and the procedure was repeated. Blood was withdrawn into heparin (10 U/mL, final concentration) and potassium fluoride (5% final concentration), the latter to prevent ex vivo metabolism of aspirin by plasma esterases. The plasma was separated immediately and stored at −70°C until analyzed. Aspirin and its metabolite, salicylic acid, were measured by NICI-GCMS with deuterium-labeled analogues as internal standards as previously described.13,25

Statistics

The data were analyzed by nonparametric ANOVA, with subsequent paired tests where appropriate. The data are expressed as mean±SEM.

Results

Mild skin reactions (erythema, peeling) occurred in four of eight subjects at the highest dose of aspirin. No ecchymoses or petechiae were reported either at or remote from the site of application. All the volunteers continued drug application, and the symptoms promptly resolved after its withdrawal. No adverse reactions occurred with vehicle alone or with the lower dose of aspirin.

Prostaglandin Biosynthesis

Serum TXB2, an index of the capacity of platelets to generate TXA2, was within the normal range in all subjects before the study, demonstrating that none had been exposed to a cyclooxygenase inhibitor. Application of the vehicle alone had no effect on serum TXB2 in six subjects (Fig 1). With aspirin 750 mg/d (n=8), there was a progressive reduction in serum TXB2 in all but one of the volunteers. In the remaining subjects, serum TXB2 was 5±3% of baseline by day 10 of application (n=7, P=.003; Fig 1). Aspirin 250 mg/d induced a smaller fall in serum TXB2, which was 55±11% by day 10 (n=4; P<.01). After the withdrawal of aspirin, serum TXB2 increased gradually and by day 8 was 93±7% and 65±9% of baseline for aspirin 250 mg and 750 mg, respectively. This is consistent with irreversible inhibition of platelet cyclooxygenase, so that recovery parallels the appearance of new platelets. The application of aspirin 750 mg/d was repeated in two of the volunteers after recovery, and similar falls in serum TXB2 (98% and 95%, respectively) were seen.
TXA₂ biosynthesis demonstrated a similar response. Thus, there was a dose-dependent reduction in the urinary excretion of TXM. At 750 mg/d of dermal aspirin, TXM decreased gradually and was 32±7% of baseline by day 10 (n=5; P=.002) of drug application. By 8 days after drug withdrawal, excretion of the metabolite had recovered to 65±9% of the pretreatment value (Fig 2). Despite the evidence of marked inhibition of platelet cyclooxygenase in vivo, there was only a small fall in PGI₂ biosynthesis according to urinary PGI-M determinations (Fig 3). Although the changes did not achieve statistical significance (P=.074 by ANOVA), there was an apparent dose-response relation. Thus, urinary excretion of PGI-M fell to 84±4% and 76±7% of baseline on aspirin 250 mg/d and 750 mg/d, respectively (Fig 3). The peak decrease in PGI-M excretion occurred by day 4 on both doses, in contrast to TXM excretion.

**Bradykinin Stimulation Studies**

Previous studies have demonstrated that bradykinin increases PGI₂ biosynthesis by, on average, twofold to sixfold.¹²⁻¹⁴ In the four subjects studied, bradykinin induced a 5.1±1.6-fold increase in PGI-M excretion. Two subjects were treated with oral aspirin 75 mg/d for 14 days and two with dermal aspirin 750 mg/d for the same period. Both preparations caused a marked and equivalent fall in urinary TXM (147±25 to 36±5 ng/mg creatinine). Oral aspirin also resulted in a decrease in urinary PGI-M at rest (48%) and after stimulation with bradykinin (61%). In contrast, resting and stimulated PGI-M excretion was largely unaltered by dermal aspirin (data not shown).

**Plasma Aspirin and Salicylate**

Plasma aspirin and salicylate levels were determined after the application of aspirin 750 mg on day 1 and day 14 in five subjects (Fig 4). On day 1, plasma aspirin increased to a peak of 0.24±0.11 μg/mL at 3 hours and had returned to baseline at 12 hours. At 24 hours, aspirin was undetectable. Plasma salicylate peaked at 6 to 12 hours with levels of 0.79±0.14 μg/mL. At 24 hours, the plasma salicylate was 0.33±0.08 μg/mL. Similar results were obtained on day 14 of drug application, although peak levels were seen 1 hour earlier after dermal application.

**Discussion**

Low-dose, once-daily aspirin is now commonly used as prophylaxis against thromboembolic events in the belief that this increases platelet selectivity and preserves PGI₂ biosynthesis. PGI₂ is the major cyclooxygenase product of vascular endothelium and is a potent platelet inhibitor. Since its formation is increased in conditions associated with platelet activation, such as severe atherosclerosis and unstable angina, PGf₂α may play a role in regulating platelet activity in vivo. Consequently, the antiplatelet effects of aspirin may be attenuated by coincident inhibition of vascular cyclooxygenase. However, it is not possible to achieve platelet selectivity with chronic administration of standard oral aspirin. Inhibition of basal PGI₂ biosynthesis is similar over doses of 80 to 2400 mg/d,¹² and bradykinin-stimulated PGI₂ formation is abolished by aspirin 75 mg/d.¹³ Inhibition of tissue cyclooxygenase and suppression of vasodilator prostaglandins may also be undesirable in patients with congestive heart failure. Thus, aspirin 350 mg abolishes the effects of the angiotensin converting enzyme inhibitor enalapril on systemic vascular resistance and left ventricular filling pressures in this setting.¹⁸

A second goal of low-dose aspirin is to reduce the risk of GI side effects, including bleeding. The incidence of gastric mucosal injury in patients taking aspirin is clearly dose dependent.²⁻⁵ However, the risk of bleeding is evident at very low doses.³⁻⁶ Indeed, the true incidence of serious GI complaints with low-dose aspirin may be underestimated, since patients at risk are often excluded from studies.²⁰ GI bleeding is probably a direct effect of aspirin on gastric mucosa.²⁰⁻³¹ Inhibition of cyclooxygenase and loss of the cytoprotective effect of
prostaglandins may play a role. In addition, salicylate is concentrated in mucosal cells and may alter their permeability to hydrogen ions. However, aspirin’s antiplatelet effect may be a contributing factor. It is noteworthy that the increased risk of bleeding when aspirin is combined with warfarin, an approach currently under investigation for the prevention of myocardial infarction, is confined almost entirely to the GI tract. Similarly, GI symptoms other than bleeding occur at very low doses of aspirin. In the RISC study, GI symptoms were reported in 5.2% to 6.5% of patients on aspirin 75 mg/d compared with 0.7% to 1.9% on placebo.

We explored the skin as a route for selective inhibition of platelet cyclooxygenase by aspirin that avoids GI exposure. Dermal administration has proved clinically useful for a number of drugs, including scopolamine, peptides, steroids, and nitroglycerin. Animal and human studies suggest that skin is moderately permeable to aspirin and its metabolite, salicylic acid. Previous experiments with 14C-labeled aspirin have demonstrated excretion of radioactivity over a period of 96 hours after a single dermal application, with a bioavailability of 22% in humans. However, this method cannot discriminate between aspirin and its metabolite salicylic acid, which has little or no activity as a cyclooxygenase inhibitor. Moreover, there were no measurements of biological effect, such as inhibition of platelet cyclooxygenase, to confirm absorption of aspirin. Since aspirin is rapidly deacetylated in aqueous conditions, it is possible that only salicylic acid was absorbed.

In this study, we used platelet cyclooxygenase activity, in addition to plasma drug levels, as a measure of aspirin’s bioavailability. The vehicle, propylene glycol and alcohol, is used as a permeability enhancer and was chosen to avoid ex vivo deacetylation. Aspirin applied daily induced a dose-dependent inhibition of platelet cyclooxygenase, as measured by serum TXB₂. Maximum inhibition was achieved at 10 days and exceeded 95% at the highest dose. Such a degree of suppression is necessary to inhibit platelet function and TXA₂ biosynthesis in vivo. inhibition of urinary TXM followed a similar pattern. TXM is a major enzymatic metabolite of TXA₂ and largely reflects platelet TXA₂ biosynthesis in vivo. Thus, after withdrawal of therapy, TXM recovered gradually over a period of days, paralleling the formation of new platelets, a process that has a half-life of 5 days. The degree of suppression of platelet cyclooxygenase achieved with the dermal preparation has been shown to inhibit platelet activity and prolong the bleeding time. However, the antiplatelet activity of dermal aspirin was not addressed directly in these experiments.

In contrast to the marked inhibition of TXA₂, there was little inhibition of basal or stimulated PGI₂ formation. Basal PGI-M excretion, an index of in vivo PGI₂ biosynthesis, decreased 24% by day 4 on the highest dose of dermal aspirin. No further inhibition occurred despite continued application, and by day 10, PGI-M excretion remained at 81% of baseline. Moreover, the increase in PGI₂ formation in response to bradykinin was unaltered. In contrast, oral aspirin 75 mg/d suppressed basal and bradykinin-stimulated PGI-M excretion. A similar degree of inhibition of basal PGI₂ biosynthesis has been demonstrated with a platelet-selective, controlled-release oral aspirin (16% at 27 days). This may reflect the contribution to vascular PGI₂ biosynthesis of platelet endoperoxides, the immediate cyclooxygenase product of arachidonic acid and precursors of all prostanoids. Alternatively, it may reflect inhibition of PGI₂ biosynthesis at the site of drug application.

The preservation of vascular cyclooxygenase is consistent with the low systemic bioavailability of dermal aspirin. Plasma aspirin was determined by a highly sensitive assay that can measure levels in the low nanogram per milliliter range. After oral aspirin 325 mg or 162.5 mg, peak plasma aspirin levels occurred at 30 to 45 minutes and were 2.0 and 1.3 μg/mL, respectively. In contrast, after dermal aspirin, plasma levels peaked at 3 hours and were substantially lower. By 24 hours, aspirin was undetectable in plasma. Plasma salicylate peaked later and was higher, consistent with its longer half-life (3 hours at doses used for antiplatelet effects). Thus, as described for other drugs, the skin acts as a reservoir from which aspirin is slowly released into the circulation. Moreover, the persistence of the drug in plasma suggests that aspirin can remain stable for some time after application to the skin. In contrast, aspirin given orally is rapidly converted to salicylate, and by 30 minutes, only 20% to 30% remains as active drug. The marked suppression of platelet cyclooxygenase despite the low levels of aspirin in the systemic circulation implies that platelets are inhibited as they pass through the site of application. A similar localized platelet effect has been reported with standard oral aspirin. After oral aspirin, inhibition of serum TXB₂...
occurs before the appearance of aspirin systemically, suggesting that platelets are inhibited in the portal circulation. This was more evident with the controlled-release, oral aspirin preparation described by Clarke and colleagues, where there was profound suppression of platelet cyclooxygenase despite plasma aspirin levels that were barely detectable. We hypothesize that a similar local effect explains the selectivity of dermal aspirin. Since platelet cyclooxygenase cannot recover, cumulative inhibition of all platelets occurs with repeated application of the aspirin. In contrast, since aspirin is deacetylated by plasma and tissue esterases in the dermal and pulmonary circulations, vascular cyclooxygenase is protected.

Skin reactions were noted in four of eight subjects at the highest dose. These were mild and disappeared after drug withdrawal. It may be possible to reduce their frequency by modifying the preparation. Lower concentrations and smaller doses may be possible under occlusive conditions or with other permeability enhancers. Alternatively, other forms of aspirin that are less acidic, such as the lysine salt, may be better tolerated. In one individual, serum TXB₂ was not inhibited. The reason for the failure is unknown, but it is unlikely to reflect poor compliance, as erythema and peeling were reported in this case.

In conclusion, aspirin is absorbed through the skin and results in marked and selective inhibition of platelet cyclooxygenase. Whether the transdermal delivery of aspirin will reduce the risk of GI side effects is unknown. However, this novel route of administration may be useful in patients with peptic ulcer disease or when aspirin is combined with oral anticoagulants. Moreover, these data demonstrate the feasibility of influencing platelet function transdermally and may be applicable to novel platelet agents.

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References


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