Inhibition of prostacyclin and thromboxane A₂
generation by low-dose aspirin at the site of plug
formation in man in vivo


ABSTRACT In a double-blind placebo-controlled crossover study, we investigated in seven healthy
male volunteers the effect of a low-dose aspirin regimen (35 mg acetylsalicylate per day for 7 days) on
the formation of thromboxane A₂ (TxA₂) and prostacyclin (PGI₁) in blood emerging from a standard-
ized injury of the microvasculature made to determine skin bleeding time. When subjects were treated
with placebo, there was rapid and substantial generation of TxA₂ and PGI₁ at the site of platelet–vessel
wall interaction within the first 2 min after vascular injury. This was reflected by a greater than 100-fold
and greater than 10-fold increase in thromboxane B₂ (TxB₂) and 6-keto-prostaglandin F₁α (6-keto-
PGF₁α) in blood obtained from incisions made to determine bleeding time as compared with the
corresponding plasma values. Low-dose aspirin caused a significant inhibition of both TxA₂ and PGI₁
generation in blood sampled from the skin incisions, represented by a 85% and 92% and 81% and 84%
inhibition of TxB₂ and 6-keto-PGF₁α, respectively, as compared with controls. We therefore conclude
that (1) rapid activation of both platelet prostaglandin metabolism and vascular PGI₁ biosynthesis
occurs at the site of platelet–vessel wall interaction, and (2) low-dose aspirin results in a significant
inhibition of both platelet and vascular cyclooxygenase activity. Thus, our data fail to confirm the
concept of a differential effect of low-dose aspirin on platelet and vascular prostaglandin synthesis in
man in vivo.


BY ACETYLATED the active site of the enzyme
cyclooxygenase, aspirin blocks the formation of pro-
taglandin endoperoxides from arachidonic acid.¹ This
leads to inhibition of the production of thromboxane A₂
(TxA₂), a potent vasoconstrictor and platelet aggregating
agent in platelets,² ³ and of its counterpart, prosta-
cyclin (PGI₂), a potent inhibitor of platelet aggregation
and vasodilator in vascular tissue.⁴ ⁵

It has been suggested that an aspirin regimen that
selectively inhibits TxA₂ formation in platelets without
blocking PGI₂ generation in the vessel wall may im-
prove the efficacy of aspirin as an antithrombotic
agent.⁶ Whereas single doses of aspirin failed to ex-
hibit a selective reduction of platelet thromboxane
production, it has recently been shown that long-
term treatment with very low doses of aspirin sub-
stantially inhibits thromboxane B₂ (TxB₂) serum pro-
duction without affecting urinary excretion of PGI₂
metabolites.⁷ ⁸

However, one major obstacle in the search for a
solution to the “aspirin dilemma” is that both PGI₂ and
TxA₂ exert their effects on thrombus formation in the
locality where they are generated, i.e., at the site of
platelet–vessel wall interaction rather than in the gen-
eral circulation. Thus, it is doubtful that PGI₂ and TxA₂
levels in serum, plasma, and urine or the capacity of
isolated vascular tissue to produce PGI₂ truly reflect
TxA₂ and PGI₂ synthesis at an active site in vivo.

A closer experimental approach in investigating the
mechanisms leading to hemostasis in vivo has recently
been described⁹ ⁰ that consists of the measurement of
mediators of hemostasis in blood emerging from a
standardized injury of the microvasculature made to
determine skin bleeding time.

We have adapted this technique to evaluate the ef-
effect of a low-dose aspirin regimen on the formation of
PGI₁ (measured as 6-keto-prostaglandin F₁α [6-keto-
PGF₁α]) and TxA₂ (measured as TxB₂) at the site of
platelet–vessel wall interaction in man in vivo.
Materials and methods

Study design. The study was conducted as a double-blind, placebo-controlled, crossover trial. Seven healthy male volunteers, 23 to 35 years old, who had not ingested any drugs for at least 2 weeks before the study and did not take any during the study period were investigated. They did not smoke and refrained from coffee consumption throughout the study period. Each subject received either 35 mg of aspirin (Bayer, Leverkusen, F.R.G.; acetylsalicylate powder) or an oral placebo (starch powder) dissolved in water daily for 7 days (day 1 to day 7). The drug was taken by each volunteer at 8:00 P.M. Skin bleeding time was determined and venous blood and blood obtained from incisions made to determine bleeding time were sampled 12 hr after the last dose of aspirin (day 8, 8:00 A.M.). After washout period of at least 2 weeks, subjects crossed over to the alternate treatment. The study protocol was approved by the Hospital Ethics Committee and the volunteers gave written informed consent.

Bleeding time. The skin bleeding time was determined according to the technique originally described by Mielke et al. A sphygmomanometer cuff was placed on the upper arm and inflated to 40 mm Hg. Two incisions, 5 mm long × 1 mm deep, were placed on the lateral aspect of the volar surface of the forearm parallel to the antecubital crease with use of a disposable standard device (Simplate II, General Diagnostics, NJ). The procedure was carried out by the same investigator each time.

Sampling of blood from incisions made to determine bleeding time. Blood emerging from the incision made for the skin bleeding time study was sampled as described recently. Briefly, the blood was collected at 30 sec intervals directly from the edge of the skin wound into calibrated polythene cannulas (Portex 90 tubing, inner diameter 0.86 mm, outer diameter 1.27 mm, Portex Ltd., Hythe, U.K.). Nine volumes of blood were passed into 1 volume of an anticoagulant mixture consisting of 100 mM ethylene-diamine tetracetic acid, 60 μM indomethacin (Sigma Chemical Co. Ltd., Poole, U.K.), and 300 mM 6-oxo-prostaglandin E1 (kindly provided by Dr. John Westwick, Department of Pharmacology, Royal College of Surgeons, London, U.K.). After mixing, the tubes were centrifuged at 12,000 g for 1 min. The supernatant was removed, frozen, and stored at −80°C until assay.

Venous blood. Blood was collected by a clean puncture of a large antecubital vein without tourniquet by the use of a 19-gauge needle. For the measurement of 6-keto-PGF1α and TXB2 in plasma, blood was drawn into 1/10 vol of the anticoagulant solution described above and immediately centrifuged at 2000 g for 15 min at 4°C. The supernatants were removed, frozen, and stored at −80°C until assay. For determination of 6-ketoPGF1α and TXB2 serum levels, nonanticoagulated blood was incubated for 90 min in glass tubes at 37°C. After centrifugation at 2000 g for 20 min, the supernatants were removed, frozen, and stored at −80°C until assay.

Assays. 6-keto-PGF1α and TXB2 concentrations were measured by the use of radioimmunoassay procedures described in detail previously. Rabbit anti-TXB2 antiserum and sheep anti-6-keto-PGF1α antiserum, TXB2, and 6-keto-PGF1α were provided by Dr. John Westwick, Department of Pharmacology, Royal College of Surgeons, London, U.K. The rabbit anti-TXB2 antibody has the following cross-reaction at 30% displacement: TXB2, 100%, PGD2, 3.5%, PGE2 less than 0.2%, PGE1 less than 0.1%, 6-oxo-PGF1α less than 0.01%, 6,15-dioxo-PGF1α less than 0.1%, 15-keto-PGF2α less than 0.001%, 13,14-dihydro-PGE2 less than 0.001%, and arachidonic acid less than 0.001%. The sheep anti-6-keto-PGF1α antibody has the following cross-reaction at 10% displacement: 6-keto-PGF1α 100%, PGE2 less than 0.15%, PGE1 less than 0.1%, PGF1α less than 0.08%, PGE2 less than 0.007, 6,15-dioxo PGF1α less than 0.22%, 13,14-dihydro-6,15-dioxo-PGF1α less than 0.1%, 15-oxo-E2 less than 0.007%, TXB2 less than 0.007%, 13,14-dihydro-15-oxo-PGF2α less than 0.007%, 15-oxo-PGF2α less than 0.007%, and 15-oxo-PGF2β less than 0.007%. [3H]-TXB2 (100 to 140 mCi/mmol) and [3H]-6-keto-PGF1α (100 to 140 mCi/mmol) were purchased from New England Nuclear, Southampton, U.K. The limit of sensitivity of both assays is 20 pg/ml. When TXB2 or PG1 concentrations were below the limit of sensitivity of the assays, 20 pg/ml TXB2 or PG1 was assumed in order to carry out statistical evaluation of the data.

Statistical analysis was performed by the use of Wilcoxon's matched-pairs signed-ranks test.15

Results

Effect of low-dose aspirin on bleeding time. The mean bleeding time was 348 ± 31 sec when the subjects were treated with placebo. A statistically significant prolongation to 410 ± 29 sec was seen after administration of a low-dose aspirin regimen (p < .05).

Effect of low-dose aspirin on 6-keto-PGF1α and TXB2 plasma and serum concentrations. 6-keto-PGF1α and TXB2 plasma levels were below 20 pg/ml after placebo and after aspirin treatment. 6-keto-PGF1α and TXB2 serum concentrations after aspirin were inhibited by 85% (decrease from 139.4 ± 34.5 pg/ml to less than 20 pg/ml 6-keto-PGF1α, p < .05) and 97.6% (decrease from 236 ± 43.3 to 5.65 ± 1.45 ng/ml TXB2, p < .05) compared with those in control subjects.

Effect of low-dose aspirin on TXB2 generation in blood obtained from the incisions made to determine bleeding time. When subjects were treated with placebo, 2.8 ± 1 ng/ml TXB2 was found in blood sampled from the skin incisions during the first minute (figure 1). A peak value of 5.3 ± 0.6 ng TXB2/ml was found in samples from the third minute. Low-dose aspirin treatment caused a marked reduction in TXB2 generation in blood obtained throughout the 4 min study period, with a peak value of 0.62 ± 0.25 ng/ml at the fourth minute. Similarly, aspirin caused a reduction in the mean TXB2 peak values in blood obtained during the bleeding time studies (from 6.0 ± 0.7 ng/ml in the control subjects to 0.7 ± 0.3 ng/ml after aspirin, p < .05).

Effect of low-dose aspirin on 6-keto-PGF1α generation in blood obtained from incisions made to determine bleeding time. When the volunteers were treated with placebo, 6-keto-PGF1α in blood sampled from the skin incisions rose rapidly from 38 ± 6 pg/ml in the first minute to 197 ± 66 pg/ml in the second minute. No further increase was seen in the third and fourth minute (figure 2). The 6-keto-PGF1α concentrations in blood samples obtained from the incisions in aspirin-treated volunteers were similar to the values found in the placebo group in the first minute. No further increase, but rather a decrease, was found after aspirin treatment

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within the second, third, and fourth minutes. Aspirin caused a reduction in the mean 6-keto-PGF₁α peak values from 271 ± 44 pg/ml in the control subjects to 39 ± 11 pg/ml after drug treatment (p < .05).

Discussion

It has recently been shown that the measurement of platelet- and coagulation-derived products in blood emerging from a local injury of the microvasculature induced to determine skin bleeding time is a suitable technique for examining the mechanisms leading to primary hemostasis in man in vivo.²⁻¹¹ We have shown that marked platelet activation and considerable generation of thrombin occur in the early stages of plug formation, as indicated by a 50-fold, 30-fold, and 12-fold increase in TxB₂, β-thromboglobulin, and fibropeptide A in blood obtained from incisions made to determine bleeding time as compared with the corresponding plasma values.¹⁰,¹¹ The present study was designed to investigate the amount and time course of the generation of PGI₂, a potent antiaggregatory agent and vasodilator, at the site of platelet–vessel wall interaction and the effect of a low-dose aspirin regimen on this process.

Levels of 6-keto-PGF₁α in blood obtained from the skin incisions made to determine bleeding time within 4 min after vascular injury were at least 10-fold higher than plasma concentrations. Since superficial incisions such as those performed in our experiments mainly transect the capillary network of the skin and to a lesser extent vessels of the horizontal subpapillary plexus, which consist of both arterioles and postcapillary venules, we can assume that 6-keto-PGF₁α in blood from the incision made to determine bleeding time reflects PGI₂ biosynthesis in the microvasculature rather than in large blood vessels.¹⁶ Evidence has been presented that PGI₂ exhibits its effects on plug formation locally at the site of vessel injury rather than in the general circulation.¹⁷ It has also been reported that under resting conditions, levels of 6-keto-PGF₁α in plasma are in the very low picogram range¹⁸ and pulmonary release of PGI₂ is not detectable.¹⁹ Furthermore, inhibition of PGI₂ synthesis does not render intact endothelium adherent for platelets,²⁰ but does increase the number of platelets that become adherent to subendothelial structures.²¹ Thus, our observation of a rapid generation of substantial amounts of PGI₂ in the microvasculature during primary hemostasis are in good agreement with these reports suggesting that PGI₂ is an important mediator of hemostasis in the vicinity of plug formation, but does not act as a circulating hormone under physiologic conditions in man.

Repetitive administration of a low dose of aspirin

![FIGURE 1](http://circ.ahajournals.org/)

**FIGURE 1.** Effect of low-dose aspirin (35 mg daily for 7 days) on the formation of TxB₂ in blood obtained from the incisions made to determine bleeding time. The values are plotted as the mean ± SEM from seven volunteers before aspirin (●) and after (○) aspirin.

![FIGURE 2](http://circ.ahajournals.org/)

**FIGURE 2.** Effect of low-dose aspirin (35 mg daily for 7 days) on the formation of 6-keto-PGF₁α in blood obtained from the incisions made to determine bleeding time. The values are plotted as the mean ± SEM from seven volunteers before aspirin (●) and after (○) aspirin.
(35 mg per day for 7 days) caused significant inhibition of the generation of TxB₂ at the site of plug formation, as reflected by a 83% to 92% reduction in blood sampled from skin incisions within the first 4 min after injury of the microvasculature. This is in good agreement with previous reports demonstrating marked reduction of TxB₂ generation in vitro by low-dose aspirin, as indicated by a greater than 90% inhibition of TxB₂ serum production and absent or substantially decreased aggregation responses to arachidonate, ADP, collagen, and epinephrine. Thus, blockade of platelet cyclooxygenase by low-dose aspirin leads to an impairment of platelet function in vivo, as reflected by reduction of β-thromboglobulin and TxB₂ in blood from the incision made to determine bleeding time. and an increase in the blood loss from the incision. According to the TxA₂-PGI₂ balance theory, an aspirin dosing regimen that blocks platelet thromboxane production without inhibiting vascular PGI₂ synthesis may increase the efficacy of aspirin as an antithrombotic agent. It has been shown that long-term administration of very low doses of aspirin per day results in cumulative inhibition of TxB₂ generation in vitro and of urinary excretion of 6-keto-TxB₂, without significant changes in urinary 6-keto-PGF₁α and 2,3-dinor-6-keto-PGF₁α, indexes of renal and systemic PGI₂ production, respectively. However, significant (>80%) reduction of PGI₂ biosynthesis was found in vascular tissue obtained from healthy subjects treated with a similar aspirin regimen (40 mg of aspirin for 4 days). Furthermore, low-dose aspirin (20 mg per day for 1 week) caused a 50% inhibition of PGI₂ generation in both aortic and venous tissue in patients with atherosclerosis. Therefore, excretion of urinary PGI₂ metabolites and 6-keto-PGF₁α production by vascular specimens ex vivo may not necessarily reflect the actual production rates of PGI₂ in vivo at the site of platelet–vessel wall interaction.

In view of these discrepant findings, we have studied the effect of low-dose aspirin on PGI₂ biosynthesis in a recently developed preparation that may be more relevant to PGI₂ generation in the microcirculation: the measurement of 6-keto-PGF₁α in blood emerging from a template incision made to determine bleeding time. Under these experimental conditions, low-dose aspirin caused a significant reduction in 6-keto-PGF₁α in blood sampled in this way, as reflected by a greater than 80% inhibition between the second and fourth minute after injury. This strongly indicates that repeated doses of aspirin, even those as low as 35 mg/day, induce significant inhibition of cyclooxygenase, not only in platelets but also in the microvasculature in vivo. It is of further interest to investigate whether our observation of a substantial blockade of both platelet and vascular cyclooxygenase by low-dose aspirin holds true for subjects with an abnormal platelet–vessel wall interaction, such as diabetics and patients with atherosclerosis. This is currently under investigation.

In conclusion, we have shown that rapid and substantial generation of both TxA₂ and PGI₂ occurs at the site of platelet–vessel wall interaction. Treatment with low-dose aspirin leads to a significant inhibition of PGI₂ and TxA₂ formation at the site of plug formation. Thus, our results fail to confirm the hypothesis of a differential effect of low-dose aspirin on platelet and vascular prostaglandin biosynthesis in man in vivo.

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