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 (PGI₂) in blood emerging from a standardized 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 PGI₂ 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 F₁₃α (6-keto-PGF₁₃α) 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 PGI₂ generation in blood sampled from the skin incisions, represented by a 85% and 92% and 81% and 84% inhibition of TxB₂ and 6-keto-PGF₁₃α, respectively, as compared with controls. We therefore conclude that (1) rapid activation of both platelet prostaglandin metabolism and vascular PGI₂ 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.

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 (days 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 \( \times \) 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 tetraacetic acid, 60 \( \mu \)M indomethacin (Sigma Chemical Co. Ltd., Poole, U.K.), and 300 mM 6-oxo-prostaglandin E \(_1\) (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 \(-80^\circ\) 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-PGF\(_{1\alpha}\) and TxB\(_2\) 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\(^\circ\) C. The supernatants were removed, frozen, and stored at \(-80^\circ\) C until assay. For determination of 6-keto-PGF\(_{1\alpha}\) and TxB\(_2\) serum levels, nonanticoagulated blood was incubated for 90 min in glass tubes at 37\(^\circ\) C. After centrifugation at 2000 g for 20 min, the supernatants were removed, frozen, and stored at \(-80^\circ\) C until assay.

Assays. 6-keto-PGF\(_{1\alpha}\) and TxB\(_2\) concentrations were measured by the use of radioimmunoassay procedures described in detail previously. Rabbit anti-TxB\(_2\) antiserum and sheep anti-6-keto-PGF\(_{1\alpha}\) antiserum, TxB\(_2\), and 6-keto-PGF\(_{1\alpha}\) were provided by Dr. John Westwick, Department of Pharmacology, Royal College of Surgeons, London, U.K. The rabbit anti-TxB\(_2\) antibody has the following cross-reaction at 30% displacement: TxB\(_2\) 100%, PGD\(_2\) 3.5%, PGE\(_2\) less than 0.2%, PGE\(_1\) less than 0.1%, 6-oxo-PGF\(_{1\alpha}\) less than 0.01%, 6,15-dioxo-PGF\(_{1\alpha}\) less than 0.01%, 15-keto-PGE\(_2\) less than 0.001%, 13,14-dihydro-PGE\(_2\) less than 0.01%, and arachidonic acid less than 0.001%. The sheep anti-6-keto-PGF\(_{1\alpha}\) antibody has the following cross-reaction at 10% displacement: 6-keto-PGF\(_{1\alpha}\) 100%, PGE\(_2\) less than 0.15%, PGE\(_1\) less than 0.1%, PGF\(_{1\alpha}\) less than 0.08%, PGE\(_2\) less than 0.007, 6,15-dioxo PGF\(_{1\alpha}\) less than 0.22%, 13,14-dihydro-6,15-dioxo-PGF\(_{1\alpha}\) less than 0.1%, 15-oxo-E\(_2\) less than 0.007%, TxB\(_2\) less than 0.007%, 13,14-dihydro-15-oxo-PGF\(_{2\alpha}\) less than 0.007%, 15-oxo-PGF\(_{2\alpha}\) less than 0.007%, and 15-oxo-PGF\(_{2\alpha}\) less than 0.007%. [\(\text{H}\)]-TxB\(_2\) (100 to 140 mCi/mmol) and [\(\text{H}\)]-6-keto-PGF\(_{1\alpha}\) (100 to 140 mCi/mmole) were purchased from New England Nuclear, Southhampton, U.K. The limit of sensitivity of both assays is 20 pg/ml. When TxB\(_2\) or PGI\(_2\) concentrations were below the limit of sensitivity of the assays, 20 pg/ml TxB\(_2\) or PGI\(_2\) 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.

Results

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

Effect of low-dose aspirin on 6-keto-PGF\(_{1\alpha}\) and TxB\(_2\) plasma and serum concentrations. 6-keto-PGF\(_{1\alpha}\) and TxB\(_2\) plasma levels were below 20 pg/ml after placebo and after aspirin treatment. 6-keto-PGF\(_{1\alpha}\) and TxB\(_2\) serum concentrations after aspirin were inhibited by 85% (decrease from 139.4 \pm 34.5 pg/ml to less than 20 pg/ml 6-keto-PGF\(_{1\alpha}\), p < .05) and 97.6% (decrease from 236 \pm 43.3 to 5.65 \pm 1.45 ng/ml TxB\(_2\), p < .05) compared with those in control subjects.

Effect of low-dose aspirin on TxB\(_2\) generation in blood obtained from the incisions made to determine bleeding time. When subjects were treated with placebo, 2.8 \pm 1 ng/ml TxB\(_2\) was found in blood sampled from the skin incisions during the first minute (figure 1). A peak value of 5.3 \pm 0.6 ng TxB\(_2\)/ml was found in samples from the third minute. Low-dose aspirin treatment caused a marked reduction in TxB\(_2\) generation in blood obtained throughout the 4 min study period, with a peak value of 0.62 \pm 0.25 ng/ml at the fourth minute. Similarly, aspirin caused a reduction in the mean TxB\(_2\) peak values in blood obtained during the bleeding time studies (from 6.0 \pm 0.7 ng/ml in the control subjects to 0.7 \pm 0.3 ng/ml after aspirin, p < .05).

Effect of low-dose aspirin on 6-keto-PGF\(_{1\alpha}\) generation in blood obtained from incisions made to determine bleeding time. When the volunteers were treated with placebo, 6-keto-PGF\(_{1\alpha}\) in blood sampled from the skin incisions rose rapidly from 38 \pm 6 pg/ml in the first minute to 197 \pm 66 pg/ml in the second minute. No further increase was seen in the third and fourth minute (figure 2). The 6-keto-PGF\(_{1\alpha}\) 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
within the second, third, and fourth minutes. Aspirin caused a reduction in the mean 6-keto-PGF\(_{1\alpha}\) 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.\(^9\)\(^{-}\)\(^1\)\(^1\) 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 TxB\(_2\), β-thromboglobulin, and fibrinopeptide A in blood obtained from incisions made to determine bleeding time as compared with the corresponding plasma values.\(^1\)\(^0\)\(^,\)\(^1\)\(^1\) The present study was designed to investigate the amount and time course of the generation of PGI\(_2\), 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-PGF\(_{1\alpha}\) 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-PGF\(_{1\alpha}\) in blood from the incision made to determine bleeding time reflects PGI\(_2\) biosynthesis in the microvasculature rather than in large blood vessels.\(^1\)\(^6\) Evidence has been presented that PGI\(_2\) exhibits its effects on plug formation locally at the site of vessel injury rather than in the general circulation.\(^1\)\(^7\) It has also been reported that under resting conditions, levels of 6-keto-PGF\(_{1\alpha}\) in plasma are in the very low picogram range\(^1\)\(^8\) and pulmonary release of PGI\(_2\) is not detectable.\(^1\)\(^9\) Furthermore, inhibition of PGI\(_2\) synthesis does not render intact endothelium adherent for platelets,\(^2\)\(^0\) but does increase the number of platelets that become adherent to subendothelial structures.\(^2\)\(^1\) Thus, our observation of a rapid generation of substantial amounts of PGI\(_2\) in the microvasculature during primary hemostasis are in good agreement with these reports suggesting that PGI\(_2\) 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 TxB\(_2\) 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-PGF\(_{1\alpha}\) 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 a significant prolongation of the bleeding time, and an increase in the blood loss from the incision.

According to the TxA₂-PGI₁ balance theory, an aspirin dosing regimen that blocks platelet thromboxane production without inhibiting vascular PGI₁ 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 PGI₁ production, respectively. However, significant (>80%) reduction of PGI₁ 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 PGI₁ generation in both aortic and venous tissue in patients with atherosclerosis. Therefore, excretion of urinary PGI₁ metabolites and 6-keto-PGF₁α production by vascular specimens ex vivo may not necessarily reflect the actual production rates of PGI₁ 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 PGI₁ biosynthesis in a recently developed preparation that may be more relevant to PGI₁ 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 PGI₁ occurs at the site of platelet–vessel wall interaction. Treatment with low-dose aspirin leads to a significant inhibition of PGI₁ 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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