The Effect of PGI₂ and Theophylline on the Response of Platelets Subjected to Shear Stress

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A specially designed rotational viscometer was used to investigate the effects of the antiplatelet agent PGI₂ in combination with theophylline on the response of human platelets subjected to shear stress. Samples of citrated platelet-rich plasma (PRP) were exposed to shear stress in the viscometer for a period of 5 min at 23°C. The levels of stress studied ranged from 50 to 300 dynes/sq cm. Pretreatment of the platelets with 0.01 μM PGI₂ and 500 μM theophylline before exposure to shear stress caused a large reduction in shear-induced platelet aggregation.

However, it was also observed that the PGI₂-theophylline pretreatment concomitantly caused a large increase in shear-induced platelet lysis and serotonin release at stress levels equal to or greater than 150 dynes/sq cm. This observed increase in platelet fragility may have important implications for clinical applications of PGI₂. The results are discussed and compared to those obtained in prior work in which platelets were pretreated with acetylsalicylic acid or with PGE₁.

The major biochemical effect of PGI₂ is to stimulate the platelet membrane enzyme adenyl cyclase, which converts platelet ATP to cyclic-AMP. Cyclic-AMP (cAMP) is a potent inhibitor of platelet aggregation. Theophylline inhibits the platelet enzyme phosphodiesterase and so prevents the breakdown of cAMP into AMP. The effects of theophylline alone are small, but in combination with PGI₂, theophylline maintains a high level of cAMP in the platelets and so potentiates the effects of PGI₂.

MATERIALS AND METHODS

Sample Preparation

Fresh venous blood was collected from healthy fasting donors (no medication in last 10 days) and mixed with sodium citrate anticoagulant (final concentration 0.38% by weight). Platelet-rich plasma (PRP) was prepared by centrifuging the anticoagulated blood at 150 g for 12 min at 23°C. Platelet-poor plasma (PPP) was prepared by centrifuging the remaining red-cell-plasma suspension at 1000 g for 10 min at 23°C. The PPP was used to dilute the PRP to a platelet count of 300,000/μl.

PRP was incubated for 1 hr at 23°C with [3H]-serotonin [5-hydroxy (side chain-2-14C) tryptamine creatinine sulphate, 58 mCi/mmol, Amersham/Searle, Arlington City, Ill., final concentration 1.0 μM]. The resulting labeled PRP was divided into two equal aliquots. A solution containing an antiplatelet agent was added to one aliquot, and the corresponding buffer solution was added to the other (control) aliquot.

The PGI₂ stock solution was prepared by adding 1.0 g of PGI₂ (Upjohn Company, Kalamazoo, Mich.) to a buffer solution that consisted of 2.674 liters of 0.05 M Tris base dissolved in water (pH 9.25) so that the stock solution had a concentration of 1.00 × 10⁻³ M PGI₂. The theophylline stock solution was prepared by dissolving 1.0 g of theophylline (Sigma Chemical Company, St. Louis, Mo.) in 0.327 liters of phosphate-buffered saline (0.0026 M KH₂PO₄, 0.0107 M Na₂HPO₄, 0.123 M NaCl, pH 6.9) to yield a concentration of 1.70 × 10⁻³ M theophylline. Both antiplatelet stock solutions were stored at -20°C.

Theophylline stock solution was first added to the labeled PRP to give a final concentration of 500 μM theophylline. The PGI₂ stock solution was then added to give a final concentration of 0.01 μM PGI₂. Subsequently, the PRP was incubated at 23°C for 5 min before exposure to shear stress. Preliminary studies using an aggregometer were carried out to ascertain that the preparation proce-
Shearing Procedure

After preparing the PRP samples, specimens were subjected to a well defined shear field produced by a specially designed rotational viscometer that has been described in detail elsewhere. Briefly, the principal components of the viscometer are a stationary inner bob and a rotating outer cup. The central portion (74% by volume) consists of concentric cylinders, the bottom portion (13% by volume) forms a cone-and-plate region, and the top portion (13% by volume) forms a cone-and-cone region that minimizes the specimen-air interface. The viscometer is designed to produce a uniform level of shear stress in all three regions.

The exposure of the PRP to shear stress was carried out in the viscometer at 23°C for a period 5 min. PRP samples that had been subjected to the same conditions as the sheared specimens, except for the exposure to shear stress, were set aside to provide controls. All shear experiments were paired, that is, each PRP sample pretreated with antiplatelet agents had a corresponding control sample to which buffer solutions were added.

Indices of Platelet Response

Platelet response was characterized by the following measurements made before and after exposure to shear stress.

1. The particle count and the particle size distribution were determined by an electronic particle counter (Coulter Electronics, Hialeah, Fla, Model ZBI). In the counting procedure, all individual particles in the size range 3 μm to 30 μm were counted as one. In normal PRP most of the particles were single platelets (average volume 8-10 μm), but platelet doublets, triplets, as well as platelet fragments larger than 3 μm were also counted.

2. The level of 14C-radiolabeled serotonin present in the plasma was determined by use of a scintillation counter (Beckman Instruments, Fullerton, Calif, Model LS-133). Increased levels of serotonin in the sheared supernatant plasma were interpreted as evidence of the release of platelet dense granules and/or nonspecific release associated with platelet lysis.

3. The level of lactic dehydrogenase (LDH) present in the plasma was determined by the method of Wroblewski and LaDue in which the rate of conversion of pyruvate to lactate is measured by use of a spectrophotometer (Beckman Model ACTA II). LDH is an enzyme contained in the platelet cytoplasm. Increased levels of LDH in the sheared supernatant plasma were interpreted to be a measure of platelet lysis.

4. The aggregation response to added ADP (final concentration 2 μM) and to low-dose collagen (1.44 mg/liter, Hormon Chemie, Munich, Germany) were determined with an aggregometer (Sienco, Morrison, Colo., Model DP-247E).

RESULTS

As shown in Fig. 1C, relatively little platelet lysis occurs for stress levels in the range of 150 dynes/sq cm and lower. However, there is a marked reduction in the particle counts of the control samples after exposure to these stress levels, as shown in Fig. 1A. It has been demonstrated that under these experimental conditions, bulk shear stress effects are dominant, while the interaction of platelets with the viscometer surface is of minor importance. Therefore, at stress levels below about 150 dynes/sq cm, the reduction in the apparent particle count can be attributed largely to shear-induced platelet aggregation rather than lysis or platelet adhesion.

The particle counts of the control samples decreased to about 30% of the initial count after exposure to shear stress, while the particle counts of the PGI2-theophylline-treated samples were not less than 78% of the initial count at any level of stress (Fig. 1A). Thus, these particle count results indicate that PGI2 in combination with theophylline significantly inhibited shear-induced platelet aggregation (paired “t” test, p < 0.01).

After exposure to shear stresses of 50 and 75 dynes/sq cm, the particle counts of the PGI2-theophylline-treated samples actually became slightly greater than the initial counts. The increased counts after exposure to 50 and 75 dynes/sq cm reflect the capacity of PGI2 to not only inhibit shear-induced platelet aggregation, but to promote disaggregation. Previous studies involving the use of a transmission electron
microscope and Coulter Channelizer have show that the apparent increase in the particle counts that is observed when comparing the counts at stress levels of 200 dyne/sq cm and higher to the counts at 100 and 150 dyne/sq cm is due to the formation of platelet fragments as well as disaggregation caused by the shear field.5

The threshold stress level for shear-induced serotonin release of platelets in normal PRP is in the range 75–100 dyne/sq cm (Fig. 1B), while the stress threshold for LDH release is in the range 100–125 dyne/sq cm (Fig. 1C). Both serotonin release and LDH release increased monotonically with shear stress, as expected.3,5 The PG12–theophylline pretreatment significantly increased both shear-induced serotonin release and LDH release over the stress range 150–300 dyne/sq cm (p < 0.01). The serotonin release includes serotonin in the supernatant fluid due to platelet lysis as well as that due to the platelet release reaction. From the platelet lysis results (Fig. 1C) it can be inferred that a large proportion of the serotonin release in the presence of PG12 and theophylline was due to shear-induced lysis.

The PG12–theophylline pretreatment before exposure to shear stress caused a large suppression of the response of platelets to exogenous ADP and collagen. Since the platelet response to ADP and collagen was greatly diminished by the pretreatment, exposing the treated samples to shear stress had essentially no additional effect.

DISCUSSION

The results reported here for the PG12–theophylline pretreatment are very similar to those obtained previously for PGE1–theophylline pretreatment.10,11 The similarity of the results of the PG12 study and PGE1 study was expected, since PG12 and PGE1 exert their effects by essentially the same biochemical mechanism (increase in intracellular cyclic-AMP level). However, PG12 is much more potent than PGE1. The concentration of PG12 used here (0.01 μM) is estimated to be a concentration that can be attained transiently, or locally, in vivo.22

Theophylline used with PG12 potentiates the effects of PG12, and so minimizes transient effects of PG12. Platelets in PRP recover relatively quickly from inhibition caused by PG12 alone. Theophylline (500 μM) alone has essentially no effect on the platelet response to shear stress.23

PG12 (or PGE1 and theophylline largely inhibited shear-induced platelet aggregation. It has previously been shown that pretreatment of platelets with ASA before exposure to shear stress does not significantly inhibit shear-induced platelet aggregation.10,12 In addition to suppressing the platelet aggregation response to a mechanical stimulus (shear stress), PG12 (or PGE1) and theophylline suppressed the platelet aggregation response to chemical stimuli (ADP and collagen). The suppressed aggregation response was retained over the entire range of stress studied. Since ASA causes only a small reduction in ADP-induced platelet aggregation,10,24 PG12 (or PGE1) and theophylline are much more effective than ASA in inhibiting platelet aggregation over a wide range of conditions.

An unexpected and possibly very significant finding was that the PG12 (or PGE1–theophylline pretreatment caused a large increase in shear-induced platelet lysis, serotonin release, and β-thromboglobulin release at stress levels equal to or greater than 150 dyne/sq cm. This result indicates that PG12 (or PGE1) and theophylline cause increased platelet fragility, that is, increased susceptibility to shear-induced lysis. The observed increase in platelet fragility may have important implications for clinical applications involving the use of PG12 or PGE1 as antiplatelet agents.

REFERENCES

PLATELET RESPONSE TO SHEAR STRESS


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