Prostacyclin (Prostaglandin I₂, PGI₂) Inhibits Platelet Adhesion and Thrombus Formation on Subendothelium

By Harvey J. Weiss and Vincent T. Turitto

Prostaglandin I₂ (prostacyclin, PGI₂), a substance synthesized in the wall of blood vessels, has been previously shown to inhibit the aggregation of platelets in stirred platelet-rich plasma. We used a method in which segments of deendothelialized rabbit aorta were perfused at arterial shear rates with human blood and found that both platelet adhesion and thrombus formation on subendothelium was inhibited in blood containing 10 nM PGI₂. PGI₂ appears to reduce adhesion by inhibiting platelet spreading. These findings suggest that PGI₂ could regulate the deposition of platelets on vascular surfaces.

The platelets in circulating blood do not adhere to each other or to the intact endothelium of blood vessels. However, if the endothelium becomes detached, the platelets can adhere and spread on the subendothelial surface, and these adherent platelets can recruit others to produce a platelet thrombus. Platelet-induced thrombosis, as well as thromboxane A₂, serotonin, a mitogenic factor, and other substances released by platelets, may mediate a variety of pathologic processes. Hence an understanding of the mechanisms that regulate the deposition of platelets on vascular surfaces is important.

Recent studies have shown that the intima and other layers of the vessel wall produce a potent inhibitor of platelet aggregation. The structure of this inhibitor, prostacyclin or prostaglandin I₂ (PGI₂), has been determined as 9-deoxy-6,9\(\alpha\)-epoxy-\(\Delta^9\)-PGF₆₉, an intermediate in the transformation of prostaglandin endoperoxides into the stable end-product 6-keto-PGF₁₀. The platelet-inhibitory properties of PGI₂ have been demonstrated by showing that extracts or microsomal fractions of the vessel wall can inhibit arachidonic acid-induced platelet aggregation in platelet-rich plasma (PRP). Extracts of endothelial cells, skin fibroblasts, and arterial smooth muscle cells grown in cell culture also inhibit platelet aggregation and secretion. The above observations have led to the hypothesis that PGI₂ may be a naturally occurring substance that prevents the formation of platelet thrombi on injured blood vessels. Whether or not PGI₂ could, in addition, prevent platelet adhesion could not be determined with the test systems used.

Baumgartner has developed an ex vivo perfusion chamber for studying the interaction of human platelets in flowing citrated whole blood with the subendothelium of rabbit aorta at shear rates comparable to those in the arterial circulation. With this technique, both platelet adhesion and thrombus formation...
on the subendothelial surface can be observed and quantified, and recent studies by Tschopp et al. showed that the deposition patterns of human platelets on rabbit and human subendothelium are similar. The method has been used to identify various defects of platelet adhesion and thrombus formation in patients with bleeding disorders. For example, platelet thrombus formation is specifically decreased in thrombasthenia, whereas adhesion is decreased in Bernard-Soulier syndrome and von Willebrand's disease.

We used this technique to examine the effects of PGI₂ on platelet adhesion and thrombus formation. The studies were performed on citrated human blood to which varying concentrations of PGI₂ had been added. We also prepared PRP and studied platelet aggregation in the aggregometer.

**MATERIALS AND METHODS**

*PGI₂*. A generous gift from Dr. K. C. Nicolaou, was synthesized from the methyl ester of prostaglandin F₂₀ as previously described. A stock solution containing 1 mM PGI₂ in a vehicle (1 mM NaCl, 3 mM sodium ethoxide, and 95% EtOH, pH 11.0) was stored at −20°C. Prior to use, vehicle and PGI₂ were diluted 1/10 with 0.15 M NaCl:0.15 M Tris (pH 9.0) (2:1, v/v). Further dilutions of the PGI₂ were in this Tris-saline buffer.

*Blood*. Venous blood from normal human subjects was mixed with 0.1 vol 3.2% sodium citrate. For the adhesion studies, the citrate concentration was adjusted to 19.7 mM in plasma. The blood was incubated for 5 min at 37°C with either nothing or 1:99 (v/v) of the following: normal saline (NS), a 1:10 dilution of vehicle (V), or PGI₂.

*Platelet adhesion and thrombus formation*. Everted segments of rabbit aorta, 14 mm in length, from which the endothelium had been removed by balloon catheter were mounted on the inner core of an annular perfusion chamber (either our standard chamber or a modified chamber with smaller dimensions). Blood maintained at 37°C was circulated in a closed system through the chamber at a flow rate of either 160 ml/min for 10 min (standard chamber, calculated wall shear rate of 800/sec) or 40 ml/min for 5 min (small chamber, wall shear rate of 2600/sec). In one study, we also used everted vessel segments previously digested with α-chymotrypsin, which produces a surface whose high thrombogenicity is due to the exposure of fibrillar collagen. After perfusion, segments were removed from the chamber for further fixation, processing, and embedding. Vessel sections 0.8 μm in thickness were evaluated morphometrically around the entire circumference of the vessel at 10 μm intervals as either contact (C, platelets contact subendothelium but do not spread upon it), spread (S, platelets spread upon subendothelium), or thrombus (T, platelet thrombus 5 μm or more in height superimposed on spread platelets). Two sections approximately 3 and 10 mm from the proximal end of the vessel segment were evaluated for each segment, and the values of C, S, and T were averaged. Platelet adhesion is defined as C + S; 100T/S is a measure of the extent to which thrombi have formed on spread platelets and is an index of platelet-to-platelet interaction (aggregation).

*Platelet aggregation*. The effects of PGI₂ on platelet-subendothelium interaction were compared at various concentrations of the drug, with its effect on platelet aggregation studied by the more conventional technique using the aggregometer. Blood was centrifuged at 20°C and 1500 g for 135 sec to obtain PRP. Platelet aggregation in the aggregometer was induced with final concentrations of the following agonists (added in 0.05 vol): 5 μM adenosine diphosphate (ADP) (Sigma Chemical, St. Louis, Mo.); 5 μM epinephrine; 1.5 mg/ml ristocetin (Abbott Pharmaceutical, N. Chicago, Ill.); and two dilutions of a collagen suspension (the dilute collagen suspension induces platelet aggregation that can be inhibited by aspirin, whereas aspirin does not inhibit aggregation by the more concentrated suspension).

**RESULTS**

*Platelet aggregation in the aggregometer*. As seen in Fig. 1, 10 nM PGI₂ (in the blood from which the PRP was prepared) inhibited the change in platelet shape that normally precedes aggregation induced by ADP or collagen. Shape change was inhibited even (as in Fig. 1D) when aggregation was not. Figure 2 depicts the
Fig. 1. Effect of PGI₂ on platelet shape change and aggregation. Platelet aggregation in PRP prepared from blood that contained either vehicle (V) or 10 nM PGI₂. (A) 5 μM ADP; (B) 5 μM epinephrine; (C) dilute collagen; (D) concentrated collagen. Note that PGI₂ inhibited platelet shape change (SC) even where (as in D) aggregation was not inhibited.

Fig. 2. Dose-response inhibition of platelet aggregation by PGI₂. Platelet aggregation in PRP prepared from blood that contained either normal saline (NS), vehicle (V), or PGI₂. ▲, ADP (5 μM); ■, epinephrine (5 μM); ●, dilute collagen; ●, concentrated collagen; ×, ristocetin (1.5 mg/ml).
dose-response curves for PGI₂ and platelet aggregation by various agonists. Addition of PGI₂ to blood in concentrations of 10–20 nM resulted in a 90% or greater reduction in platelet aggregation induced by ADP or a dilute suspension of collagen. Higher concentrations of PGI₂ were needed to inhibit aggregation induced by ristocetin, the more concentrated suspension of collagen, and epinephrine (which is usually considered to be a weak agonist for platelet aggregation).

Platelet interaction with subendothelium. We found that PGI₂ strongly inhibited the formation of platelet thrombi, as shown in the photomicrograph of Fig. 3 and in Tables 1 and 2. The most striking inhibition was observed in the studies with the thrombogenic α-chymotrypsin-digested subendothelium (Table 2). Addition of PGI₂ to blood in a concentration of 10 nM resulted in a reduction of 100T/S values from 59.1% ± 8.7% (observed with vehicle-treated blood) to 6.1% ± 2.8%. With 100 nM PGI₂, this value was reduced further to 2.5% ± 1.1%, and thrombi were eliminated completely with 1000 nM PGI₂. Results of studies using (undigested) subendothelium are shown in Table 1 and Fig. 3. PGI₂ (10 nM) virtually
Table 1. Platelet Interaction With Subendothelium

<table>
<thead>
<tr>
<th>Addition</th>
<th>800 sec⁻¹, 10 min</th>
<th>2600 sec⁻¹, 5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C+S (Adhesion) (%)</td>
<td>100T/S (Aggregation)</td>
</tr>
<tr>
<td>None</td>
<td>78.8 ± 4.0</td>
<td>9.0 ± 7.8</td>
</tr>
<tr>
<td>Vehicle</td>
<td>79.7 ± 8.6</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>PG1 10 nM</td>
<td>65.9 ± 4.2 (NS)</td>
<td>3.5 ± 1.1</td>
</tr>
<tr>
<td>PG1 100 nM</td>
<td>54.8 ± 13.1 (NS)</td>
<td>12.7 ± 5.4</td>
</tr>
<tr>
<td>PG1 1000 nM</td>
<td>45.2 ± 7.5*</td>
<td>9.1 ± 3.5</td>
</tr>
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Studies performed on citrated blood from each of four normal subjects C, S, and T denote percentage surface coverage with contact platelets (C), spread platelets (S), and thrombi (T). 100T/S reflects the ability of contact platelets to spread on the subendothelium. Statistical significance of C+S values (compared with vehicle) are shown NS, not significant; *p < 0.05, **p < 0.01 (Student’s t test)

Values are the mean ± SE

Table 2. Platelet Interaction With α-Chymotrypsin-Digested Subendothelium (Fibrillar Collagen)

<table>
<thead>
<tr>
<th>Addition</th>
<th>800 sec⁻¹, 10 min</th>
<th>2600 sec⁻¹, 5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C+S (Adhesion) (%)</td>
<td>100T/S (Aggregation)</td>
</tr>
<tr>
<td>Nothing</td>
<td>22.9 ± 2.6</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>24.1 ± 4.8</td>
<td>1.3 ± 0.3</td>
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<tr>
<td>PG1 10 nM</td>
<td>16.1 ± 3.2 (NS)</td>
<td>4.7 ± 1.4</td>
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<tr>
<td>PG1 100 nM</td>
<td>9.7 ± 3.8 (NS)</td>
<td>5.8 ± 2.9</td>
</tr>
<tr>
<td>PG1 1000 nM</td>
<td>4.1 ± 3.3*</td>
<td>7.1 ± 4.2</td>
</tr>
</tbody>
</table>

Studies and symbols as in Table 1.

eliminated platelet thrombi. However, this surface is considerably less thrombogenic (control 100T/S values only 9%-15%) than the fibrillar collagen surface of α-chymotrypsin-digested subendothelium.

PGI1 also inhibited platelet adhesion to subendothelium. This was best demonstrated by the studies performed at the higher shear rate (2600 sec⁻¹). As seen in Table 1, addition of PGI1 to blood in concentrations of 10, 100, and 1000 nM resulted in a significant reduction of platelet adhesion values from 75.9% ± 4.1% (observed with vehicle-treated blood) to 40.2% ± 7.8%, 33.6% ± 4.0%, and 32.2% ± 8.0%, respectively. At the lower shear rate (800 sec⁻¹), adhesion was reduced from 79.7% ± 8.6% to 65.9% ± 4.2%, 54.8% ± 13.1%, and 45.2% ± 7.5% by these same concentrations of PGI1 (only the last reduction was significant). The adhesion of platelets to the fibrillar collagen surface of α-chymotrypsin-digested subendothelium was also inhibited by PGI1 (Table 2).

DISCUSSION

We studied the interaction of platelets in flowing blood with subendothelium at shear rates comparable to those in the arterial circulation and found that PGI1 markedly inhibited thrombus formation (100T/S). This result was achieved at a concentration of PGI1 (10 nM) that also inhibited platelet-to-platelet interaction (aggregation) induced in the aggregometer by ADP and a concentration of collagen that induces platelet aggregation that is inhibited by aspirin. It is currently held that another prostaglandin, PGE1, inhibits platelet aggregation and shape change by stimulating adenyl cyclase, thereby increasing the basal levels of platelet
cAMP.\textsuperscript{19,20} Since PGI\textsubscript{2} is an even more potent stimulator of cAMP accumulation in platelets,\textsuperscript{21,22} its inhibitory effects on platelet-to-platelet interaction (e.g., aggregation and thrombus formation) could occur through a similar mechanism.

The results of the study also demonstrate that PGI\textsubscript{2} in a concentration of 10 nM inhibits platelet adhesion (C + S) to subendothelium. However, at no concentration was adhesion completely eliminated, and, as also reported by Higgs et al.,\textsuperscript{24} the inhibitory effect of PGI\textsubscript{2} on platelet adhesion was less pronounced than its effect on thrombus formation. The mechanism by which PGI\textsubscript{2} inhibits platelet adhesion requires further study. As previously observed with PGE\textsubscript{1},\textsuperscript{23} the values of 100C/(C + S) are increased by PGI\textsubscript{2} (Tables 1 and 2). This expression reflects the ability of platelets that have made contact with the subendothelium to change their shape and spread upon this surface. The increased values of 100C/(C + S) observed with PGI\textsubscript{2} indicate that it inhibits platelet spreading, perhaps by the same mechanism that accounts for its inhibitory effect on the shape change induced by aggregating agents (Fig. 1). Since platelets that have initially attached to a surface, but have not spread upon it, are more likely to be removed by the high shear rates at the vessel wall, it is possible that PGI\textsubscript{2} facilitates the removal of contact platelets by preventing their spreading. This might also explain the greater inhibitory effect of PGI\textsubscript{2} at the higher of the two shear rates used in this study and the failure of Higgs et al. (who used only the lower shear rate) to observe inhibition of adhesion except at PGI\textsubscript{2} concentrations of 50 nM and greater.\textsuperscript{24} In the present study, we found that 10 nM PGI\textsubscript{2} inhibited platelet adhesion by about 45\% when studied at a shear rate of 2600 sec\textsuperscript{-1}, and it might be an even more potent inhibitor of adhesion at higher shear rates. This could be of significance in view of recent studies indicating that shear rates as high as 16,000 sec\textsuperscript{-1} may exist in the microvasculature.\textsuperscript{25,26} The shear rate dependence of the platelet adhesion defect produced by PGI\textsubscript{2} is reminiscent of similar observations in patients with von Willebrand disease.\textsuperscript{11} In addition, this finding suggests that PGI\textsubscript{2}, either alone or through its synergistic effects on drugs such as dipyridamole,\textsuperscript{27} may be a more effective inhibitor of platelet adhesion in the arterial (high shear) than in the venous (low shear) circulation. Finally, since we used denuded blood vessels, the results of the study are not directly applicable to the question of platelet-endothelial interactions (or lack thereof), although inhibition of platelet spreading on surfaces could well be a general property of PGI\textsubscript{2}.

It is currently held that removal of the endothelium by a variety of mechanisms (immunologic, toxic, hemodynamic) is followed by the deposition of platelet thrombi and release from the platelets of biologically active substances. The results of this study suggest that PGI\textsubscript{2} (produced either in adjacent endothelial cells or in other cells of the vessel wall) could play a role in regulating the deposition of platelets on the subendothelium. Hence PGI\textsubscript{2} could be an important regulator of a variety of platelet-mediated biologic processes.

ACKNOWLEDGMENT

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REFERENCES

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