Inhibition of Indium-111 Platelet Accretion Onto Venous Thrombi in Dogs by Prostacyclin

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SUMMARY. The known platelet anti-aggregant effects of prostacyclin (epoprostenol) suggest that it may have therapeutic potential in conditions in which the platelet plays a pathophysiological role. The growth of venous thrombi is one such condition. We have attempted to determine, in a canine model of fresh venous thrombosis, whether prostacyclin infusion inhibits platelet accretion in vivo and how this in vivo event related to hemodynamic and in vitro platelet anti-aggregant effects. Gamma camera imaging over thrombi for accretion of indium-111-labeled platelets disclosed that prostacyclin, at an infusion rate of 50 ng/kg per min, inhibited platelet accretion in vivo and resulted in a 95 ± 4% decrease in in vitro adenosine diphosphate-induced platelet aggregation, and a decrease in mean arterial pressure to 86 ± 4% of pre-infusion values. Stepwise decrements of prostacyclin infusion demonstrated that platelet accretion occurred in vivo at infusion rates of approximately 10–20 ng/kg per min and correlated with an in vitro adenosine diphosphate-induced aggregation of 54 ± 13% of control values. Thus, prostacyclin, in a dose that causes only a mild decrease in systemic pressure, can completely inhibit platelet uptake onto fresh venous thrombi in the dog, and this inhibition correlates closely with in vitro adenosine diphosphate-induced platelet aggregation. The potential therapeutic implications of these findings are discussed. (Circ Res 53: 830-833, 1983)

PROSTACYCLIN (PGI2), the major product of arachidonic acid metabolism in the vascular endothelium, is a potent inhibitor of platelet aggregation and vasodilator (Bunting et al., 1976; Johnson et al., 1976; Kadowitz et al., 1978). As such, prostacyclin has potential as a therapeutic tool in pathophysiological states involving platelets. Indeed, recent reports have suggested the usefulness of PGI2 infusion in disease states such as pulmonary embolism (Utsunomiya et al., 1980), coronary artery thrombosis (Aiken et al., 1979a, 1981; Romson et al., 1981), hemodialysis (Woods et al., 1978; Zusman et al., 1981), cardiopulmonary bypass (Coppe et al., 1979; Koshal et al., 1981; Longmore et al., 1979), and endotoxic shock (Demling et al., 1981; Fletcher and Ramwell, 1980; Krausz et al., 1981). However, systemic hypotension, secondary to vasodilation, may limit the clinical applicability of prostacyclin. Most reports in dogs which demonstrate a therapeutic benefit of PGI2 have used infusion rates of from 100 to 500 ng/kg per min (Coppe et al., 1979; Demling et al., 1981; Fletcher and Ramwell, 1980; Koshal et al., 1981; Krausz et al., 1981; Longmore et al., 1979; Romson et al., 1981; Utsunomiya et al., 1980; Woods et al., 1978), doses known to cause substantial reductions in systemic arterial pressures.

To date, no investigations have been reported regarding the ability of prostacyclin infusion to inhibit the growth of venous thrombi. In a canine model of venous thrombosis, we have examined the effects of prostacyclin infusion upon the growth of fresh venous thrombi, as monitored by gamma camera detection of accretion of indium-111-labeled platelets. The questions addressed were: at what level of prostacyclin infusion does platelet accretion onto venous thrombi occur; what relationship, if any, exists between in vitro measures of platelet aggregability (Czer and Moser, 1983) and the onset of platelet accretion; and at the infusion rates of PGI2 that prevent platelet accretion, what degree of systemic hypotension occurs?

Methods

Experimental Venous Thrombi

Mongrel dogs weighing 18–23 kg were anesthetized with sodium pentobarbital, 25 mg/kg, and intubated, but allowed to breathe spontaneously. Venous thrombi were induced without intimal damage, as previously described (Moser et al., 1980). Briefly, a modified Swan Ganz catheter was introduced through a venous cutdown proximal to the paw and the tip advanced to the femoral triangle. The balloon was then inflated with 1 cc of air. Two minutes later, 10 units of topical thrombin were injected into an orifice created proximal to the balloon and flushed with 2 ml of saline. The balloon was deflated 30 minutes later, but the catheter was left in place. All thrombi were, therefore, 30-minutes-old when re-exposed, by balloon deflation, to venous blood flow. Contrast venography has previously shown that balloon deflation is associated with restoration of blood flow over the thrombus, but not embolization.

Hemodynamic Measurements

A femoral arterial catheter was placed in the contralateral leg for blood sampling and measurement of mean
arterial pressure using a Statham-Gould P23db pressure transducer.

Preparation of Indium-111-Labeled Platelets

Indium-111 oxine complex was formed by a modification of Thakur’s method (Thakur et al., 1976) which has been previously described in detail elsewhere (Fedullo et al., 1982).

Using this procedure, from 3 to 6 x 10^8 autologous platelets were labeled with 1-3 mCi of indium-111. Measurement of cell-bound and platelet-bound indium-111 (Fedullo et al., 1982) were done with each preparation, and overall results were: cell-bound = 95 ± 3%, platelet-bound = 93 ± 3% (n = 7).

Nuclear Imaging

The scintillation camera used was equipped with a medium energy, parallel hole collimator, and the pulse height analyzer was set to include both the 173 keV and 247 keV photopeaks of indium-111. Data were acquired and stored on a Nuclear Medicine dedicated computer system, and consisted of anterior static images acquired for approximately 200,000 counts every 5 minutes. Scintiphotos were also taken every 10 minutes to allow more immediate analysis of thrombus platelet uptake. Regions of interest over the thrombus, the contralateral vein, and a background area were outlined with a light pen and subjected to computer analysis to construct time-activity curves. The time at which thrombus platelet uptake occurred (clot visualization) was determined to be the point at which the slope of the time-activity curve rose.

Preparation of “Platelet-Rich Plasma” (PRP)

Blood was drawn into a plastic syringe containing a 3.8% sodium citrate solution, pH 6.5 (blood:anticoagulant ratio of 9:1, and resulting pH = 7.30). This was processed as previously described (Czer and Moser, 1983). The time from blood drawing to platelet aggregometry was 2½ minutes ± 10 seconds.

Platelet Aggregometry

Platelet aggregometry was performed in a Payton Aggregometer after the method of Weiss (1972). PRP (0.45 ml) was pipetted into an aggregometer tube, stirred at 800 rpm, and pre-incubated at 37°C for exactly 1 minute before the addition of adenosine diphosphate (ADP) (Sigma Chemical Co). The changes in light transmission were recorded on a Houston Instruments Omniscribe recorder. Prior to prostacyclin infusion, the highest concentration of ADP that evoked reversible platelet aggregation was determined. Platelet aggregation was induced in each subsequent sample with this ADP concentration, and results were expressed as percent of the control (pre-infusion) value.

Study Protocol

A solution of prostacyclin was prepared from prostacyclin sodium salt (1.14 mg of this containing 1.00 mg of free prostacyclin) (kindly provided by Dr. John Pike, Upjohn Company, Kalamazoo, Michigan). The PGI2 was dissolved in a 20 mM glycine buffer, pH 10.5, and infused through a central venous line placed via the internal jugular vein. Two hours after balloon deflation, and immediately after initial platelet studies and mean arterial pressure (MAP) measurements, prostacyclin infusion was begun at 50 ng/kg per min. Twenty minutes thereafter, a blood sample was drawn for platelet aggregation studies, MAP was recorded, and the indium-111-labeled platelets were injected. After 1 minute, gamma camera data acquisition was begun, with one frame recorded every 5 minutes as described above. Prostacyclin infusion was continued at 50 ng/kg per min for 20 minutes, then decreased to 12 ng/kg per min for 20 minutes and finally decreased to 6 ng/kg per min for 20 minutes, after which the infusion was stopped. Samples of blood were taken for platelet studies and MAP measurements made every 10 minutes during this sequence. Data acquisition continued for 50 minutes after scintiphoto evidence of thrombus platelet accretion. We evaluated the extent of thrombus formation at postmortem examination, noting the thrombus diameter, as well as proximal and distal extension.

Previous studies have shown that platelet accretion onto venous thrombi occurs within 5 minutes after indium-111 platelet infusion in the absence of any anticoagulant (Moser et al., 1980). Preliminary studies in dogs with PGI2 infused at 50 ng/kg per min. over 6 hours have shown no change in platelet aggregability (measured in vitro) over the interval.

Statistical Analysis

Data are expressed as mean ± sd. The paired t-test was used to determine statistical significance at P < 0.05.

Results

The prostacyclin infusion rate of 50 ng/kg per min reduced in vitro ADP-induced aggregation to 5 ± 4% of control (pre-infusion) values (Table 1). This was associated with a small (14 ± 4%, P < 0.05) decline in mean arterial pressure from 134 ± 24 to 116 ± 20 mm Hg (Table 2). During the 20-minute period of prostacyclin infusion after injection of the radiolabeled platelets, there was no demonstratable uptake of indium-111-labeled platelets in seven of the eight animals. Platelet uptake occurred in one animal despite complete inhibition of platelet aggregation demonstrated in vitro. This same animal had the most extensive clot formation at postmortem examination.

The results of a typical experiment (animal #8) are shown in Figure 1. The decremental prostacyclin

<table>
<thead>
<tr>
<th>Animal</th>
<th>Platelet aggregation at visualization*</th>
<th>Platelet aggregation at visualization*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>—†</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>60</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>54 ± 13</td>
</tr>
</tbody>
</table>

*See Methods and Figure 1.
†Clot visualization occurred during 50 ng/kg per min PGI2 infusion.
infusion chosen was associated with a smooth recovery of in vitro platelet aggregation. In the seven animals that demonstrated no platelet uptake during the 50 ng/kg per min PGI$_2$ infusion, clot visualization occurred at in vitro platelet aggregation of 54 ± 13% of control values (Table 1).

The hemodynamic and platelet observations during decremented prostacyclin infusion are summarized in Table 2. The 12 ng/kg per min and 6 ng/kg per min infusions resulted in no statistically significant declines in arterial pressure but resulted in 58 and 34% inhibition of in vitro platelet aggregation, respectively.

**Discussion**

This study demonstrates that in vivo accretion of platelets onto fresh canine venous thrombi can be inhibited by prostacyclin at infusion rates that cause only a mild decrease in mean arterial pressure. Furthermore, our data indicate that this in vivo inhibition of platelet function correlates with in vitro measurement of ADP-induced platelet aggregation. Vascular instability and hypotension are present in many of the disease states in which PGI$_2$ might be useful (endotoxic shock, hemodialysis, cardio-pulmonary bypass, and pulmonary embolism). Therefore, the ability to achieve favorable in vivo effects at dose regimens that avoid systemic hypotension is an important therapeutic consideration.

Aiken et al. (1979a), using a canine model of coronary arterial obstruction, have demonstrated that a prostacyclin infusion rate of 50 ng/kg per min completely prevented thrombus formation, whereas a rate of 15 ng/kg per min did so in only 33% of animals. In rabbits, prostacyclin at 100–200 ng/kg per min has been shown to inhibit thrombus formation in electrically stimulated carotid arteries while MAP was minimally decreased (Utatuba et al., 1979). These findings parallel ours, despite the very different local conditions (high blood flow arterial site vs. low flow venous site). Taken together, these results suggest that, in different pathophysiological states, a therapeutic window may exist wherein platelet function can be inhibited without inducing clinically significant systemic hypotension.

Using a rapid platelet isolation technique that minimized the time from blood drawing to platelet aggregometry, we also found that the onset of platelet accretion onto venous thrombi correlated with a narrow range of in vitro ADP-induced platelet aggregation (54 ± 13% of control values) in seven of eight animals. In the remaining animal, platelet accretion occurred despite 100% inhibition of platelet function demonstrated in vitro. There are several possible explanations for the behavior of this one animal. First, inter-animal variation could exist in the correlation between the in vivo and in vitro measures of platelet function. This seems unlikely in view of the narrow range seen in the other animals. Second, local conditions around the thrombus may have caused prostacyclin to be less effective. This particular animal had the most extensive thrombus formation of the group, which may have led to a much decreased local rate of blood flow. Since PGI$_2$ is very unstable in vivo, with a half-life of less than 3 minutes, stasis may have led to significant PGI$_2$ degradation locally, resulting in decreased platelet anti-aggregant effect and allowing thrombus platelet uptake.

In prior studies in which in vitro platelet aggregometry was used to reflect in vivo prostacyclin activity, the inhibition of in vitro platelet aggregation has been highly variable (Aiken et al., 1979a; Koshal et al., Szczeklik et al., 1978; Whittle et al., 1980; Zusman et al., 1981). Such variability appears to reflect the variability in platelet isolation and aggregation techniques used—in particular, the time from blood collection to platelet aggregometry, which ranged from 2 (Whittle et al., 1980) to 40 (Aiken et

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**TABLE 2**

<table>
<thead>
<tr>
<th>Infusion rate (ng/kg per min)</th>
<th>Mean arterial pressure (% control)*</th>
<th>Platelet aggregation (% control)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>86 ± 4†</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>12</td>
<td>95 ± 5</td>
<td>42 ± 15</td>
</tr>
<tr>
<td>6</td>
<td>101 ± 5</td>
<td>66 ± 10</td>
</tr>
</tbody>
</table>

* Data obtained 20 minutes after infusion change; $n = 7$.
† $P < 0.05$.
al., 1979b) minutes. Considering the lability of prostacyclin (Johnson et al., 1976; Pifer et al., 1981), it is likely that a technique which minimizes that time, such as the one used here, more closely reflects the in vivo state of platelet aggregability. In fact, we obtained a close correlation.

Interspecies differences exist not only in the sensitivity to given infusion rates of prostacyclin, but also in the relationship between the hemodynamic and anti-platelet effects (Whittle et al., 1980). A rapid, simple in vitro technique which could predict the minimum prostacyclin infusion rate necessary to achieve inhibition of platelet function in vivo would, therefore, be useful. In man, infusions of prostacyclin at rates exceeding 10–20 ng/kg per min are associated with profound hypotension. It is not yet known if lower infusion rates causing less hypotension can achieve potentially therapeutic platelet anti-aggregant effects in vivo. Our data demonstrate that, in the dog, a 50 ng/kg per min infusion rate led to a mild decline in arterial pressure, and the lower infusion rates were not associated with statistically significant declines.

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References

References


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