In Vivo Release and Turnover of Secreted Platelet Antiheparin Proteins in Rhesus Monkey (Macaca Mulatta)

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Human and rhesus monkey platelets secrete at least two antiheparin proteins: platelet factor 4 (PF₄) and low affinity platelet factor 4 (LA-PF₄). Neither of these proteins showed species-related antigenic differences. As determined by radioimmunoassay, the levels of PF₄ and LA-PF₄ antigen per 10⁸ monkey platelets amounted to 10.7 and 20.3 μg, respectively. One milliliter of monkey plasma prepared from blood collected into an anticoagulant composed of EDTA, prostaglandin E₁, and theophylline solution contained 22.4 ng LA-PF₄ and 8.0 ng PF₄. Concentrations of these two platelet-specific proteins in monkeys closely resembled levels found in human platelets and plasma. Infusion of prostacyclin (PGI₂) (100 or 300 ng/kg/min) into monkeys for 15 min resulted in a significant decrease of plasma levels of LA-PF₄ antigen and of PF₄ by 40%-60% (p < 0.0001). This decrease was related to the inhibitory effect of PGI₂ on the secretion of platelets stimulated by a catheter or by venipuncture. Longer infusion of PGI₂ did not produce further significant change. The supernate obtained after aggregation of human platelets stimulated by thrombin was injected into monkeys receiving PGI₂ infusion. The disappearance of LA-PF₄ antigen in monkey plasma followed a biphasic exponential curve with half-lives for the fast and slow component of 8.4 and 63 min. PF₄ disappeared faster but followed the same pattern (half-lives for the fast and slow component of 2.1 and 70 min). Analysis of the experimental data suggests that the low levels of secreted platelet proteins in monkey plasma are related to their minimal in vivo release and to their rapid clearance.

The stimulated platelet releases a number of proteins from its α-granules. Two low molecular weight proteins with antiheparin activities—platelet factor 4 (PF₄) and low affinity platelet factor 4 (LA-PF₄)—are immunologically specific for human platelets and can be considered as platelet markers. LA-PF₄ is immunologically identical with β-thromboglobulin (βTG). LA-PF₄ is originally secreted by platelets but it can be converted to βTG upon the action of plasmin or platelet neutral protease, which splits off a tetrapeptide from the N-terminus of LA-PF₄.

The levels of PF₄ and LA-PF₄/βTG antigens in platelet-free plasma of normal human subjects amount to less than 0.3% of their levels in platelet-rich plasma. As determined in various laboratories, the values of PF₄ in platelet-poor plasma vary between 2.5 and 16 ng/ml and the values for βTG/LA-PF₄ antigen vary between 11 and 39 ng/ml. The reported values of PF₄ and LA-PF₄/βTG antigens in intact human platelets are 12.4 and 24.2 μg/10⁹ platelets or 18.0 and 17.7 μg/10⁹ platelets.

Several reports indicate that increased levels of PF₄ and LA-PF₄/βTG antigen may occur in the plasma of patients with enhanced platelet stimulation or destruction in vivo. However, the data obtained in various laboratories are still inconsistent. For instance, Chesterman et al. concluded that the raised level of PF₄ is by no means specific for thromboembolic disease and that platelet destruction is not invariably associated with abnormally increased PF₄ levels.

The level of platelet proteins in plasma depends on the rate of their release in vivo and on their half-lives in the circulation. It is not known whether release of platelet proteins occurs continuously in the flowing blood or if it is limited to the sites of injury. Reports on the half-lives of platelet proteins in plasma are preliminary. Gjesdahl and Pepper determined that the half-life of PF₄ in the plasma of the rhesus monkey was 7–11 hr. Dawes et al. injected serum into a human volunteer and determined that the clearance of PF₄ was so rapid that its half-life could not be estimated. βTG antigen was cleared with a half-life of about 100 min.

In this study, we compared immunologic properties of PF₄ and LA-PF₄/βTG antigen secreted by human and rhesus monkey platelets, and we found that these proteins did not show species-dependent antigenic differences. Therefore, the rhesus monkey appeared to be a suitable experimental model for studying release...
TURNOVER OF SECRETED PLATELET PROTEINS

in vivo and turnover of secreted platelet proteins. This article describes experiments in which we attempted to block release of PF₄ and LA-PF₄ antigen from monkey platelets in vivo by infusing prostacyclin (PGI₂) and determined the half-lives of human PF₄ and LA-PF₄ antigens in the circulation.

MATERIALS AND METHODS

Material Released From Human Platelets by Thrombin

Washed platelets were prepared from human blood according to Mustard et al. Material released from a platelet suspension (5 x 10⁷ platelets/ml) was obtained following the addition of 100 U/ml of highly purified human thrombin kindly supplied by Dr. J. Fenton (Albany, N.Y.). After 3-min incubation at 37°C with occasional shaking, the platelet pellet was removed by centrifugation and discarded. The supernate was heated for 2 min at 90°C and the resulting precipitate was removed by centrifugation. One milliliter of supernate contained 1.3 mg protein, 90 μg LA-PF₄ antigen, and 46.5 μg PF₄. As determined by combination of isoelectric focusing and radioimmunoassay, LA-PF₄, β-thromboglobulin, and platelet basic protein contributed 80%, 8%, and 12%, respectively, of the total immunoreactivity of LA-PF₄ antigen in the material. The supernate was free of any thrombin activity as tested by clotting assay. It was stored at -20°C.

Human PF₄ and LA-PF₄

Human PF₄ and LA-PF₄ were isolated as previously described, and antibodies against these proteins were raised in rabbits.

Prostacyclin (PGI₂)

Prostacyclin (PGI₂), synthesized as described previously, was dissolved in 99% ethanol (3.5 mg/10 ml) and stored at -20°C. Before infusion, a stock solution of PGI₂ was diluted with 0.05 M Tris buffer, pH 9.0.

Animals

For all experiments, female Macaca mulatta monkeys were used. Their weights ranged from 3.6 to 8.0 kg (average 5.9 kg). All animals were sedated before the inception of the experiment with 10 mg of phencyclidine hydrochloride injected intramuscularly (Sernylan, Bio-Ceutic Labs, Inc., St. Joseph, Mo.). This drug does not influence platelet aggregation. During the time of the study, an additional 2 mg of the drug was administered as often as necessary. An intravenous catheter (no. 4 French size, Cook, Inc., Bloomington, Ind.) was placed into the femoral vein via percutaneous puncture. After catheter insertion, an intravenous line was established with 0.9% saline, which was administered at the rate of 0.5 ml/min for the duration of the study.

Blood Sampling

Blood for various experiments was taken either by direct venipuncture of the femoral veins using 20-gauge siliconized needles or from the catheter via a 3-way stopcock, and it was anticoagulated by the method of Ludlam to prevent in vitro release of platelet proteins. When blood was taken from the catheter, the first 1 ml was discarded. To obtain platelet-poor plasma (PPP), 1.8 ml of blood was taken into a cooled syringe (4°C) containing 0.2 ml of 10% EDTA with 5.4 mg/ml of theophylline. Just before blood sampling, 20 μl of 10⁻³ M PGE₁ was added to the previously described mixture. Blood for PPP was then spun for 20 min at either 2000 g or 3000 g at 4°C. The resulting PPP was then removed and respun for another 20 min under the same conditions. PPP obtained in this way was transferred into another tube and a small aliquot taken for platelet counting ( Coulter-Counter, Hialeah, Fla.). Then 10 μl Triton X-100 (Sigma Co., St. Louis, Mo.) was added per 900 μl PPP, and the sample was frozen and stored at -20°C for determination of the level of platelet-specific proteins. PRP was obtained by centrifugation (15 min, 150 g, room temperature) of 9.0 ml blood, drawn into a syringe containing 1.0 ml of 3.8% sodium citrate. After platelet counting, samples for determining platelet-specific proteins were treated with Triton X-100 as above and kept frozen. Samples of platelet-rich plasma (PRP) for aggregation studies were kept at room temperature for 2–3 hr.

Immunodiffusion

Immunodiffusion was performed according to Outcherlony. Human and monkey PRPs were used to detect LA-PF₄ antigen. Anti-human PF₄ antibody showed much lower precipitating titer as compared with anti-human LA-PF₄ antibody. Using this antibody, PF₄ was not detectable in human or monkey PRP by immunodiffusion technique. In order to obtain visible precipitation lines with PF₄, the platelet pellet obtained from 1.0 ml of human or monkey plasma was resuspended in 0.2 ml 0.15 M NaCl and sonicated. This material was used as the antigen against anti-PF₄ antibody.

Determination of the Level of Platelet-Specific Proteins in PRP, PPP, and Urine

LA-PF₄ antigen and PF₄ were determined in PRP and PPP using radioimmunoassay developed in this laboratory, as previously described. Alternatively, PF₄ was determined using a commercial kit from the Abbott Company. LA-PF₄ antigen in urine was determined in 5 or 10 times concentrated material after dialysis and lyophilization of urine samples according to Dawes et al.

Platelet Aggregation

Adenosine diphosphate (ADP) induced platelet aggregation was studied using a Chrono-Log aggregometer (Chrono-Log Corporation) in 0.5 ml PRP samples prewarmed at 37°C for 1 min. In each experiment, the ADP concentration was adjusted to cause maximum aggregation corresponding to 40 chart units. In these conditions, deaggregation observed after 3 min was usually less the 10 U. The aggregometer recorder was set up in such a manner that the difference between PRP and corresponding PPP was 80 chart units.

Infusion of Prostacyclin and Heparin

After insertion of the catheter, blood samples for PPP, PRP, hematocrit, and pH determinations were obtained through a 3-way stopcock. As a rule, 2 separate samples of blood for PPP preparation were collected within 10–15 min. PGI₂ was diluted in 0.05 M Tris buffer (pH 9) and infused into the animal at a constant rate of 0.0388 ml/min. The dilution of prostacyclin was adjusted to obtain an injection dose of either 100 ng/kg/min or 300 ng/kg/min. Blood samples for PPP were taken through the catheter at 15, 30, 60, 90, 120, 150, and 180 min during PGI₂ infusion. PGI₂ was then turned off, and blood samples for PRP, hematocrit, and pH were taken. When PGI₂ in the dose of 300 ng/kg/min was injected, additional samples for PPP were obtained 30 and 60 min after PGI₂ withdrawal. Heparin was infused into the animals in the dose of 1.0 U/ml diluted in 0.9% saline at a rate of 0.5 ml/min. Heparin and PGI₂ were infused into different animals. Blood samples for determination of platelet-specific proteins were then taken as described above.
Half-life of Secreted Platelet Proteins

Material released by thrombin-stimulated platelets was injected into animals that were infused with PGI₂ (100 ng/kg/min) for 15 min. The dose injected was 100 μg/kg of body weight of LA-PF₄ antigen and 51.7 μg/kg of PF₄, respectively. Blood was then taken from the catheter at various time intervals, and PPP was obtained as previously described for determination of the level of platelet-specific proteins. In addition, during the 4-hr period after injection of human platelet proteins, urine was collected using a catheter placed into the urinary bladder.

The half-lives of platelet proteins in monkey circulation were calculated by graphic fitting of the data using a simplified two-compartment model for plasma protein kinetics. The disappearance of platelet proteins from plasma was described by the equation:

\[ C(t) = c_1e^{-k_1t} + c_2e^{-k_2t} \]

where \( t \) = time, \( c_1 \) = initial concentration of protein for the fast component, \( c_2 \) = initial concentration of protein for the slow component, \( k_1 \) and \( k_2 \) = rate constants of the two exponentials.

All statistical comparisons of data were made with Student’s t test.

RESULTS

Immunoassays of Platelet Proteins in Monkey M. Mulatta

A double immunodiffusion study with the antibody prepared against human LA-PF₄ confirmed complete immunologic identity of human LA-PF₄ and monkey LA-PF₄ antigens. Similarly, human PF₄ and monkey PF₄ showed complete immunologic identity (Fig. 1).

In the PF₄ or LA-PF₄ radioimmunoassay, all displacement curves obtained either with human or with monkey platelet proteins were identical and superimposable.

Levels of platelet-specific proteins in monkey PRP and PPP are shown in Table 1. These values were close to those reported previously for human platelets. The levels of both platelet-specific proteins in monkey PPP depended on the method of centrifugation and correlated with the residual platelet count. Since centrifugation of blood at 3000 g reduced the platelet count to a minimum and yielded the lowest values of secreted platelet proteins in plasma, this method was applied in further studies. Platelet counts in PPPs of all experimental groups did not differ significantly.

Effect of Prostacyclin (PGI₂) Infusion on the Levels of the Platelet Proteins in Platelet-Poor Plasma

We attempted to block release of platelet proteins in vivo by infusing PGI₂ into monkeys. Effects of PGI₂ were monitored by studying ADP-induced platelet aggregation in monkey PRP. PGI₂ was infused in two doses: 100 ng/kg/min and 300 ng/kg/min. Low doses of PGI₂ had a slight inhibitory effect on platelet aggregation and high doses of PGI₂ resulted in

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of Experiments</th>
<th>LA-PF₄ Antigen (ng/ml)</th>
<th>PF₄ (ng/ml)</th>
<th>LA-PF₄ Antigen: PF₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP*</td>
<td>11</td>
<td>20,270 ± 5,920</td>
<td>10,710 ± 5,470</td>
<td>1.89</td>
</tr>
<tr>
<td>PPP†</td>
<td>26</td>
<td>44.7 ± 11.3</td>
<td>22.1 ± 14.7</td>
<td>2.02</td>
</tr>
<tr>
<td>PPP‡</td>
<td>14</td>
<td>22.5 ± 9.5</td>
<td>8.0 ± 5.9</td>
<td>2.81</td>
</tr>
</tbody>
</table>

*Platelet count in PRP adjusted to 10⁶/ml.
†PPP was obtained by two times centrifugation at 1500 g (see Materials and Methods). Platelet count was 2 x 10⁶/ml (SD ± 10⁶/ml).
‡PPP was obtained by two times centrifugation at 3000 g (See Materials and Methods). Platelet count was 0.7 x 10⁶/ml (SD ± 0.43 x 10⁶/ml). The differences in platelet count, PF₄ level, and LA-PF₄ antigen levels in the 2 PPP samples were statistically significant at the level <0.001.

Fig. 1. Immunodiffusion of secreted platelet proteins in human and monkey PRP. Left central well contained rabbit anti-human LA-PF₄ serum and right central well, rabbit anti-human PF₄ serum. Peripheral wells contained human PRP (H-PRP), monkey PRP (M-PRP), and purified human PF₄ and LA-PF₄ prepared by the method of Rucinski et al. Volume of each sample was 5 μl.
before infusion
30 0 15 30 60 120 180 240
TIME, MINUTES

Fig. 2. Effect of PGI2 infusion on the levels of LA-PF4 (O—O) and PF4 (Δ—Δ—Δ) in plasma. Values refer to means and SEM. (A) Infusion of PGI2 (100 ng/min/kg) for 100 min. Mean values from 6 experiments. (B) Infusion of PGI2 (300 ng/min/kg) for 150 min. Additional samples were obtained after 180 and 210 min (i.e., 30 and 60 min after cessation of the infusion). Mean values from 5 experiments. In 6 animals receiving lower dose of PGI2 during 150 min, aggregation amounted to 70.2% ± 17.6% (SEM) of the maximal aggregation. Infusion of high dose of PGI2 resulted in a complete inhibition of ADP-induced platelet aggregation.

Infusion of PGI2 (100 ng/kg/min) into monkeys for 15 min resulted in a significant decrease of LA-PF4 antigen and PF4 in PPP (Fig. 2A). Statistical analysis revealed that there was no further decrease in the levels of both platelet proteins during longer PGI2 infusion up to 150 min. In three separate experiments (not shown), the level of these proteins was followed up to 240 min, and no apparent changes were noticed. High doses of PGI2 (300 ng/kg/min) produced a similar effect on the level of secreted platelet proteins in monkey plasma (Fig. 2B). At both doses (Fig. 2A and B), approximate decrease of LA-PF4 antigen and PF4 levels was 40% and 60%, respectively. In 6 animals (Fig. 2B), the levels of both proteins in plasma significantly increased within 60 min after cessation of the infusion of PGI2. Control experiments demonstrated that the platelet counts in PRP and PPP, hematocrit, and pH of blood were not affected by the infusion of prostacyclin.

Table 2 summarizes levels of LA-PF4 antigen in PPP prepared from monkey blood obtained by venipuncture and catheter. It can be seen that levels of both proteins were almost identical in PPP prepared from blood obtained by venipuncture and in PPP prepared from blood obtained through catheter immediately after its insertion. Longer maintenance of the catheter with continuous infusion of 0.85% NaCl resulted in the formation of small blood clots that were frequently observed after 60 min. Infusion with heparin (0.5 U/min) or with prostacyclin inhibited formation of these clots. Table 2 shows that the infusion of heparin slightly increased the average value of LA-PF4 and significantly increased the variability of results. The level of PF4 in plasma after heparin infusion could not be determined because in our hands heparin interfered with the assay. Infusion of prostacyclin resulted in a decrease of the level of LA-PF4 antigen and PF4 in plasma and a decrease of the variability of the assay.

**Half-lives of the Platelet Proteins in Plasma of Rhesus Monkey**

Figure 3 shows the levels of LA-PF4 antigen in monkey plasma at various time intervals after bolus injection of material released by thrombin-stimulated platelets into animals receiving continuous infusion of prostacyclin. The disappearance of LA-PF4 antigen from monkey plasma followed a biphasic exponential curve with half-lives for the fast and slow components of 8.4 and 63 min. As shown in Fig. 4, PF4 disappeared faster but followed the same pattern. The half-lives for the fast and slow components were 2.1 and 70 min, respectively. Less than 0.1% of the total LA-PF4 antigen injected was recovered in urine collected during the 3 hr following injection. Table 3 compares the rate of disappearance of PF4 and LA-PF4 antigen after injection of platelet proteins into the rhesus monkey. Fifteen minutes after injection, the percentages of initial LA-PF4 antigen and PF4 recovered in plasma were 17.2% and 1.2%, respectively.

**DISCUSSION**

Our data indicate immunologic identity of LA-PF4/βTG antigen in human platelets and in platelets
Table 2. Levels of LA-PF₄ Antigen and PF₄ in Monkey PPP Prepared From Blood Obtained by Venipuncture and Catheter (Mean Values and SEM)

<table>
<thead>
<tr>
<th>Method of Blood Sampling</th>
<th>Number</th>
<th>LA-PF₄ Antigen (ng/ml)</th>
<th>PF₄ (ng/ml)</th>
<th>LA-PF₄ Antigen: PF₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venipuncture</td>
<td>10</td>
<td>22.4 ± 2.44</td>
<td>8.73 ± 2.76</td>
<td>2.57</td>
</tr>
<tr>
<td>Catheter (A)</td>
<td>14</td>
<td>22.5 ± 2.63</td>
<td>8.02 ± 1.70</td>
<td>2.81</td>
</tr>
<tr>
<td>(B)</td>
<td>3</td>
<td>27.8 ± 3.25</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>(C)</td>
<td>11</td>
<td>14.2 ± 0.62</td>
<td>3.68 ± 0.35</td>
<td>3.86</td>
</tr>
</tbody>
</table>

Values in samples A do not differ statistically from values in venipuncture, and values in sample B (wide variations) do not differ from A. Values in C differ significantly from A (p < 0.0001).

ND, not determined.

of the rhesus monkey, and immunologic identity of PF₄ antigen in these two species (Fig. 1). The antigenic similarity between monkey and human LA-PF₄ has been previously reported by our laboratory and others, however, Chater et al. found decreased values of βTG antigen in monkey serum and plasma as compared with human material. In our experimental system, the levels of both antigens in monkey platelets (Table 1) and in human platelets were almost identical. The LA-PF₄:PF₄ ratio for platelets of both species is close to 2.0 (Table 1). The ratio of LA-PF₄ in PRP:LA-PF₄ in PPP for the rhesus monkey was 500–1000. Similar values were reported for human subjects. In contrast, secreted platelet antieheparin proteins from other nonprimate mammalian species are antigenically different from human platelet antieheparin proteins. Therefore, the rhesus monkey appears to be a suitable experimental model for studying in vivo release and turnover of human platelet secreted proteins.

Prostacyclin (PGI₂), one of the most potent inhibitors of platelet aggregation and release in vitro, also inhibits platelet aggregation in vivo when infused into human subjects. By infusing PGI₂ into monkeys, we intended to inhibit endogenous release of secreted platelet proteins in order to estimate the half-lives of injected PF₄ and LA-PF₄ antigen with adequate accuracy.
TURNOVER OF SECRETED PLATELET PROTEINS

Two compartmental analysis of the disappearance of antithrombin proteins from monkey plasma revealed fast and slow components in both processes (Figs. 3 and 4). Studies on the catabolism of plasma proteins suggest that the fast component may represent the rate of distribution of protein in the body fluids. Since the molecular weights of PF$_4$ and LA-PF$_4$ (βTG) are very close, their distribution rates should be similar or identical. However, the initial disappearance rates of both proteins are clearly different. There is evidence that both PF$_4$ and βTG bind to endothelial cells and that heparan sulfate present on the surface of endothelial cells is essential for PF$_4$ binding. In vitro, both PF$_4$ and LA-PF$_4$ bind to heparan sulfate; however, PF$_4$ binds with greater affinity. Therefore, it can be postulated that the fast components of the disappearance process of platelet proteins reflect their binding to the endothelial cells rather than the rate of distribution in the body fluids. The slow components most likely reflect true catabolic processes. Recent observations in our laboratory suggest that the kidney is a catabolic site of human platelet secreted proteins injected into the monkey. Hypothetical values, assuming immediate and uniform distribution of injected protein in the circulating plasma. The plasma volume was calculated on the basis of hematocrit measurement, assuming that the total amount of blood constitutes 5.6% of the body weight of the rhesus monkey.

### Table 3. Disappearance of LA-PF$_4$, Antigen and PF$_4$ From Monkey Plasma (Mean Values and SD From Six Experiments)

<table>
<thead>
<tr>
<th>Sample</th>
<th>LA-PF$_4$ Antigen</th>
<th>PF$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>3.24 ± 0.18 µg/ml</td>
<td>100.00</td>
</tr>
<tr>
<td>2 min</td>
<td>1.59 ± 0.22 µg/ml</td>
<td>49.0</td>
</tr>
<tr>
<td>15 min</td>
<td>0.56 ± 0.10 µg/ml</td>
<td>17.2</td>
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*Hypothetical values, assuming immediate and uniform distribution of injected protein in the circulating plasma. The plasma volume was calculated on the basis of hematocrit measurement, assuming that the total amount of blood constitutes 5.6% of the body weight of the rhesus monkey.

†Experimental data.

As pointed out in the introduction, LA-PF$_4$ is originally secreted by platelets, but it can be converted to βTG upon the action of plasmin or platelet neutral protease that splits off a tetrapeptide from the N-terminus of LA-PF$_4$. It is not known whether this conversion takes place in plasma, but this possibility cannot be excluded. Since LA-PF$_4$ and βTG are immunologically identical, we decided to refer in this study to half-life of LA-PF$_4$ antigen rather than to half-life of LA-PF$_4$.

The data obtained refer exclusively to the turnover of human platelet secreted proteins injected into the rhesus monkey. In view of the similarity of human and monkey platelet proteins, it can be expected that the disappearance of monkey platelet proteins in plasma would follow a similar pattern. The turnovers of rhesus monkey plasma proteins, such as kininogens and fibrinogen, were similar to those of corresponding human plasma proteins when measured in monkeys.

In agreement with Rasi, we found that the measurement of LA-PF$_4$/βTG antigens in platelet-poor plasma are greatly influenced by the presence of residual platelets. Centrifugation at 3000 g was more efficient in removal of the platelets than centrifugation at 2000 g. It reduced the platelet count to about 0.7 x 10$^8$/ml. However, the identity of residual platelets remains unclear. We succeeded in reducing the platelet count in PPP to the same level in all experimental groups. We did not succeed in removing these platelets (or platelet-like particles) completely from human plasma by centrifugation at higher speeds (10,000–100,000 g) and by filtration through 0.2-µ Millipores (unpublished data).

It is possible that both residual platelets and extracellular platelet proteins contribute to the baseline levels of PF$_4$ and LA-PF$_4$ antigen in plasma. It is known that PF$_4$ and LA-PF$_4$/βTG antigens are released in parallel during platelet stimulation in vitro. It is conceivable that following platelet stimulation in vivo, both antigens are also released in parallel. However, PF$_4$ would disappear much faster than LA-PF$_4$ antigen (Figs. 3 and 4, Table 3). Therefore, an increased LA-PF$_4$/PF$_4$ ratio in PPP may indicate an in vivo release of platelet proteins. In our experiments, LA-PF$_4$/PF$_4$ ratio for PRP was 1.89 and for PPP, 2.89 (Table 1). This indicates a possibility of a secretion of platelet proteins occurring in vivo. Recently, Nossel et al. demonstrated a decrease of fibrinogen level, increase of fibrinopeptide A, and decrease of platelet counts in patients after intrauterine infusion of hypertonic saline. In these patients, βTG antigen levels generally paralleled fibrinopeptide A levels, whereas PF$_4$ levels showed only slight change. Nossel’s observations may be explained if one assumes that both platelet proteins are released in parallel in vivo and that PF$_4$ disappears from the circulation faster as compared to LA-PF$_4$/βTG antigen (Figs. 3 and 4). It can be suggested that determination of LA-PF$_4$ antigen:PF$_4$ ratio in patient PPP can be helpful for evaluation of the results of clinical trials on radioimmunoassays of secreted platelet proteins. Low LA-PF$_4$ antigen:PF$_4$ ratio would indicate release related to inadequate blood sampling or presence of residual platelets; high LA-PF$_4$ antigen:PF$_4$ ratio would indicate in vivo release of secreted platelet proteins.

Infusion of PGI$_2$ reduced partially the levels of LA-PF$_4$ antigen and PF$_4$ in monkey plasma. The following data indicate that this was related to inhibition of the release of these proteins from platelets.
stimulated by catheter or by venipuncture rather than to the inhibitory effect of PGI$_2$ on the latent secretory activity of circulating platelets in vivo. (A) In all plasma samples after PGI$_2$ infusion, LA-PF$_4$ antigen and PF$_4$ antigens were present although reduced; LA-PF$_4$ antigen:PF$_4$ ratio in these samples increased from 2.81 to 3.86 (Table 2). If in vivo release of secreted platelet proteins was blocked, this ratio would decrease. (B) Infusion of PGI$_2$ reduced LA-PF$_4$ antigen levels from 22 to 14 ng/ml. Accumulation of 8 ng LA-PF$_4$ antigen/ml in plasma in the absence of PGI$_2$ during 15 min would indicate the rate of release of LA-PF$_4$ antigen of 46.5 ng/ml/15 min (Table 3). If platelets were releasing LA-PF$_4$ antigen continuously at this rate, they would lose all antigen stored in the granules within 2 days. This is unlikely because the average lifespan of platelets in *M. mulatta* is about 6.5 days. (C) The decreased variability of LA-PF$_4$ antigen and PF$_4$ values measured in animals infused with PGI$_2$ (Table 2) also suggests that this agent blocked release caused by varying levels of trauma related to venipuncture or catheter. Recently, Kaplan et al. described that ingestion of aspirin by volunteers reduced the level of PF$_4$ and $\beta$TG antigens in their PPs. Perhaps this effect of aspirin was also related to the inhibition of the release from platelets stimulated by venipuncture rather than to the blocking of the in vivo release.

Our study suggests that infusion of PGI$_2$ into the rhesus monkey inhibits release of antiheparin proteins from platelets stimulated by catheter or venipuncture but it does not eliminate completely in vivo release of these proteins. The mechanism whereby low levels of platelet antiheparin proteins appear in monkey plasma remains to be investigated.

**ACKNOWLEDGMENT**

The authors wish to thank Dr. Y. Ostrowski for his help in mathematical evaluation of the data, Dr. D. Paul for his help and support, John Bowen, Peter Donisi, Linda Guiod, Pranee James and Richard Pirschak for their expert technical assistance. Assistance of Joanne Convery in typing and editing of this manuscript is gratefully acknowledged.

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NOTE ADDED IN PROOF
Recently half life data for secreted platelet proteins in monkey circulation have been recalculated in our laboratory using Gauss-Newton nonlinear regression computer program. The half-lives for the fast and slow components of La-PF4 were 7.2 min ± 1.3 min (SD) and 51 min ± 7.4 min (SD). The half-lives for the fast and slow components of PF4 were 2.6 min ± 0.4 min (SD) and 92 min ± 25 min (SD).
In vivo release and turnover of secreted platelet antiheparin proteins in rhesus monkey (Macaca mulatta)

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