Effect of Platelet-Activating Factor (PAF) on Human Platelets

By Carolyn McI. Chesney, D. David Pifer, L. W. Byers, and E. Eric Muirhead

The effect of pure synthetic PAF (1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) was studied in human platelets. PAF (0.2–2.0 μg/ml) produced a dose-dependent aggregation in human platelet-rich plasma (PRP) or platelet suspension obtained by gel-filtration (GFP). In addition, PAF (0.8 μg/ml) induced secretion of 14C-serotonin (45% ± 10%; mean ± SD, n = 9) and platelet factor 4 (PF4) (12.89 ± 3.81 μg/10^9 platelets; n = 9) in PRP. Similar results were obtained in GFP. Aggregation and release of 14C-serotonin and PF4 were inhibited by the metabolic inhibitors 2-deoxyglucose (16.7 mM) and antimycin-A (8.3 μg/ml), by the membrane-active drugs mepacrine (10 μM) and chlorpromazine (0.025 mM), by PGI2 (5.34 nM), which elevates intracellular c-AMP, by indomethacin (10 μM) or aspirin (100 μM). The ADP scavengers, creatine phosphate and creatine phosphokinase (CP/CPK), inhibited the second wave of aggregation but not secretion. These data suggest that the major effect of PAF on human platelets is mediated through the cyclo-oxygenase pathway and not through a third pathway.

PLATELET-ACTIVATING FACTOR (PAF), originally described in the supernatant of a suspension of rabbit basophils after an immunologic challenge,1 has been shown to aggregate platelets from various species, including rabbit,2–5 guinea pig,3 rat,4 and man.5 Recently, the structure of PAF has been determined to be 1-0-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine.3–4 An identical structure was shown for the antihypertensive polar lipid extracted from the renal medulla,9 as originally described by Muirhead.10 Much of the original work on platelets was done in the rabbit. Studies from several groups have shown that PAF stimulates aggregation and secretion of prelabeled serotonin, which is not blocked by indomethacin or aspirin.2,8 Chignard et al.12 have suggested that PAF works through a different pathway from adenosine diphosphate (ADP), epinephrine, and collagen and in fact may be a third pathway in platelets. This group has also reported that platelets synthesize PAF de novo in response to stimulation by the calcium ionophore A23187.

To date, few studies have been done on human platelets. Conflicting results over the effect of indomethacin inhibition have been reported.13,14 We have studied the effect of pure PAF on human platelet aggregation and secretion.

MATERIALS AND METHODS

Bovine serum albumin (fraction V), ADP, epinephrine, indomethacin, chlorpromazine, mepacrine, creatine phosphate, and creatine phosphokinase were obtained from Sigma Chemical Company, St. Louis, Mo. Human fibrinogen (grade L, 95% clottable) was a product of Kabi, Stockholm, Sweden. Aspirin powder was obtained from Merck Chemical Division, Rahway, N.J. Fibrillar equine tendon collagen (collagen reagent Horm) was obtained from Horm-Chemie, Munich. Prostaglandin I2 (PGI2) was a gift of Dr. John Pike of the Upjohn Company, Kalamazoo, Mich.

PAF

For the studies reported here, pure synthetic 1-0-octadecyl-2-acetyl-sn-glycero-3-phosphorylcholine was obtained from Bachem Feinchemikalien, Bubendorf, Switzerland. Similar results were obtained with the 1-0-hexadecyl or 1-0-octadecyl derivative purified in our laboratory as previously described11 or obtained from Dr. J. Benveniste. Immediately prior to a given set of experiments, PAF in chloroform-methanol (2:1) was dried under a stream of nitrogen and weighed. Saline was added to give the desired concentration and the suspension was then sonicated in a Sonicator Cell Disruptor (Model W 185F Heat Systems—Ultrasonics, Inc., Plainview, N.Y.), at 50 W for 60 sec in four 15-sec bursts with cooling of the tube in ice between bursts.

Platelet Preparations

Platelet-rich plasma (PRP) and gel-filtered platelets (GFP) were prepared as previously described.18 Studies on GFP were done in the presence of calcium-free Tyrode's buffer containing albumin (0.35%) and fibrinogen (1.67 mg/ml).

Platelet aggregation was measured on a Payton Dual Channel Aggregometer, Payton Associates, Inc., Buffalo, N.Y., according to the method of Born.17

4C-serotonin release was performed as described by Jerushalmi and Zucker.14

Platelet factor 4 (PF4) was determined by radioimmunoassay using a commercial kit (Abbott Laboratories, North Chicago, Ill.).

Platelet factor V was assayed by a one-stage assay, as previously described.16

RESULTS

PAF-Induced Platelet Aggregation

PAF showed a dose-dependent response in PRP (Fig. 1). First and second waves of aggregation were
PLATELET-ACTIVATING FACTOR

Fig. 1. Effect of PAF on platelet aggregation in PRP. PAF is added at arrow. This is a representative study. Similar results were obtained in 8 experiments.

Fig. 2. Effect of PAF on platelet aggregation in GFP in calcium-free Tyrode's buffer containing albumin and fibrinogen. PAF was added at arrow. This is a representative study. Similar results were obtained in five experiments.

were observed with GFP. Data for inhibition by indomethacin and aspirin are shown in Table 2. There was minimal secretion of $^{14}$C-serotonin by PAF in the presence of mepacrine. Metabolic inhibitors and PGI$_2$ totally inhibited PAF-induced secretion of PF4 in PRP. Aspirin and indomethacin inhibited PF4 secretion by 80% and 95%, respectively, in PRP (Table 1) and by 100% in GFP (Table 2). Inhibition of factor V secretion by PAF was also demonstrated in GFP (Table 2). Though the ADP scavenger creatine phosphate/creatine phosphokinase (CP/CPK) inhibited the second wave of aggregation in both PRP and GFP, they did not cause significant inhibition of release of $^{14}$C-serotonin or PF4 (Tables 1 and 2) in concentrations that totally inhibit secretion of these components by ADP (10 $\mu$M).

DISCUSSION

PAF produced dose-dependent aggregation in PRP or GFP. The GFP were sensitive to smaller doses of PAF than the same platelets in plasma, suggesting the presence of an inhibitor and/or degradative enzyme in plasma, as has been described in rabbit plasma.\(^\text{19}\)

PAF-stimulated secretion from dense granules and

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Table 1. Effect of Inhibitors on PAF Induced Secretion in PRP

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Number</th>
<th>$^{14}$C-Serotonin (%)</th>
<th>PF4 (pg/10$^9$ Platelets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inhibitor</td>
<td>9</td>
<td>45 ± 10</td>
<td>12.89 ± 3.81</td>
</tr>
<tr>
<td>PGI$_2$, 5.34 nM</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aspirin, 100 $\mu$M</td>
<td>5</td>
<td>0</td>
<td>1.49 (0.49–2.41)</td>
</tr>
<tr>
<td>Indomethacin, 10 $\mu$M</td>
<td>5</td>
<td>0</td>
<td>0.38 (0.00–0.76)</td>
</tr>
<tr>
<td>Mepacrine, 10 $\mu$M</td>
<td>5</td>
<td>4 (0–6.2)</td>
<td>1.33 (0.00–2.58)</td>
</tr>
<tr>
<td>2-deoxyglucose (16.7 mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ antimycin-A (8.3 $\mu$g/ml)</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Creatine phosphate (4 mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ creatine phosphokinase (8 U/ml)</td>
<td>5</td>
<td>41 (34–51)</td>
<td>15.11 (8.73–18.62)</td>
</tr>
</tbody>
</table>

Platelets prelabeled with $^{14}$C-serotonin were preincubated with PGI$_2$ (30 sec), aspirin (15 min), indomethacin (5 min), mepacrine (5 min), 2-deoxyglucose + antimycin-A (30 min), and CP + CPK (2 min). PAF (0.8 $\mu$g/ml) was added and stirred in aggregometer cuvette. Aggregation was recorded for 5 min. PRP was then centrifuged for 3 min at 10,000 g. Aliquots of supernatant were assayed for PF4 and $^{14}$C-serotonin.

*Results for $n = 9$ are expressed as mean ± SD. Results for $n = 5$ are expressed as mean and range.
Platelets were preincubated with saline or inhibitor under the
α-granules. This effect was seen in both PRP and GFP. Though the data reported in this paper were obtained with the pure synthetic 1-0-octadecyl-2-acetyl-sn-glycero-3-phosphorylcholine, similar results were seen with pure 1-0-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine and with a mixture of these natural lipids extracted from the renal medulla. Most of the results reported in the literature have been obtained with a mixture of the compounds purified from rabbit buffy coat containing sensitized basophils.12711

Studies employing inhibitors give some insight into the mechanism of action of PAF on human platelets, which previously have not been extensively studied. In contrast, many data exist for rabbit platelets. PAF-induced aggregation and secretion require metabolic energy, thus confirming a physiologic process rather than nonspecific aggregation and/or lysis. PGI₂, which elevates intracellular c-AMP, also has a profound inhibitory effect in human platelets. Identical results have been reported for rabbit platelets.2 In addition, membrane-active drugs such as mepacrine and chlorpromazine inhibited PAF-induced aggregation and secretion, as has also been reported in rabbit platelets.2 In the human system, the ADP scavengers CP/CPK inhibit the second wave of aggregation induced by PAF but fail to significantly inhibit secretion of 14C-serotonin or PF4. This finding has been reported in human PRP14 as well as in rabbits.2,12 In these respects, the human platelet response to PAF parallels that of the rabbit.

However, the role of the cyclo-oxygenase pathway seems to be different in these species. A number of authors have published data showing no inhibition of PAF-induced platelet aggregation and release by indomethacin in the rabbit.23,12 We have confirmed these results in the rabbit using our pure synthetic compounds. On the other hand, we repeatedly see inhibition by indomethacin or aspirin in the human system. Concentrations of PAF as high as 2 μg/ml fail to overcome this inhibition, which can be demonstrated in both PRP and GFP. In contrast, McManus et al.14 reported that indomethacin inhibited the second wave

Table 2. Effect of Inhibitors on PAF-Induced Aggregation and Secretion in GFP

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>n</th>
<th>Percent</th>
<th>Character</th>
<th>14C-Serotonin (%)</th>
<th>PF4 (μg/10⁶ Platelets)</th>
<th>Factor V* (U/10⁶ Platelets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inhibitor</td>
<td>5</td>
<td>77</td>
<td>1st and 2nd wave</td>
<td>38</td>
<td>13.46</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(72–86)</td>
<td></td>
<td>(30–54)</td>
<td>(9.4–17.93)</td>
<td></td>
</tr>
<tr>
<td>Mepacrine, 10 μM</td>
<td>2</td>
<td>30</td>
<td>1st wave with</td>
<td>2.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25–34)</td>
<td>disaggregation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin, 10 μM</td>
<td>4</td>
<td>39</td>
<td>1st wave with</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20–40)</td>
<td>disaggregation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin, 100 μM</td>
<td>2</td>
<td>39</td>
<td>1st wave with</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(21–57)</td>
<td>disaggregation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine phosphate (4 mM) + creatine phosphokinase (8 U/ml)</td>
<td>1</td>
<td>62</td>
<td>1st wave with</td>
<td>26</td>
<td>8.5</td>
<td>—</td>
</tr>
</tbody>
</table>

Platelets prelabeled with 14C-serotonin were incubated with inhibitor as described in Table 1. PAF (0.8 μg/ml) was added and stirred at 900 rmp in an aggregometer cuvette. Aggregation was recorded. After 5 min, the sample was centrifuged for 3 min at 10,000 g. One-tenth milliliter was immediately assayed for factor V. 0.1 ml was frozen for PF4 assay, and 0.1 ml was transferred to a scintillation vial and counted. Results are expressed as the mean and range.

*Factor V activity was studied only once.
of aggregation in human platelets but did not inhibit secretion of $^{3}$H-serotonin or PF4. The explanation for these differences is not apparent. Our data, therefore, suggest that PAF induces the second wave of aggregation as well as secretion mainly via the cyclo-oxygenase pathway. Secondary release of ADP from dense granules appears to be important for the second wave of aggregation but not for secretion. If there is a third pathway for this lipid mediator, it does not appear to be predominant under the conditions of study, which are the standard ones used in platelet physiology.

REFERENCES


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