The Effect of Prophylactic Anti-Asthma Drugs on PAF-Induced Platelet Accumulation in the Thorax of the Guinea Pig

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Abstract—Intravenous infusion of platelet activating factor (PAF) causes an accumulation of platelets within the thorax of the guinea pig that is accompanied by increased sensitivity of the airways to spasmogens. A crystal scintillation detector has been used for measurement of intrathoracic accumulation of 111Indium-labeled platelets during responses to PAF. PAF-antagonists inhibit both development of airway hyperreactivity and the accumulation of 111Indium-labeled platelets. Prophylactic anti-asthma drugs, which are known to inhibit development of airway hyperreactivity, do not diminish platelet accumulation in response to an intravenous infusion of PAF. It is therefore concluded that intrathoracic platelet accumulation per se is not the determinant of increased airway reactivity.

Platelet activating factor (PAF) has been proposed as a mediator of asthma because of its capacity to mimic a range of asthma symptoms (1). In guinea pigs, the effects of intravenous infusion of PAF include development of increased sensitivity of the airways to spasmogens (2) and intrathoracic accumulation of both platelets and neutrophils (3). Prior treatment with selective anti-platelet anti-serum prevents the development of airway hyperreactivity in response to intravenous infusion of PAF (2). This observation raises the possibility that prophylactic anti-asthma drugs could influence the development of airway hyperreactivity by limiting intrathoracic platelet accumulation during responses to PAF.

Materials and Methods

Animals: Male Dunkin-Hartley guinea pigs, 400–600 g body weight, have been used throughout. Test animals were anesthetized with urethane (1.4 g/kg, i.p.), and an external jugular vein or dorsal foot vein was cannulated for administration of isotopically-labeled materials, drugs or agents that cause platelet activation.

Materials: Adenosine diphosphate (ADP) (Sigma), heparin (Roche) (5 U/ml), bombesin (Bachem) and urethane (Siegfried) were diluted in physiological saline (0.9% w/v); collagen (equine) (Hormon-Chemie) was diluted in isotonic glucose buffer (pH 2.7); platelet activating factor (PAF) (1-0-hexadecyl-2-0-acetyl-sn-glycero-3-phosphorylcholine) (NovoBiochem) was dissolved in ethanol (1 mg/kg) and stored at -18°C, which was diluted in a solution of bovine serum albumin (Sigma) (0.25% w/v in saline) as required; citrate buffer, for separation and labeling of platelets or erythrocytes, contained trisodium citrate (2.8%), sodium dihydrogen phosphate (0.015%) and glucose (0.2%) (all reagent grade) and was autoclaved at 15 lb/inch² for 30 min (4); 111Indium oxine (37 MBq/ml) was obtained from Amersham International plc (Amersham, England). Sodium cromoglycate (Sandoz), Gingkolide B (9H-1a-(epoxymethano)-1H,6aH-cyclopenta(c)fur-2,3,4-cyclopenta(1,2-d) furan-5,9,12-(4H)-trione,3-tert-butyloxacyclohexahydro-8-ethylphenyl-8-methoxypinyloxyethylquinolinium hydroxide, inner salt) (Sandoz), aminophylline (Siegfried) and prednisolone (Sigma) were dissolved in physiological saline.

Preparation of 111Indium-labeled platelets: Blood (18 ml) was collected in 2 ml of citrate
buffer by cardiac puncture from an ether-anesthetized guinea pig (4) and placed in polystyrene tubes. After centrifugation (200xg for 10 min), platelet-rich plasma (PRP) was removed and mixed with an equal volume of citrate buffer. Following a second centrifugation (1000xg for 10 min), the supernatant was discarded and platelets were resuspended in 1 ml of citrate buffer. Platelet numbers were determined using an automated platelet counter (Cell-dyn 100, Sequoia-Turner, U.S.A.). After addition of $^{111}$Indium oxine (1.5 MBq), the platelet suspension was incubated for 5 min at room temperature and then centrifuged (1000xg for 10 min). Unbound $^{111}$Indium oxine in the supernatant was discarded, and the surface of the platelet pellet was washed twice with citrate buffer to remove unbound $^{111}$Indium associated with the meniscus. Platelets were resuspended (5.0–9.0×10^8/ml) in 1 ml of citrate buffer, and 0.3 ml aliquots of the platelet suspension were injected intravenously via a jugular vein or a dorsal foot vein into recipient guinea pigs; 1 ml of heparinized saline was injected to maintain patency of the cannula. $^{111}$Indium-labeled platelets that were injected into recipient animals comprised between 2 and 8% of the circulating platelet population (5).

Monitoring of $^{111}$Indium-labeled platelets: Anesthetized, spontaneously breathing guinea pigs (groups of 3 to 4) were placed supine, and a collimated sodium iodide crystal scintillation detector (1 inch crystal, DM1-1, Nuclear Enterprises, England) was placed above the thorax and abdomen of each animal. NIMS series amplifier/analyzers (Nuclear Enterprise 4697) were used to amplify and select signals from each detector. Pulses from the NE 4697 modules were logged at up to 5 MHz by an eight channel co-processor (AIMSplus, Mumed Ltd., England) within a host computer (IBM AT3), and count rates (cpm) were displayed graphically in real time as an index of intrathoracic platelet content (5).

Immediately following injection of platelets, count rates over the thorax were stable (circa 10,000/sec); nevertheless, a period of 5–10 min was allowed to elapse before commencing an experiment. PAF (600 ng/kg/hr) was infused in a graded fashion over a one-hour period (30 ng/kg for 10 min, followed by 120 ng/kg for 20 min, followed by 450 ng/kg for 30 min) so that the bulk of the PAF was given in the final 30 min. Recording commenced 4 min before infusion of PAF, and count rates were recorded over 90 successive 60-sec periods.

Drug administration: When testing for inhibition of platelet accumulation, drugs or putative inhibitors were injected intravenously as a bolus 1 min prior to the infusion of PAF and concomitantly with the PAF infusion; i.e., ketotifen (1:1 mg/kg) indicates that ketotifen was injected as a bolus (1 mg/kg) 1 min prior to infusion of PAF and as an infusion (1 mg/kg) together with the PAF infusion.

Results

Anti-asthma drugs and platelet accumulation: Intravenous infusion of PAF (300 ng/kg/hr) did not cause noteworthy intrathoracic accumulation of $^{111}$Indium-labeled platelets (maximal increase above pretreatment count rates was 1.6±3%; n=5). Intravenous infusion of PAF (600 ng/kg/hr) caused an intrathoracic accumulation of $^{111}$Indium-labeled platelets (maximal increase above pretreatment count rates was 50±9%, n=6) that was associated with development of airway hyperreactivity (Fig. 1). Ketotifen (1:1 mg/kg), cromoglycate (10:10 mg/kg), aninophylline (10:10 mg/kg) and prednisolone (10:10 mg/kg) did not diminish intrathoracic platelet accumulation (Fig. 2), even though at these doses, development of airway hyperreactivity was effectively inhibited (preceding paper). The inability of these drugs to inhibit platelet accumulation due to PAF can be contrasted with the capacity of these drugs to inhibit airway hyperreactivity (Table 1).

PAF antagonists and platelet accumulation: Two competitive inhibitors of PAF receptor binding sites on isolated platelets (SRI 63-441 and Gingkolide B) (6) were used in this study. Both Gingkolide B (1:1 mg/kg) and SRI 63-441 (1.1 mg/kg) strongly inhibited intrathoracic platelet accumulation due to infusion of PAF as illustrated in Fig. 3. At this dosage, both compounds fully inhibited the
induction of airway hyperreactivity by such an infusion of PAF (Table 1).

Discussion

PAF may evoke pronounced biological effects as consequences of platelet activation or by mechanisms that are independent of platelet activation (6). For instance, the increased airway resistance and decreased dynamic compliance that follow intravenous injection of PAF are no longer detectable in animals depleted of platelets (7, 8). By way of contrast, increased vascular permeability in guinea pig skin is not influenced by depletion of platelets (9).

Development of airway hyperreactivity in guinea pigs is a platelet-dependent phenomenon, for there is no change in airway reactivity when animals that have been depleted of platelets are exposed to PAF (2). Ac-
Accumulation of \(^{111}\)Indium-labeled platelets within the thorax during an infusion of PAF (600 ng/kg) (at arrow) for one hour. Drugs were administered as an intravenous injection 1 min prior to PAF infusion and as an intravenous infusion throughout the exposure to PAF. Treatments include vehicle (open circles) or a) ketotifen (1 mg/kg and 1 mg/kg/hr), b) cromoglycate (10 mg/kg and 10 mg/kg/hr), c) aminophylline (10 mg/kg and 10 mg/kg/hr) and d) prednisolone (10 mg/kg and 10 mg/kg/hr) (open squares). Each group is comprised of 6 animals and bars depict S.E.M.

Table 1. The effect of prophylactic anti-asthma drugs and PAF antagonists upon the airway hyperreactivity and platelet accumulation that results from an infusion of PAF

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>% Inhibition airway hyperreactivity*</th>
<th>% Inhibition platelet accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketotifen</td>
<td>1.0</td>
<td>73 (n=10)</td>
<td>28 (n=7)</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>10.0</td>
<td>91 (n=10)</td>
<td>0 (n=4)</td>
</tr>
<tr>
<td>Cromoglycate</td>
<td>10.0</td>
<td>65 (n=10)</td>
<td>27 (n=4)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>10.0</td>
<td>40 (n=10)</td>
<td>0 (n=4)</td>
</tr>
<tr>
<td>SRI 63-441</td>
<td>1.0</td>
<td>65 (n=5)</td>
<td>90 (n=5)</td>
</tr>
<tr>
<td>Gingkoxide B</td>
<td>1.0</td>
<td>76 (n=5)</td>
<td>81 (n=5)</td>
</tr>
</tbody>
</table>


Accordingly, PAF receptor antagonists, which cause a pronounced inhibition of platelet accumulation within the thorax in response to PAF infusion, also inhibit development of airway hyperreactivity in such animals. However, it does not necessarily follow that accumulation of platelets within the thorax determines increased airway hyperreactivity, because other platelet activation stimuli (such as thrombin or ADP) induce comparable platelet accumulation without influencing airway reactivity (8, 10, 11). Nonetheless, it has been proposed that platelet activation is necessary in order that eosinophils might accumulate within the airway of laboratory animals during responses to either PAF or
allergen (12, 13). Furthermore, preliminary findings implied that prophylactic anti-asthma drugs could be effective as a consequence of their ability to inhibit intrathoracic platelet aggregation (14). More detailed study has not confirmed these preliminary findings, since the present study reveals that whereas established prophylactic anti-asthma drugs effectively inhibit development of airway hyperreactivity, they do not signifi-
cantly diminish platelet accumulation.

The proposal that PAF may be a central agent in asthma exacerbation implied that platelet changes should be a feature of asthma. An extensive study of non-symptomatic asthmatics has substantiated this prediction (15). It does not follow that platelet aggregation in the lung must be a feature of asthma exacerbation, so that failure to demonstrate such gross events in allergic asthma (16) is not at variance with the concept that PAF and platelet activation participate in asthma. The present studies in the guinea pig are consistent with the dissociation of platelet aggregation from development of airway hyperreactivity, since prophylactic anti-asthma drugs inhibited the development of airway hyperreactivity following exposure to PAF without influencing platelet aggregation.

Recent evidence implies that the action of ketotifen and prednisolone to achieve inhibition of airway hyperreactivity may be attributed to an inhibition of the effects upon the airways of a platelet derived hyperreactivity factor (17) that is generated when platelets interact with PAF (18). These observations are consistent with the interpretation that platelet aggregation within the airways is not a determinant of airway hyperreactivity.

References