The Effect of Inhalation of Platelet-activating Factor on the Pulmonary Clearance of ^{99m}Tc-DTPA Aerosol

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Platelet-activating factor (PAF) is a short-acting, lipid-soluble autocoid, inhalation of which causes an immediate pulmonary vascular sequestration of granulocytes and a peripheral neutropenia. We investigated the effect of PAF inhalation on the pulmonary clearance rate of inhaled ^{99m}Tc-DTPA in order to test the hypothesis that the pulmonary sequestration of granulocytes results in acute lung injury. In nine normal nonsmoking adults, the rate of clearance of DTPA, corrected for background activity, was 1.5 (SD 0.7) %/min over the first 10 min after inhalation. Inhalation of 4.8 μg PAF abruptly increased the clearance rate to a mean value of 2.3 (1.4) %/min ($p < 0.05$). No increase in clearance was observed in four nonsmoking subjects who inhaled vehicle only. The mean overall increase after PAF was 87% of the baseline clearance, significantly different ($p < 0.05$) from the corresponding change in the control group, which was −17%. After PAF, the clearance rate returned to baseline values within 10 min in all subjects. In all subjects who inhaled PAF, but in none who inhaled vehicle, there was an immediate neutropenia of 51 (SD 25) % of the baseline value ($p < 0.01$). This neutropenia persisted longer than the corresponding accelerated DTPA clearance and was still 74 (36) % of the baseline value at 10 min. Furthermore, there was no correlation between the increase in DTPA clearance induced by PAF inhalation and the decrease in peripheral blood granulocyte count. We conclude that PAF inhalation results in an increase in pulmonary DTPA clearance, probably not mediated by pulmonary vascular granulocyte sequestration. **Mason GR, Peters AM, Myers MJ, Ind PW, Hughes JMB. The effect of inhalation of platelet-activating factor on the pulmonary clearance of ^{99m}Tc-DTPA aerosol. Am J Respir Crit Care Med 1995;151:1621-4.**

Platelet-activating factor (PAF) is an endogenously produced, short-acting, lipid-soluble autocoid that is not appreciably stored, is produced locally by multiple different cells and tissues, and is rapidly metabolized (1). Purification and synthesis of PAF has provided evidence that this autocoid activates inflammatory cells (2), is chemotactic in animals (3), and increases microvascular permeability (3). Sufficient doses of PAF given intravenously will cause permeability edema in the lungs (4).

In humans, inhalation of PAF results in an immediate neutropenia, followed several hours later by a rebound neutrophilia (5). Tam and coworkers, imaging ^{111}In-labeled granulocytes, showed that this neutropenia is the result of rapid neutrophil sequestration in the pulmonary vascular bed (6).

There is considerable experimental evidence implicating neutrophils in acute lung injury (7–12). We questioned whether inhalation of PAF, with subsequent pulmonary vascular neutrophil sequestration, was associated with any evidence of lung injury, and employed the rate of clearance from the distal airway of an inhaled aerosol of ^{99m}Tc-DTPA as a sensitive technique for the assessment of lung injury (13–17). The transfer of this hydrophilic solute from the airway to pulmonary capillary blood is rate-limited by airway epithelial permeability, which is much less than the permeability of the pulmonary capillary endothelium (18). The aim of the study, therefore, was to identify lung injury from the rate of DTPA clearance induced by PAF inhalation and to compare its time course with that of the peripheral neutropenia known to result from PAF inhalation.

METHODS

Subjects

Thirteen normal nonsmoking adult volunteers (seven women, six men) 22 to 62 yr of age were recruited. All gave informed written consent to the study, which was approved by the Royal Postgraduate Medical School Local Research Ethics Committee and by the Administration of Radioactive Substances Advisory Committee of the United Kingdom. None were taking medication prior to the study. Nine received PAF, and four received vehicle alone. The PAF subjects had received PAF on a previous occasion more than 2 wk earlier with no decrement in FEV_1, vital capacity, or airway resistance.

PAF Inhalation

PAF (C16-1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) (Bachem Inc., Torrance, CA) was stored in aliquots at a concentration of 10 mg/ml in 100% ethanol at −20° C. On the morning before the study, PAF was diluted to 2 mg/ml in 0.9% saline, containing heat-treated human serum

(Received in original form October 12, 1993 and in revised form October 24, 1994)

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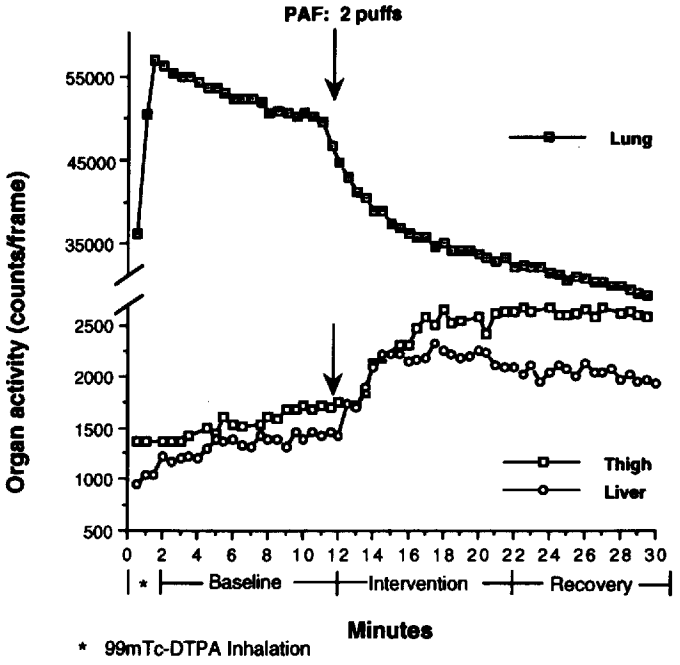

Figure 1. An example of the kinetics of ^{99m}Tc-DTPA lung disappearance and appearance in liver and thigh after PAF inhalation. After the 2-min administration of ^{99m}Tc-DTPA aerosol, the baseline clearance rate for 10 min was 1.69%/min, during which there was slow accumulation in the liver and thigh tissues. After PAF inhalation, there was an abrupt and transient increase in the lung clearance rate (3.39%/min) and a rapid increase in the liver activity followed by an increase in the thigh activity. The liver activity began to decline while the thigh activity continued to rise (see text for discussion). The duration of the effect of PAF was < 10 min. Clearance rate of DTPA for the final 10 min was 1.70%/min.

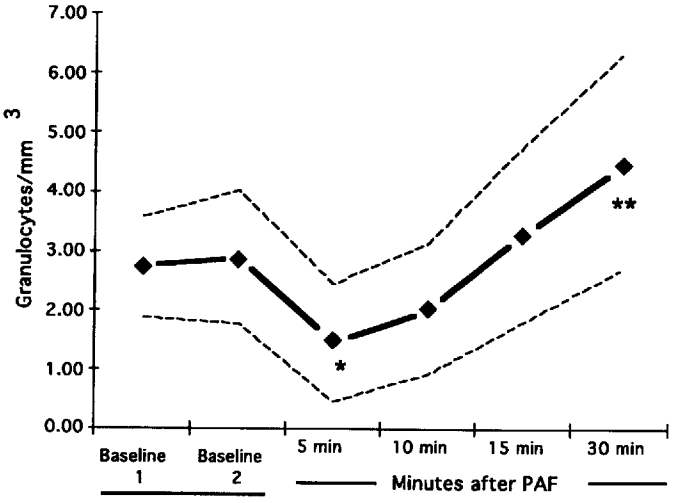

Figure 2. The amount of circulating neutrophils (± SD) after PAF inhalation. Each subject developed neutropenia and a rebound neutrophilia. From prior studies, the neutropenia results from pulmonary sequestration. The duration of neutropenia was longer than the effect of PAF on DTPA clearance. *$p < 0.01$. **$p < 0.05$.

albumin at a final concentration of 0.04%. Each subject received 48 μg of PAF (two puffs of 24 μg; particle size, 90% < 5 μm) delivered by nebulizer connected to a dosimeter (Mefar, Brescia, Italy), which was driven by compressed air at a pressure of 1.5 kg/m².

^{99m}Tc-DTPA Aerosol Clearance

An aerosol of ^{99m}Tc-DTPA (pentetate; Amersham International, Amersham, UK) was generated as previously described, particle size 1.8 μm (14), using an acorn nebulizer (OEM, Richmond, VA). Wearing a noseclip, the supine subject inhaled through a mouth piece at tidal volume for 2 min. Radioactivity was monitored during inhalation and for 30 min after with a scintillation camera positioned over the anterior chest and abdomen. Regions of interest were drawn over the right lung and over the liver.

A total of 20 MBq of ^{99m}Tc-DTPA was given intravenously for background correction, before aerosol inhalation.

Study Design

The subject was supine beneath a gamma camera (IGE 400 A or T) online to a computer (MDS A²). Ten minutes after inhaling DTPA, the subject was asked to inhale an aerosol of PAF. Data were continuously recorded dynamically at a frame rate of 2 frames/min for 10 min after inhalation of DTPA and for a further 20 min after inhalation of PAF. The peripheral circulating neutrophil count was measured from venous blood samples 5 and 10 min before PAF inhalation (i.e., duplicate baseline values) and 5, 10, 15, and 30 min after PAF inhalation. Four normal volunteers followed the same protocol but inhaled nebulized vehicle instead of PAF.

Statistics

Comparisons of aerosol clearance rates were made using the nonparametric Friedman's test of repeated measures, and the neutrophil kinetics with ANOVA for repeated measures. The Student-Newman-Keuls post hoc test was used to determine critical differences (19). The PAF group was compared with the vehicle-only group with an unpaired *t* test.

RESULTS

All subjects but one experienced a minor cough that was less than 15 s in duration; most noted a sensation of warmth, often with flushing after PAF. No other adverse effects were noted, and there was no tachypnea or observable increase in respiratory effort. The control subjects reported no symptoms.

In the four control subjects, the mean rates of ^{99m}Tc-DTPA clearance, corrected for background, over the three 10-min periods were 1.6 (SD 0.7), 1.2 (0.4), and 1.3 (0.3) %/min. Thus, inhalation of vehicle did not increase the clearance rate. Indeed, during the second period of 10 min, the clearance rate slowed to about 80% of that over the initial 10 min and remained at this level during the third period of 10 min.

The mean clearance of DTPA over the first period of 10 min in the subjects inhaling PAF was 1.5 (SD 0.7) %/min, not significantly different from that of the control subjects. Inhalation of PAF 10 min after inhalation of DTPA caused an acceleration in the rate of DTPA clearance in six of the nine subjects, to a mean value for the group of 2.3 (SD 1.4) %/min over the period of 10 min after PAF (Figure 3). This is an increase, compared with the baseline, of 87% ($p < 0.05$) and also is significantly different ($p < 0.05$) from the corresponding change in the control group, which was −17%.

The increase in clearance after PAF was immediate and apparent in the first frame of data acquired after the two puffs of PAF (Figure 1). However, it appeared to be short-lived and was highly variable between the subjects. The mean clearance rate of DTPA during the third 10-min period was 1.1 (SD 0.6) %/min. The largest change in an individual subject was from 0.96 to 5.2%/min. Three subjects had no response to PAF, and two of these had the highest and third highest baseline DTPA clearance of all 13 subjects, 2.5 and 2.0%/min, respectively.

The peripheral neutrophil count decreased immediately after PAF inhalation from duplicate baseline values of 2.6 (SD 0.8) and 2.8 (1.0) to 1.4 (0.9) ($p < 0.05$ versus average baseline) at 5 min and 1.9 (1.1) × 10^9/L at 10 min after PAF. There was then a re-

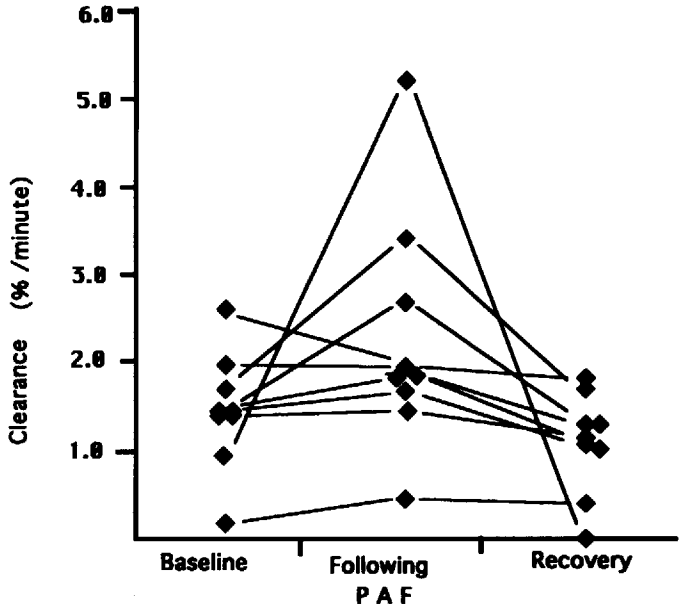

Figure 3. The response of DTPA clearance to PAF inhalation. Each period represents the DTPA clearance rate calculated over a 10-min interval. The PAF clearance (2.3 %/min) was greater than baseline (1.5%/min) and recovery (1.1%/min). The overall response was a significant increase in clearance ($p < 0.05$). The response to PAF was variable, despite neutropenia, that occurred in each subject. Slowing of clearance was found after inhalation of vehicle alone (see text).

bound neutrophilia to 3.1 (1.5) at 15 min and 4.1 (2.0) ($p < 0.05$ versus average baseline) $\times$ 10^9/L at 30 min after PAF (Figure 2). The change in DTPA clearance induced by PAF did not correlate either with the average baseline neutrophil count or the change in count between the baseline value and the value at 5 min after PAF.

PAF inhalation had differing effects on the count rates recorded over the liver and thigh (Figure 1). During the baseline 10-min period after DTPA inhalation, liver and thigh count rates slowly increased, reflecting the slow transfer rate of DTPA from distal airway to pulmonary blood. After PAF inhalation, the liver and thigh count rates both increased abruptly. However, while the thigh count rate continued to slowly increase, the liver count rate soon started to decrease, reflecting the differing kinetics of equilibration of DTPA between the intravascular and extravascular spaces of these two tissues.

DISCUSSION

Neutrophils have been implicated in the etiology of acute lung injury. Experimental acute lung injury induced by several insults, including microembolization (7), endotoxemia (4), and hyperoxic lung injury (9), is dependent on neutrophils. It was therefore tempting to speculate that the apparent lung injury induced by PAF inhalation, as reflected by an increase in the pulmonary clearance of ^{99m}Tc-DTPA, was mediated through neutrophils sequestered in the pulmonary vascular bed. PAF inhalation caused an immediate increase in the clearance of DTPA. Although the neutrophil count decreased abruptly, it is difficult to link the affect of PAF to pulmonary neutrophil sequestration because the effect of PAF was so short-lived. DTPA clearance had returned to baseline at a time when the peripheral blood neutrophil count was still depressed and presumably when neutrophils were still sequestered in the lung. Tam and coworkers (6) have shown that after PAF inhalation, the concentration of ^{111}In-labeled neutrophils in the lung increased, did not begin to decrease until at least 5 min after PAF inhalation, and was still elevated 20 min after PAF.

The mechanism whereby PAF increases epithelial permeability remains unclear from this study. We do not know whether inhaled PAF acts directly on the pulmonary endothelium to induce neutrophil sequestration or indirectly after uptake into pulmonary vascular blood and activation of neutrophils in transit through pulmonary capillaries. Because of its lipophilicity, PAF is likely to be rapidly transferred from distal airways to pulmonary blood (20). Furthermore, if PAF acted directly on pulmonary vascular endothelium, its effect is unlikely to be mediated through leukocyte adhesion molecules, since there is a significant latent period required for the *de novo* synthesis of these molecules after challenge with proinflammatory cytokines (21). It is unlikely that there is an indirect effect of PAF on clearance mediated through a change in lung mechanics or from increased respiratory effort, as these subjects had minimal symptoms and no change in lung function. Large changes in lung volume are required to effect DTPA clearance and large increases in inspiratory effort may not affect clearance at all (22).

The reason for the variable response to PAF is unclear. It does not relate to sex, smoking status, age, or previous lung damage. It was unrelated to baseline clearance, though the two subjects with the fastest baseline clearance did not have an increase after PAF. The dose of PAF actually inhaled is not known but was adequate to cause neutropenia in all subjects. There was no correlation between effect on clearance and the baseline or decrement in circulating neutrophil count. It is unlikely that the effect of PAF represents a measurement artifact. The effect, when it occurred, was immediate and definite. Baseline clearances were in the normal range previously reported by us and others (13–18) and did not differ between the two groups of subjects.

The differing responses to PAF inhalation between thigh and liver count rates illustrate the differing kinetics of equilibration of ^{99m}Tc-DTPA between the intravascular and extravascular spaces of these two tissues. After a brief sharp increase resulting from PAF inhalation, the thigh count rate continued to increase even though the blood level of DTPA was decreasing, a finding that has been consistent with direct intravenous injection of ^{99m}Tc-DTPA (unpublished observations). This is because of the thigh's large extravascular space, the volume of which determines the rate at which soluble hydrophilic tracers, like DTPA, equilibrate between plasma and interstitial fluid (23). In contrast, after an increase following PAF inhalation, liver counts decreased, responding rapidly to the decreasing blood level as a result of rapid equilibration of tracer between plasma and the relatively small interstitial space of the liver.

The four control subjects, given vehicle only, illustrate that the DTPA clearance curve is not mono-exponential, even after appropriate background correction. The use of the liver for background correction illustrates that the normal clearance curve is a multi-exponential curve, a fact obscured by the use of the thigh for background correction because of an increasing thigh signal and a consequent progressive overestimation of background. Although background correction, appropriate or not, is probably irrelevant over the first 10 min of routine clearance study, it is important when the effect of an intervention on a curve already in progress is studied. The reason for the multi-exponential form of the aerosol clearance curve, normally only seen in severe lung injury when it is not obscured by oversubtraction of background, is uncertain, but may be the result of back-diffusion of DTPA into the alveolar fluid.

In conclusion, the most significant new finding from this study is the change in permeability to DTPA after PAF inhalation. Although the mechanism is not clear, a short-lived pathophysiologic affect of PAF is likely to be involved and should provide fruitful ground for the study of PAF antagonists. Because the pulmonary sequestration of neutrophils is not decreasing when the permeability changes are resolving, it is unlikely that the increased permeability is caused by neutrophils. Further studies, correlating the pulmonary sequestration of radiolabeled granulocytes with DTPA clearance after PAF inhalation, may throw further light on the role of the neutrophil.

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