Inhibition of PAF-Induced Eosinophil Accumulation in Pulmonary Airways of Guinea Pigs by Anti-Asthma Drugs

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Abstract—Intraperitoneal (i.p.) injection of platelet activating factor (PAF) in guinea pigs caused a dose-related increase in the number of eosinophils recovered from bronchoalveolar lavage fluid (BALF). The prevalence of eosinophils in BALF had significantly increased within 1 hr of i.p. injection of PAF (10 μg/animal) and was maximal after 24 hr. Subcutaneous osmotic mini-pumps were used to administer drugs for 5 days prior to i.p. injection of PAF (10 μg/animal) and for the subsequent 24 hr. The percentage increase of eosinophils in BALF, due to PAF, was inhibited in animals treated with dexamethasone, aminophylline, cromoglycate, tranilast or ketotifen, but not in animals treated with oxatomide, azelastine, amlexanox, ibudilast or AA-861. These results suggest that inhibition of pulmonary eosinophilia may be a necessary property of prophylactic anti-asthma drugs and provide indirect evidence favoring a role for PAF in eosinophilia of asthma.

It has been proposed that the actions of platelet activating factor (PAF) could account for the development and exacerbation of asthma that can result from allergic or non-allergic processes (1). In this context, it has been established that systemic administration of PAF will induce airway hyperreactivity (2) and that prophylactic anti-asthma drugs can prevent development of such airway hyperreactivity (3). More recently, it has been established that inhalation (4) or intratracheal instillation (5) of PAF can also induce a selective accumulation of eosinophils that can be prevented by ketotifen (5, 6) and other prophylactic anti-asthma drugs (6). These observations raise the possibility that inhibition of eosinophil accumulation may be a property common to anti-asthma drugs.

The present study demonstrates the capacity of intraperitoneal injection of PAF to effect eosinophil accumulation in the airways and has defined the capacity of prophylactic anti-asthma drugs to inhibit this process.

Materials and Methods

Materials: Dunkin-Hartley guinea pigs (400–600 g) were used throughout. Platelet activating factor (PAF) (1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphorylcholine) (NovoBiochem) was dissolved in ethanol (1 mg/ml) and stored at -18°C as a stock solution. PAF was diluted in saline (0.9% w/v) containing bovine serum albumin (BSA, Fluka, 0.25% w/v). Sodium pentobarbitone (Siegfried Zofinger AG), Türk’s solution (Merck) and Leishman’s stain (Merck) were commercial preparations. Sodium cromoglycate (Sandoz), ketotifen (Sandoz), amlexanox (Takeda), azelastine (Sandoz), tranilast (Kissei), oxatomide (Janssen), ibudilast (Kyorin), dexamethasone (Sigma) and amnophylline (Siegfried) were dissolved in saline; and AA-861 (2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadinyl) -1,4-benzoquinone) (Takeda) was dissolved in 10% tris-buffered saline.

Bronchoalveolar lavage and eosinophil determination: Bronchoalveolar lavage (BAL) was performed in guinea pigs after i.p. injection of an overdose of pentobarbitone (100 mg/kg). When respiration was no longer evident, the trachea was exposed and cannulated. Aliquots (6 x 10 ml) of buffered, modified Tyrode’s solution (Composition: 11.9 mM NaHCO₃, 136.9 mM NaCl, 2.7 mM KCl, 0.4 mM Na₂HPO₄, 5.6 mM glucose and
19.8 mM EDTA; Protein % w/v: Gelatin, 0.1 and BSA, 0.5; pH adjusted to 7.4 by adding 2M NaOH) were introduced successively and aspirated by gently compression of the lung tissue. Total fluid recovery exceeded 80%.

Cell suspensions were concentrated by low speed centrifugation (200×g for 10 min), and the cell pellet was resuspended in 1 ml modified Tyrode. Ten microliters of cell suspension were diluted in 90 μl of Türk’s fluid for total cell counts. Differential cell counts were made from smears fixed in methanol (100%) and stained with Leishman’s stain in order to differentiate cell types. A total of ca. 500 cells per smear were counted at 1000-fold magnification.

Time and dose-related increases in bronchial eosinophils: To determine the optimal time for bronchial eosinophil accumulation, guinea pigs were injected with PAF (10 μg/animal in 5 ml of BSA-saline, i.p.); and 1, 4, 8, 16, 24 or 48 hr later, bronchial eosinophil counts were determined.

To determine whether responses to PAF were dose-related, increasing concentrations of PAF (0.1–10 μg/animal, i.p.) were injected, and bronchial eosinophil counts were determined at 24 hr. Injections of BSA-saline alone acted as the control for all experiments.

Inhibition of eosinophil accumulation in pulmonary airways: Drugs were administered to guinea pigs as a subcutaneous infusion over a 6 day period, using osmotic mini-pumps (Alzet 2001, 2 ml). Pumps were implanted subcutaneously in the nuchal region under ether anesthesia, 5 days prior to intraperitoneal injection of PAF. Twenty-four hours after injection of PAF, BAL was performed and eosinophil counts were determined.

Results

The time course of eosinophil accumulation in BALF was examined following i.p. injection of 10 μg/animal PAF. There was an increase in the percentage of eosinophils 1 hr after i.p. injection of PAF or BSA-saline (Fig. 1). However, the percentage of eosinophils in BALF of the PAF treated animals gradually increased and was maximal at 24 hr (Fig. 1), whereas no such increase was observed in the BSA-saline treated animals (Fig. 1).

Intraperitoneal injection of PAF (0.1–10 μg/animal) induced a dose-related increase in the total number and percentage of eosinophils in BALF at 24 hr (Fig. 2), although no increase in total leukocyte number was observed (Table 1).

The effect of drug treatment for 5 days prior to exposure to PAF is shown in Table 2. There was a significant decrease in the percentage of eosinophils in BALF from animals treated with dexamethasone, cromoglycate, tranilast or ketotifen. Treatment with equivalent doses of oxatomide, azelastine, amlexanox, ibudilast or AA-861 failed to diminish eosinophil accumulation. Aminophylline inhibited eosinophil accumulation when administered at 10 mg/kg/day.

Discussion

Infiltration of the bronchial wall and airways by eosinophils is a characteristic of...
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asthma, being evident not only when asthma patients die in status asthmaticus (7), but also death is due to causes unrelated to asthma (8). Bronchial eosinophilia has also been observed in biopsy studies on specimens from asthma patients (9). The association between increased eosinophil counts and severity of asthma is strengthened by the observations that bronchial eosinophil counts increase when there is a late-onset asthmatic reaction following aerosol challenge with the provocative antigen (10) and the presence of a positive correlation between blood eosinophil numbers and increased airway reactivity (11). Major basic protein (MBP) and eosinophil cationic protein (ECP) are present within eosinophil granules. The concentrations of such toxic proteins are elevated in bronchial tissue at sites of epithelial damage (8) and in the sputum (12) and blood (13) of asthma patients. MBP is known to damage bronchial epithelium under laboratory conditions (14) within the concentration range that can be found in asthmatic sputum (15). It has been proposed that damaged epithelium could lead to increased airway reactivity either by exposing underlying nerve endings (16) or by removing the protective effect of an epithelial-derived relaxant factor (17). Recent studies have confirmed that epithelial damaged with MBP can lead to increased contractility of airway smooth muscle in vitro (18).

Despite the importance of eosinophils in the pathology of asthma, the actions of anti-asthma drugs on eosinophil function have not been studied extensively. Studies in the rat (19), guinea pig (6) and mouse (20) indicate that drugs such as ketotifen or cromoglycate may achieve inhibition irrespective of the stimulus used to induce eosinophil accumulation. There are few clinical reports on the effect of anti-asthma drugs on eosinophils in asthma. Treatment with steroids is an effective means of suppressing eosinophilia in peripheral blood (21, 22), and both ketotifen and theophylline have been reported to diminish eosinophil counts in peripheral blood (23, 24). Cromoglycate has been reported to diminish the number of eosinophils recovered from airway lavage fluid in asthma (25). Recently, the activation state of eosinophils in allergic diseases has attracted interest, and it

Table 1. Comparison of bronchial cell populations after i.p. injection of increasing doses of PAF

| Treatment (µg/animal) | n   | Total cell (×10⁶) | Macrophage | Eosinophil (% cell population) | Neutrophil | Lymphocytes |
|-----------------------|-----|------------------|------------|-------------------------------|------------|-------------|
| BSA (0.25%)           | 7   | 15.7±2.5         | 65.1±4.9   | 8.6±2.0                      | 4.6±4.5    | 9.3±3.1     |
| PAF 0.1               | 10  | 18.4±1.2         | 73.4±2.2   | 11.3±1.6                     | 2.5±0.5    | 7.1±1.7     |
| PAF 1.0               | 5   | 19.0±2.9         | 65.4±4.5   | 17.0±1.4                     | 0.6±0.1    | 16.2±4.3    |
| PAF 10.0              | 10  | 23.2±3.9         | 61.1±4.7   | 31.4±4.0                     | 1.3±0.5    | 4.0±1.4     |

*P<0.01, assessed by Student's t-test vs. BSA treatment.
has been reported that ketotifen treatment decreased the number and proportion of hypodense (light density, 26) eosinophils in the peripheral blood of patients with atopic dermatitis (27).

In the present study, marked inhibition of airway eosinophilia was achieved by dexamethasone, aminophylline, cromoglycate, tranilast and ketotifen. Other drugs tested in this study exhibit a range of properties that could lead to anti-allergic activity including mast cell stabilization and histamine (H1) antagonism (azelastine and oxatomide), leukotriene antagonism (amlexanox and ibudilast) and lipoxygenase inhibition (AA-861). None of these compounds influenced eosinophil accumulation in the airway lumen of guinea pigs, although it has been reported elsewhere that oral administration of ibudilast inhibits pulmonary eosinophilia due to antigen (28).

The capacity of prophylactic anti-asthma drugs to inhibit eosinophilia in the airways due to PAF cannot be associated with the known biological effects of these drugs. An effect of the drugs on platelets might be anticipated, since cromoglycate, ketotifen, aminophylline and steroids inhibit the development of airway hyperreactivity in response to i.v. infusion of PAF, a process known to be platelet-dependent (2), and there have reports of effects of such drugs upon platelet activation (29). However, in contrast to airway hyperreactivity in the guinea pig, PAF-induced eosinophil accumulation is not subordinate to platelet activation (4). The conclusion that induction of airway hyperreactivity and eosinophil accumulation by PAF are distinct events is reinforced by the evidence that tranilast inhibits eosinophil accumulation but not airway hyperreactivity (30), whereas ibudilast inhibits PAF-induced hyperreactivity (30) but not eosinophil accumulation.

The findings of the present study are consistent with the concept that PAF formation may determine accumulation of eosinophils in asthma. These results suggest that inhibition of eosinophil accumulation in the airways is an essential component of the pharmacological action of prophylactic anti-asthma drugs and that the guinea pig treated with PAF may be an appropriate model for prophylactic anti-asthma drug evaluation.

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**Table 2. The effect of anti-asthma drugs on PAF-induced eosinophil accumulation in pulmonary airways of the guinea pig**

| Drug             | Dose mg/kg/day | n  | Total white cell count in BAL (x10^6) | Eosinophils (%) | Probability* vs. saline |
|------------------|----------------|----|--------------------------------------|----------------|------------------------|
| Saline           | —              | 20 | 17.7±1.0                             | 20.6±1.4       | 0.0005                 |
| Cromoglycate     | 1.0            | 11 | 17.2±1.5                             | 11.1±1.5       | 0.00005                |
| Ketotifen        | 1.0            | 20 | 14.9±2.1                             | 10.0±1.3       | 0.0005                 |
|                  | 0.1            | 4  | 9.5±1.3                              | 13.3±1.4       | 0.024                  |
| Tranilast        | 1.0            | 10 | 17.7±1.7                             | 13.9±1.4       | 0.005                  |
| Oxatomide        | 1.0            | 7  | 18.6±1.9                             | 17.2±3.1       | 0.15                   |
| Azelastine       | 1.0            | 8  | 14.6±1.1                             | 20.8±3.7       | 0.52                   |
| Ibudilast        | 1.0            | 10 | 19.3±1.6                             | 17.3±1.8       | 0.10                   |
| AA-861           | 1.0            | 10 | 18.1±1.5                             | 16.7±1.6       | 0.06                   |
| Amlexanox        | 1.0            | 8  | 15.7±1.9                             | 19.8±3.5       | 0.40                   |
| Dexamethasone    | 1.0            | 11 | 21.0±3.9                             | 9.8±1.5        | 0.0005                 |
| Aminophylline    | 10.0           | 11 | 14.9±0.9                             | 11.7±1.4       | 0.0005                 |

*Probability by Student's t-test.
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