Ozone-induced pulmonary inflammation and epithelial proliferation are partially mediated by PAF

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Longphre, M., L.-Y. Zhang, J. R. Harkema, and S. R. Kleeberger. Ozone-induced pulmonary inflammation and epithelial proliferation are partially mediated by PAF. J. Appl. Physiol. 86(1): 341–349, 1999.—Ozone (O₃) exposure stimulates airway inflammation and epithelial sloughing in a number of species, including mice. Platelet-activating factor (PAF) is a lipid mediator released by activated mast cells, macrophages, and epithelial cells and causes pulmonary inflammation and hyperpermeability. We hypothesized that the activation of PAF receptors is central to the development of inflammation and epithelial injury induced by acute O₃ exposure in mice. To test this hypothesis, O₃-susceptible C57BL/6J mice were treated with a PAF-receptor antagonist, UK-74505, or vehicle either before or immediately after 3-h exposure to O₃ (2 parts/million) or filtered air. Bronchoalveolar lavage (BAL) fluids were collected 6 and 24 h after exposure. Differential cell counts and protein content of the lavage were used as indicators of inflammation in the airways. O₃-induced epithelial injury was assessed by light microscopy, and DNA synthesis in epithelium of terminal bronchioles was estimated by using a bromodeoxyuridine-labeling index. Intercellular adhesion molecule 1 (ICAM-1) expression was also examined in the lung by immunohistochemical localization. O₃ caused significant increases in polymorphonuclear leukocytes and protein in the BAL fluid, increased pulmonary epithelial proliferation, and increased epithelial expression of ICAM-1 compared with air-exposed, vehicle-treated control mice. Relative to O₃-exposed, vehicle-treated control mice, UK-74505 before exposure significantly (P < 0.05) decreased BAL protein, polymorphonuclear leukocytes, and epithelial cells. O₃-induced inflammation was similarly attenuated in mice treated with UK-74505 after exposure. These experiments thus support the hypothesis that activated PAF receptors contribute to O₃-induced inflammation and epithelial damage in the airways of O₃-susceptible C57BL/6J mice. We indirectly examined the role of PAF by interfering with the binding of PAF to PAF receptors. In the present study, mice were treated with the PAF-receptor antagonist UK-74505 or vehicle either before or after exposure to characterize the time course of PAF action and also to evaluate the usefulness of this antagonist as a therapeutic intervention in this model of acute lung injury.

METHODS

General

Male (6- to 8-wk old, 20–25 g) C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). They were housed in the animal care facilities of The Johns Hopkins School of Hygiene and Public Health at 22°C with a constant 14:10-h light-dark photoperiod. Water and mouse chow (Agway Pro-Lab RMH 1000, Waverly, NY) were provided ad libitum. Sentinel animals in the room were examined periodically (titer and necropsy) to ensure that the animals had remained free of infection. Mice were handled in accordance with the standards established by the US Animal Welfare Act set forth in National Institutes of Health guidelines and by The Johns Hopkins University School of Hygiene and Public Health Animal Care and Use Committee.

O₃ Exposure

Mice were placed in wire cages set inside glass chambers and were exposed to O₃ (2 parts/million (ppm)) or filtered air for 3 h. Chamber air was pretreated by passage through an activated charcoal column, a silica gel drying column, and a high-efficiency, particulate-filtered air filter before it was directed to either chamber. A split-stream airflow design ventilated both chambers at a rate of 18–20 changes·h⁻¹, and the pressure within each chamber was +1.15 cmH₂O. O₃ was generated by passing a constant flow (2 l/min) of filtered air through a glass bottle that contained a mercury bulb (BHk, Monrovia, CA). A portion of this flow was then mixed with the stream of filtered air (35 l/min) that flowed into the 70-liter glass chamber. The proportion of the generator output that flowed into the exposure chamber was regulated by a computer-monitored control system. The O₃ concentration in the exposure chamber was continuously monitored by a Chem-Lab spectrophotometer (Bar Harbor, ME) located in a separate room near the exposure chamber. The O₃ concentration in the exposure chamber was continuously monitored by a Chem-Lab spectrophotometer (Bar Harbor, ME) located in a separate room near the exposure chamber.

PLATELET-ACTIVATING FACTOR (PAF) is a potent proinflammatory lipid mediator that has been associated with the etiology of asthma, pulmonary hypertension, sarcoidosis, shock, and respiratory distress syndrome. Instilled or aerosolized PAF causes pulmonary sequestration of polymorphonuclear leukocytes (PMNs) (30), vascular hyperpermeability in the lung, and increases in reactivity to bronchoconstrictive agonists (27). The pulmonary effects of exogenous PAF are similar to those observed after ozone (O₃) exposure in some animal models (12) and in humans (14).

Acute O₃ exposure causes an inflammatory response in the mouse that is characterized by PMN infiltration that peaks 4–8 h after exposure (12) and by regional expression of intercellular adhesion molecule 1 (ICAM-1; CD54) that peaks 4–8 h after exposure (12) and by regional expression of intercellular adhesion molecule 1 (ICAM-1; CD54) and are exposed to O₃ [2 parts/million (ppm)] or filtered air for 3 h. Chamber air was pretreated by passage through an activated charcoal column, a silica gel drying column, and a high-efficiency, particulate-filtered air filter before it was directed to either chamber. A split-stream airflow design ventilated both chambers at a rate of 18–20 changes·h⁻¹, and the pressure within each chamber was +1.15 cmH₂O. O₃ was generated by passing a constant flow (2 l/min) of filtered air through a glass bottle that contained a mercury bulb (BHk, Monrovia, CA). A portion of this flow was then mixed with the stream of filtered air (35 l/min) that flowed into the 70-liter glass chamber. The proportion of the generator output that flowed into the exposure chamber was regulated by a computer-monitored control system. The O₃ concentration in the exposure chamber was continuously monitored by a Chem-Lab spectrophotometer (Bar Harbor, ME) located in a separate room near the exposure chamber.
monitored with an oxidant meter (Dasibi Environmental, Glendale, CA) that was calibrated regularly against a standard O₃ source (Dasibi Environmental). A parallel stream of air (35 l/min) flowed through a second identical chamber for simultaneous exposure of control animals to filtered air.

After exposure the animals were returned to filter-topped cages for either 6 or 24 h and exposed to only room air. Some mice designated for euthanasia 24 h after exposure were injected 2 h before death with a thymidine analog, bromodeoxyuridine (BrdU; 50 mg/kg body wt ip). BrdU incorporation into the DNA of dividing cells was used as a marker of cell proliferation.

**Bronchoalveolar Lavage (BAL)**

Mice were killed by cervical dislocation. BAL (35 ml/kg, 4 times) was performed in situ with Hanks’ balanced salt solution through a polyethylene catheter inserted into the trachea. Recovered lavage fluids from each mouse were immediately cooled to 4°C and centrifuged (500 g). Total protein content of the first lavage from each mouse was measured (Pierce BSA kit, Rockford, IL) and used as an indicator of lung permeability. Total numbers of cells from the total pooled lavage were estimated as described previously (12). Cell pellets from the four lavages were combined and resuspended in 1 ml of lavage fluid. Aliquots (30 μl) of this suspension were centrifuged (Shandon Southern Products, Cheshire, UK) and stained with Diff Quik (AHS, del Caribe, PR) for differential analysis of cells by standard cytological techniques. Differential cell counts and protein content in the lavage fluids were used as markers of inflammation. Epithelial cell number in the lavage fluids was used as an indicator of epithelial death but may also include cells undergoing apoptosis.

**Tissue Preparation and Histological Analyses**

The lungs of air- and O₃-exposed mice were excised and fixed by perfusion through the trachea with zinc Formalin fixative (PolySciences, Warrington, PA) at a constant pressure of 25 cmH₂O. After 2-h perfusion-fixation, the lungs were immersed in a large volume of the same fixative. After fixation, the right caudal lobes were removed and cut sagittally into three pieces. Tissues were embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin.

Lung sections were also immunohistochemically stained for the presence of ICAM-1 by using a polyclonal goat IgG (Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:200 dilution and Vectastain ABC Peroxidase kit (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions. Endogenous peroxidase activity was quenched after deparaffinization with 0.3% hydrogen peroxide in methanol for 30 min. Control slides were treated as the experimental slides without the primary antibody. Slides were incubated in the peroxidase substrate diaminobenzidine peroxidase for 2 min and rinsed. The reaction product formed a brown color that deposited in those areas of the tissue that had bound anti-ICAM-1. Slides were counterstained with hematoxylin.

Serial sections from lung tissues of some of the O₃-exposed mice were immunohistochemically stained for BrdU as previously described (18) and counterstained with hematoxylin and lithium carbonate. All slides were coded and counted without knowledge of the treatment group. BrdU-stained sections were imaged on a digitizing tablet by camera lucida. Terminal bronchioles were the focus of study in the lungs because O₃ exposure causes histologically evident lesions in these areas in other species (8). By using ×400 magnification, all BrdU-labeled and nonlabeled epithelial cells lining the terminal bronchioles were counted throughout each section. A terminal bronchial profile in a lung section was defined as the first branch of a conducting airway proximal to the alveolar ducts. This bronchiolar airway is bounded by a continuous wall, which is lined by a cuboidal epithelium. Only terminal bronchioles that were continuous with the proximal alveolar ducts in the section were used to determine the labeling index. The epithelium lining the luminal surface of the bronchioles was identified on the basis of the presence of a basal lamina supporting cuboidal cells. No distinction was made between nonciliated and type 2 pneumocytes, and luminal macrophages and interstitial fibroblasts were not included in the BrdU count. A mean of 816 ± 122 epithelial cells and 10 ± 1 bronchial profiles were counted per mouse. The labeling index was determined by dividing the number of labeled cells by the total number of cells counted. The linear length of the basal lamina was also calculated from a contour tracing of the bronchioles by using Sigma Scan (Jandel Scientific, Corte Madera, CA). The data for each group were expressed as the mean number of BrdU-labeled nuclei divided by total cells ± SE (n = 5–10 mice/group). Epithelial labeling, when expressed as the number of BrdU-labeled nuclei per millimeter of basal lamina (data not shown), was also measured and led to similar conclusions.

**Experimental Protocol**

To test the hypothesis that PAF contributed to inflammation and epithelial damage in the airways of mice after O₃ exposure, animals were injected intraperitoneally with 5 mg/kg of the PAF antagonist (UK-74505) or vehicle 30 min before exposure. The mice were exposed to filtered air or 2 ppm O₃ for 3 h, and the resultant airway inflammation, epithelial proliferation, and alveolar histology were assessed as described above and compared between treatment groups. To further evaluate the time course of PAF contribution to O₃-induced inflammation and epithelial injury, groups of mice were treated with UK-74505 or vehicle 10 min after exposure to O₃ or air. The inflammatory responses of the treatment groups were compared 6 and 24 h after exposure to assess the effects of postexposure treatment.

**Drugs**

On the day of the experiment, UK-74505 was prepared in saline containing 0.005 M HCl. UK-74505 was generously provided by Pfizer (Groton, CT). PAF (L-α-phosphatidylcholine, β-acetyl-γ-O-alkyl; Sigma Chemical, St. Louis, MO) was prepared daily in 0.25% BSA (Sigma Chemical) in physiological saline.

**Statistics**

The effects of pharmacological treatment (UK-74505 or vehicle), exposure (air or O₃), and recovery (6 or 24 h) on differential cell counts, lavage protein, and epithelial proliferation in the lung were assessed by three-factor ANOVA. Student-Newman-Keuls analyses were used for post hoc comparison of means. Statistical significance was accepted at P < 0.05.

**RESULTS**

**O₃ Exposure and PAF-Antagonist Treatment**

BAL. The BAL of vehicle-treated mice reflected an effect of O₃ exposure on inflammatory markers in the lung compared with air-exposed control mice. There
were significant increases in BAL PMNs, macrophages, and epithelial cells 6 h after exposure (P < 0.05, n = 5–10 mice/group, Figs. 1 and 2). BAL macrophages and total protein were increased in the BAL 6 and 24 h after exposure (P < 0.05, Figs. 1–3).

UK-74505 administered before exposure significantly decreased the mean numbers of lavageable PMNs and epithelial cells 6 h after O₃ relative to vehicle-treated controls (P < 0.05, Fig. 1). There were no significant changes in the numbers of BAL macrophages in mice treated with UK-74505 either 6 or 24 h after O₃ (P > 0.05, Fig. 1). The antagonist caused a statistically significant decrease in the O₃-induced change in BAL protein only at 24 h (P < 0.05, Fig. 3A).

Postexposure treatment with UK-74505 produced a similar attenuation in O₃-induced inflammation. There were statistically fewer BAL PMNs and epithelial cells 6 h after O₂ compared with vehicle-treated controls (P < 0.05, n = 5–10 mice/group, Fig. 2). The numbers of BAL macrophages and lymphocytes in the post-O₃, UK-74505-treated group were not statistically different from those in vehicle-treated control mice (P > 0.05, Fig. 2). The antagonist also decreased the O₂-induced change in BAL protein 24 h after exposure (P < 0.05, Fig. 3B).

Epithelial proliferation. O₃ significantly increased the epithelial labeling index in vehicle-treated mice compared with air-exposed control mice (P < 0.05, Fig. 4). Epithelial proliferation was significantly decreased by >30% in the centriacinar regions of the UK-74505-pretreated animals after O₃ exposure relative to vehicle-pretreated mice (P < 0.05, Fig. 4A). Approximately 10.2 ± 1.9% of the cells in terminal bronchioles were labeled after exposure in the vehicle-treated mice compared with 7.2 ± 1.3% labeling in mice pretreated with UK-74505. A small but nonsignificant decrease in epithelial proliferation was observed in the centriacinar regions of mice that were treated with UK-74505 after exposure to O₃ (P > 0.05, Fig. 4B).

Histopathology. In vehicle-treated mice, multifocal epithelial and inflammatory lesions were evident in the pulmonary conducting airways (i.e., main axial bronchi, preterminal and terminal bronchioles) and proximal alveolar ducts within centriacinar regions of the lung 6 h after O₃ (Fig. 5). PMNs were particularly noticeable at these inflammatory lesions. No exposure-related inflammatory or epithelial lesions were evident in the distal lung parenchyma. Lung sections that were immunohistochemically stained for ICAM-1 showed a definite association of this adhesion molecule with the epithelium of the distal and terminal bronchioles in O₃-exposed lungs but not air-exposed controls (Fig. 6). The degree of pulmonary ICAM-1 staining was greater in the O₃-exposed, vehicle-treated mice compared with antagonist-treated mice. There was no obvious staining...
in the endothelium of the vessels associated with these airways in any of the treatment groups.

The distribution of O₃-induced epithelial alterations in the respiratory tract of vehicle-treated mice killed 24 h after exposure was similar to those described for 6-h postexposure, although the character of the airway injury at this time was different. The airway epithelium was mildly hyperplastic, with hypertrophy of individual epithelial cells and a conspicuous increase in the number of mitotic figures and of cellular debris along the length of the terminal bronchi (Fig. 5). In addition, there was a mild accumulation of alveolar macrophages in the airway lumens of some centriacini in these O₃-exposed lungs. ICAM-1 staining in the vehicle treated, O₃-exposed lungs was still present in the terminal bronchiolar epithelium but to a lesser degree compared with 6-h, post-O₃ lungs (Fig. 6).

In contrast, there were fewer exposure-related epithelial or inflammatory lesions in the airways of mice treated with UK-74505 either before or after exposure compared with vehicle-treated control animals. This effect was most noticeable 6 h after exposure. Specifically, fewer PMNs were observed (Fig. 5). Air-exposed control animals of both UK-74505- and vehicle-treated mice were completely free of pulmonary histopathology or ICAM-1 immunoreactivity (Figs. 5 and 6). ICAM-1 staining in the epithelium of the terminal bronchioles from UK-74505-treated mice, although present after O₃ exposure, was less intense than that of the vehicle-treated lungs, suggesting less expression of this protein.

**DISCUSSION**

PAF has been implicated in the pathogenesis of a number of acute lung injury models, including sepsis (see Ref. 13 for review) and smoke inhalation (9). Acute exposures to O₃ also induce acute lung injury (12). Inasmuch as macrophages and epithelial cells are targets of O₃, and these cells are known to produce PAF, we hypothesized that this proinflammatory lipid mediator, or activation of its receptor, was responsible, in part, for O₃-induced inflammation and epithelial damage in murine airways.

Compared with vehicle controls, the PAF-receptor antagonist UK-74505 markedly attenuated the mean number of lavageable PMNs 6 h after O₃. UK-74505 also significantly decreased BAL protein recovered 24 h after exposure to O₃ and inhibited O₃-induced DNA synthesis in terminal bronchioles (i.e., epithelial proliferation). These observations are consistent with those of Kaneko et al. (10), who found that the PAF-receptor antagonist E-6123 significantly attenuated O₃-induced airways hyperpermeability as measured by Evans blue extravasation in a guinea pig model. In a similar study, Tan and Bethel (31) were unable to blunt O₃-induced increases in permeability and BAL PMNs in guinea pigs with another PAF-receptor antagonist, L-659989.
Our laboratory has also been unable to reproducibly antagonize PAF receptors in an O₃ model with L-659989 (unpublished observations). The discrepancy in the results of the two studies may be due to the dosing regimen used. L-659989 has a relatively short in vitro half-life (30 min) and may not have reached an effective concentration for complete antagonism over the course of exposure and recovery in both studies. UK-74505, in comparison, has a much longer half-life because it irreversibly binds to receptors and may be more appropriate for in vivo studies like those presented here. Although a direct comparison of L-659989 and UK-74505 has not been undertaken in a whole-animal model, UK-74505 has been shown to be 10–30 times more potent than WEB-2086, a PAF-antagonist similar to L-659989 in affinity, in several in vivo models (21). Similarly, E-6123 is a PAF antagonist with a longer half-life (4.77 h in a guinea pig model; Ref. 16), making it also more appropriate for in vivo assessment of PAF-receptor activation.

The mechanism(s) through which PAF receptors mediate O₃-induced PMN influx and epithelial injury is not certain. PAF may be released from, and receptors are located on, a number of cells, including epithelial cells, macrophages, endothelial cells, and mast cells, and may act directly as a chemoattractant for PMNs. PAF has been shown to induce selective in vitro and in vivo PMN chemotaxis and activation (1, 5, 30). In vitro studies of human PMNs have suggested that PAF may act as an important “cofactor” required in combination with other chemoattractants [i.e., interleukin-8 (IL-8)] for proper migration (15). Kuijpers et al. (15) showed that PMN migration across an endothelial barrier in culture could be partially inhibited either by a PAF antagonist or by addition of antibodies to IL-8 but could be completely blocked by the addition of both of these agents. We have been able to demonstrate a decrease in O₃-induced IL-8 release from human epithelial cells after treatment with UK-74505 in vitro (17). This observation suggests that activation of PAF receptors on epithelial cells is necessary for the release of IL-8, a

Fig. 3. Effect of O₃ (2 ppm, 3 h) and filtered air on BAL total protein 6 and 24 h after exposure in mice treated with either vehicle (0.005 M HCl in saline ip) or PAF antagonist UK-74505 (5 mg/kg ip) either before (A) or 10 min after exposure (B). Protein means ± SE are presented; n = 5–10 mice/group. Data for filtered air control mice were not statistically different at 6 and 24 h recovery and have been pooled. Statistical comparison of O₃ exposure with filtered air control, *P < 0.05; statistical comparison of vehicle with antagonist treatment, +P < 0.05.

Fig. 4. Effect of O₃ (2 ppm, 3 h) and filtered air on epithelial cell proliferation in mice treated with either vehicle (0.005 M HCl in saline ip) or PAF antagonist UK-74505 (5 mg/kg ip) either before (A) or just after exposure (B). Percentage of epithelial cells in terminal bronchioles that stained positively for uptake of bromodeoxyuridine (BrdU) is shown. Values are means ± SE; n = 5 mice/group. Statistical comparison of O₃ exposure with filtered air control, *P < 0.05. Statistical comparison of vehicle with antagonist treatment, +P < 0.05.
critical chemotactic factor involved in the recruitment of PMNs.

Effects of PAF on blood flow in the pulmonary circuit may also result in changes in local inflammatory responses. In a study by Hellewell et al. (6), instillation of C5a in rabbits and guinea pigs resulted in decreased pulmonary blood flow (i.e., induced pulmonary hypertension), an effect that was reversed with PAF-receptor antagonism. The authors suggested that the increased pulmonary arterial pressure was a protective mechanism that diverted blood flow and leukocyte recruitment. We did not measure pulmonary arterial pressure in the present study, but from the results we can only conclude that, if PAF were altering blood flow, treatment with a receptor antagonist would lead to an enhancement of PMNs in the lavage, not a decrease.

PAF receptors may affect PMN chemotaxis by acting as a regulator of adhesion molecules thought to be important in O₃-induced inflammation in mice [e.g., ICAM-1; Ref. 29]. PAF can cause loss of reciprocal connections between endothelial cells to facilitate the attachment and migration of PMNs from the vasculature to the air spaces (3). In addition, when PAF binds to receptors on the surface of PMNs, it can cause increased oxidant production (11, 28) and “priming” for responsiveness to other stimuli (33). Although expressed in the epithelium of both vehicle- and UK-74505-treated lungs after O₃ exposure in the present

Fig. 5. Light micrographs of centriacinar region of zinc Formalin-fixed lung tissue from air (A)- and O₃-exposed (B-E) mice. Vehicle-treated, air-exposed mouse (A) shows normal lung architecture. Lung sections from O₃-exposed mice that were pretreated with vehicle are shown after 6 (B)- and 24-h (D) exposure. Comparable lung sections from mice that were pretreated with PAF antagonist UK-74505 (5 mg/kg ip) are also shown after 6 (C)- or 24-h (E) exposure. Influx of neutrophils (thick arrows) and focal areas of epithelial necrosis (thin arrows) are evident in preterminal and terminal bronchioles (tb) of same specimens. e, Bronchial epithelium; a, alveolus; ad, alveolar duct. Sections were stained with hematoxylin and eosin; magnification ×200. Epithelium is also shown at ×400. Bar, 30 µm.
study, ICAM-1 was much less conspicuous in the antagonist-treated mice. Given a residual expression of ICAM-1 in the epithelium despite the dramatic decrease in lavagable PMNs, we suggest that the PAF antagonist may be disrupting the PMN recruitment signaling pathways at other points, as well as the expression of ICAM-1.

PAF is also thought to cause increases in permeability by affecting calcium flux and microtubule stability in the endothelium and cause "rounding up" of endothelial cells. Northover and Northover (20) observed increased trapping of colloidal carbon within the blood vessel walls of rat small intestine that was treated with PAF. This weakening of cell-cell tight junctions may be responsible for increased permeability. Similarly, PAF may affect epithelial cell loss by directly binding to epithelial cells and altering the integrity of the cell-cell connections. This potential mechanism may account for the significant effect of PAF antagonism on the number of epithelial cells recovered in lavage fluid.

PAF may also promote transcriptional activation of primary response genes that contribute to the pathogenesis of lung injury (2, 25). For example, PAF has been shown to upregulate inducible cyclooxygenase gene expression (2, 32). Cyclooxygenase products of arachidonic acid metabolism may have an important role in the pathogenesis of O3-induced acute lung injury (19). PAF also modulates proinflammatory cytokines such as...
tumor necrosis factor (TNF-α) (26). A significant role for PAF as a secondary mediator of inflammation and lung injury induced by TNF-α, IL-2, and interferon-γ has also been reported (e.g., 7, 24) and may have a potential role in O₃ toxicity.

To further understand the role of PAF and PAF receptors in O₃-induced acute lung injury, C57BL/6J mice were treated with UK-74505 immediately after O₃ exposure. Relative to vehicle control mice, UK-74505 significantly attenuated the number of lavageable PMNs and epithelial cells, total BAL protein, and DNA synthesis induced by O₃. Compared with mice that were treated with UK-74505 before O₃ exposure, there were some differences in BAL cell profile and protein content. Regardless of these small differences, the degree of attenuation by the antagonist was similar (although not identical) between pre- and posttreated mice.

Results of these antagonist experiments may suggest that the proinflammatory events mediated by PAF receptors occur, not as a direct effect of O₃ stimulation, but rather as a secondary process initiated by O₃ or another proinflammatory mediator. This observation is consistent with the “cascade” hypothesis of O₃ toxicity, in which cells are stimulated by lipid ozonation products derived from O₃-lipid interactions in epithelial cell membranes or lipids present in the epithelial lining fluid (22). The source of PAF in this scenario is uncertain, but epithelial cells, macrophages, or subepithelial mast cells may be potential candidates. Although the preexposure treatment results could potentially be explained by alteration in PAF baseline through disruption of PAF homeostasis, we think this possibility is unlikely because of the timing of treatment. Circulating levels of PAF were probably not altered in <30 min; however, because we did not measure circulating PAF, this remains a possible explanation. In any case, our experiments may also suggest that PAF-antagonist treatment may be a viable option for therapeutic intervention in similar models and clinical scenarios of acute lung injury.

It is important to recognize that a significant amount of O₃-induced lung hyperpermeability and epithelial proliferation was not attenuated by UK-74505. Other lipid mediators (e.g., leukotrienes, prostaglandins), cytokines (e.g., macrophage inflammatory protein 2 and TNF-α), and enzymes such as mast cell-derived tryptase may contribute to these O₃ effects.

In conclusion, O₃-induced airways inflammation and epithelial damage are mediated, in part, by activation of PAF receptors after exposure. Antagonism of PAF receptors may be a useful intervention in oxidant-induced lung injury.

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