Pentoxifylline Does Not Alter the Response to Inhaled Grain Dust*

Paul J. Jagiello, MD; Janet L. Watt, BA; Timothy J. Quinn, BS; Howard R. Knapp, MD, PhD; and David A. Schwartz, MD, MPH, FCCP

Pentoxifylline (PTX) has been shown to reduce sepsis-induced neutrophil sequestration in the lung and inhibit endotoxin-mediated release of tumor necrosis factor-α (TNF-α). Previously, we have shown that endotoxin appears to be the principal agent in grain dust causing airway inflammation and airflow obstruction following grain dust inhalation. To determine whether PTX affects the physiologic and inflammatory events following acute grain dust inhalation, 10 healthy, nonsmoking subjects with normal airway reactivity were treated with PTX or placebo (PL) followed by corn dust extract (CDE) inhalation (0.08 mL/kg), using a single-blinded, crossover design. Subjects received PTX (1,200 mg/d) or PL for 4 days prior to CDE inhalation and 400 mg PTX or PL on the exposure day. Both respiratory symptoms and declines in FEV₁ and FVC occurred following CDE exposure in both groups, but there were no significant differences in the frequency of symptoms or percent declines from baseline in the FEV₁ and FVC at any of the time points measured in the study. Elevations in peripheral blood leukocyte and neutrophil concentrations and BAL total cell, neutrophil, TNF-α, and interleukin-8 concentrations were measured 4 h following exposure to CDE in both the PTX- and PL-treated subjects, but no significant differences were found between treatment groups. These results suggest that pretreatment with PTX prior to inhalation of CDE, in the doses used in this study, does not alter the acute physiologic or inflammatory events following exposure to inhaled CDE.

(CHEST 1997; 111:1429-35)

Key words: airway inflammation; endotoxin; grain dust; pentoxifylline

Abbreviations: CDE=corn dust extract; CRC=clinical research center; IL=interleukin; PTX=pentoxifylline; TNF-α=tumor necrosis factor-α

Occupational exposure to grain dust has been shown to cause a variety of clinical syndromes, including chronic disease, acute bronchitis, bronchial hyperreactivity, and progressive, irreversible airflow obstruction. Prior epidemiologic studies suggest that acute changes in airflow across a workshift or work week are consistently associated with longitudinal declines in lung function. Therefore, identification of the initial biological and physiologic events associated with acute grain dust exposure may be relevant in developing interventions leading to the prevention of the chronic manifestations of this environmental lung disease.

The acute inflammatory events following grain dust inhalation appear to be mediated through non-immunologic mechanisms. Using a model of acute grain dust inhalation, we have demonstrated that healthy, nonasthmatic volunteers without prior exposure to grain dust develop acute respiratory symptoms and significant declines in FEV₁ following grain dust inhalation. Furthermore, these subjects develop a neutrophilic inflammatory response in the lower respiratory tract following inhalation chal-
challenged, associated with increases in BAL tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-8 and increased expression of messenger RNA for IL-8 in the bronchial airway epithelial cells.  

There is growing evidence suggesting that endotoxin is the primary constituent in grain dust responsible for inflammation in the lower respiratory tract. For example, respiratory symptoms and airflow obstruction among grain workers are strongly related to the concentration of endotoxin in the workplace bioaerosol. Endotoxin responsiveness appears to be required for the development of the inflammatory response following inhalation of grain dust. Reductions in the concentration of endotoxin in grain dust result in a substantial decline in grain dust-induced inflammation. Moreover, both grain dust and endotoxin inhalation produce similar respiratory symptoms, equivalent decreases in FEV₁, and similar increases in BAL concentrations of inflammatory cells and inflammatory cytokines.

Given the nature of the inflammatory events resulting from grain dust inhalation, one may consider altering the inflammatory response using specific agents that decrease endotoxin-mediated cytokine release and neutrophil recruitment. Pentoxifylline (PTX), a methylxanthine derivative that increases cellular cyclic adenosine monophosphate, has been shown by others to possess anti-TNF-α properties and to reduce lung inflammation in animal models, decrease human neutrophil adherence in vitro, and inhibit endotoxin-mediated release of TNF-α.

The purpose of the present investigation is to determine whether PTX administration prior to inhalation of grain dust alters the physiologic and inflammatory events in healthy volunteers. We hypothesize that pretreatment of subjects with PTX prior to grain dust inhalation will reduce the physiologic and inflammatory response.

**Materials and Methods**

We used a single-blinded crossover design to determine whether pretreatment with PTX reduces the physiologic and inflammatory response to inhalation of corn dust extract (CDE) in normal volunteers.

**Study Subjects**

Ten healthy, nonasthmatic, never-smoking subjects without any history of cardiopulmonary disease or occupational exposure to grain dust were recruited. The study protocol used was approved by our human subjects review committee and carried out at the University of Iowa National Institutes of Health General Clinical Research Center. To be considered eligible for participation, study subjects were required to have normal results of physical examination, 12-lead ECG, chest radiograph, and pulmonary function tests (spirometry, lung volumes, diffusing capacity, and arterial blood gases). Skin testing for 10 common aeroallergens was performed in all study subjects. Two of the 10 subjects tested positive for ragweed, corn, and wheat. An abbreviated histamine challenge test was performed on each subject, and subjects demonstrating a 20% or greater fall in their baseline FEV₁ compared with diluent were excluded. Using this protocol, a negative histamine challenge corresponds to a provocative concentration of substance causing a 20% fall in FEV₁ (PC₂₀) of >16 mg/mL or PC₂₀ >85 cumulative breath units of histamine.

**Protocol**

Study subjects underwent two separate inhalation challenges, with exposures being separated by at least 3 weeks. Based on previous kinetic studies performed in our laboratory, this interval of time is sufficient for lung function and lavage parameters to return to baseline values. Prior to the first inhalation challenge, study subjects received oral placebo three times daily for 4 days. However, prior to the second challenge, each study subject received oral PTX (400 mg) three times daily for 4 days. Study subjects were blinded to the intervention. PTX was purchased (from Hoechst-Roussel) as a controlled-release tablet (400 mg per tablet). On the day of the exposure, subjects received one dose 2 h prior to the exposure. To ensure compliance, two of the three daytime doses were given under direct observation in the clinical research center (CRC). The third (evening) dose was self-administered and confirmation of taking the study drug was made by telephone to a nurse in the CRC. Vital signs and pulmonary function were measured prior to and following each inhalation exposure. Bronchoscopy was performed 4 h following completion of the inhalation challenge. Phlebotomy was performed prior to and 4 h following each exposure for measurement of peripheral blood leucocyte count and neutrophil counts. In addition, serum was collected and frozen at −70°C for measurement of PTX levels.

**Preparation of the CDE**

Corn dust used in this study was obtained from the air filtration system at an Eastern Iowa grain facility. CDE was prepared by mixing 3 g of dust in 30 mL of sterile, pyrogen-free Hank's balanced salt solution without calcium or magnesium (0.1% solution), vortexing for 2 min, and shaking for 1 h at 4°C. The mixture was centrifuged at 800×g for 20 min, and the supernatant solution was collected, resulting in the CDE. The CDE solution underwent filter sterilization through a 0.45-μm filter (Sterifill-D GV Filter Unit; Millipore; Bedford, Mass). All solutions used for inhalation were derived from a stock solution, which underwent sterility testing (bacteria and fungi) and endotoxin assay prior to separation into individual aliquots. These aliquots were stored at −70°C until being used. Endotoxin concentration was measured by the end-point chromogenic Limulus amebocyte lysate assay (QCL-1000; Whittaker Bioproducts; Walkersville, Md). The measured endotoxin concentration in the CDE prepared by this method was 2.6 μg/mL.

**Inhalational Challenge**

The solutions were administered via a nebulizer (DeVilbiss 646) and dosimeter (DeVilbiss: DeVilbiss Health Care Inc; Somerset, Pa), operated at 20 psi air pressure. Subjects controlled the timing of each nebulized dose and were instructed to inhale through the mouthpiece of the nebulizer and exhalate through their nose. Using this delivery system and technique, a precise dose of inhalant was delivered, obtaining maximal airway...
mucosal exposure. For each exposure, subjects were administered 0.1 mL of inhalant per kilogram of body weight, given over a 60-min period.

**Pulmonary Function Testing**

The pulmonary function tests consisted of serial spirometry using a spirometer (Spirotech S-600; Graseby Anderson; Atlanta). These maneuvers were performed using standard protocols and the American Thoracic Society guidelines. The spirometer was calibrated prior to each visit. Spirometry was performed with noseclips, in a sitting position preexposure, and at the following time points postexposure: 10, 20, and 30 min, and 1, 2, 3, 4, and 24 h.

**Bronchoscopy**

Bronchoscopy was performed 4 h following each inhalation exposure, in accordance with the standards established by the American Thoracic Society for bronchoscopy in asthmatics. A fiberoptic bronchoscope (Olympus P-10; Olympus: Lombard, Ill) was introduced into the chosen lung segment for lavage and wedged. Twenty milliliters of sterile 0.9% saline solution (37°C) was injected through the bronchoscope and then collected. This was performed five more times for a total lavage volume of 120 mL. The return of the first 20-mL aliquot was separated from the remaining lavage fluid. Lung segments chosen for BAL alternated between a subsegment of the right middle lobe following the first exposure and a subsegment of the lingula following the second exposure.

**Processing of Specimens**

Immediately following bronchoscopy, the BAL samples were processed according to methods described previously. The BAL supernatant was frozen at −70°C for subsequent use. After washing the cells twice with Hank’s balanced salt solution, the cell pellet was suspended in RPMI-1640 medium and cell counts were performed. Cytospin preparations were made from the lavage cell resuspension, stained with a Giemsa-type stain (Diff-Quik; Baxter Scientific Products; Miami), and cell differential counts were quantified counting 200 cells. TNF-α and IL-8 were measured in the BAL supernatant fluid using commercially available enzyme immunoassays (R&D Systems; Minneapolis).

**Measurement of Serum PTX**

PTX was measured in serum and BAL fluid essentially using the extraction method described by Lambert et al and the high-performance liquid chromatographic conditions of Graseila and Rocci. In brief, samples were spiked with known amounts of isobutyl methylnitramine (Aldrich Chemical Co; Milwaukee) as an internal standard, extracted, and analyzed using a high-performance liquid chromatographic system (ISCO; Lincoln, Neb) equipped with a V4 UV detector, monitoring at 273 nm. The column was a 4.6 mm (internal diameter) × 25 cm long, 5 μm particle size, LC 8-DB (Supelco; Bellefonte, Pa), and quantitation was achieved by comparing the ratio of the analyte peak height with that of the internal standard with the standard curve ($R^2=0.9989$ over the sample concentration range).

**Statistics**

CDE-induced changes in lung function and biological measures of inflammation (BAL cellularity and cytokines) following treatment with PTX were compared with these CDE-induced responses following treatment with placebo. Analysis of data was performed using paired, nonparametric statistics (Wilcoxon signed rank test). $χ^2$ analysis was performed in comparing symptom frequencies following treatment with PTX vs placebo.

**Results**

Ten subjects, seven men and three women (mean age, 30 years; range, 22 to 40 years) participated in the study. There were no significant side effects reported with taking the PTX or placebo over the 5-day study period. PTX was easily measured in the serum of subjects 2 h following ingestion of PTX and prior to inhalation exposure to CDE (mean±SEM: 191.1±42.1 ng/mL; range, 22 to 384 ng/mL). Low amounts (8 to 20 ng/mL) of PTX were also detected in the BAL fluid on occasions when PTX was administered as expected from the dilution inherent in the procedure.

Acute respiratory symptoms were noted following exposure to CDE for both the PTX- and placebo-pretreated exposures (Table 1). Following pretreatment with placebo, acute inhalation of CDE resulted in the development of chest tightness, cough, dyspnea, and sputum production. Other less frequently reported symptoms included nasal congestion, chills, fever, and myalgias. Similarly, subjects pretreated with PTX developed acute respiratory symptoms and constitutional symptoms. However, the frequency in which these symptoms were reported was not significantly different between the PTX and placebo pretreatment.

Prechallenge spirometric parameters for individuals did not change significantly between exposures. Following pretreatment with placebo or PTX, inhalation challenge with CDE resulted in clinically significant reductions in the FEV1 and FVC, occurring as early as 10 min postexposure (data not shown) and persisting for at least 4 h, prior to bronchoscopy (Fig 1). In subjects pretreated with placebo, the

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>PTX (n=10)</th>
<th>Placebo (n=10)</th>
<th>p Value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest tightness</td>
<td>9</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Cough</td>
<td>5</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>4</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>Phlegm</td>
<td>1</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>3</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Chills</td>
<td>4</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Fever (temperature &gt;38°C)</td>
<td>0</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Myalgia</td>
<td>1</td>
<td>1</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$Values represent actual number of subjects reporting symptoms.

$^1$NS = p value > 0.05.

CHEST / 111 / 5 / MAY, 1997 1431
maximal mean percent decline from baseline in the FEV₁±SEM over the 4-h period following inhalation of CDE was 16.5±3.7%, while pretreatment with PTX prior to inhalation exposure caused a maximal mean percent decline of 19.9±3.5%. Over this same time period, pretreatment with placebo caused a maximal mean percent decline in the FVC±SEM of 11.9±3.3%, while the PTX-pretreated exposure resulted in a mean percent decline of 14.5±3.3%. Overall, there were no significant differences in the percent declines from baseline in the FEV₁ or FVC following inhalation challenge to CDE between the PTX- and placebo-treated subjects (p>0.05 at all time points measured).

Exposure to CDE resulted in acute increases in BAL total cell and neutrophil concentrations for both the placebo- and PTX-pretreated exposures (Fig 2). However, pretreatment with PTX did not appear to significantly alter the magnitude of the BAL inflammatory cells in response to CDE inhalation. In subjects treated with placebo, CDE inhalation resulted in elevations in BAL total cell and neutrophil concentrations. Similarly, in subjects pretreated with PTX, both BAL total cell and neutrophil concentrations were elevated, and these increases were not significantly different from those measured in the placebo-pretreated exposure (p>0.05 for each).

Increased levels of TNF-α and IL-8 were measured in the BAL fluid of subjects following inhalation of CDE (Fig 3). In subjects pretreated with placebo, the group mean BAL concentrations±SEM for TNF-α and IL-8 were 127.8±50.2 pg/mL and 569.5±76.1 pg/mL, while in the PTX-treated group, these were 119.3±35.7 pg/mL and 639.5±82.3 pg/mL, respectively. The concentrations of TNF-α and IL-8 in the BAL fluid following inhalation of CDE were not significantly different between the PTX- and placebo-pretreated exposures (p>0.05).

Baseline peripheral blood leukocyte counts were in the normal range and were not significantly different between subjects prior to inhalation exposure. Four hours following inhalation challenge to CDE, both peripheral blood leukocyte and neutrophil counts were significantly higher than preexpo-
sure values (data not shown) for both the PTX- and placebo-pretreated subjects. The increase in the leukocyte count was principally due to elevations in the number of neutrophils. The mean percent change from baseline in the leukocyte and neutrophil counts for each pretreatment group are shown in Figure 4. Following exposure to CDE, the mean total leukocyte count increased by at least a factor of two and the neutrophil count increased by a factor of three for both the placebo- and PTX-treated subjects. However, the increases in the total leukocyte and neutrophil counts were not significantly different between treatment groups (p>0.05 for each).

**DISCUSSION**

Our results indicate that PTX, in the dose given in this study, does not alter the physiologic and inflammatory events following exposure to CDE. In our model of acute grain dust exposure, both placebo and PTX treatment prior to inhalation challenge with CDE resulted in a similar frequency of respiratory symptoms, such as chest tightness, cough, and dyspnea. In addition, for both treatment groups, similar reductions in the FEV\(_1\) and FVC and equivalent increases in the BAL concentrations of total cells, neutrophils, TNF-\(\alpha\), and IL-8 were demonstrated. Furthermore, the systemic inflammatory response, as measured by elevations in peripheral blood leukocyte and neutrophil counts, was not significantly different. These findings indicate that PTX does not appear to show a beneficial effect in altering the inflammatory events associated with acute grain dust inhalation.

These findings were not expected, given the previous studies demonstrating the anti-inflammatory properties of PTX. In animal models, PTX has been shown to inhibit endotoxin-induced release of TNF-\(\alpha\).\(^{24}\) as well as to prevent TNF-\(\alpha\)-induced acute lung injury.\(^{25}\) PTX inhibits the recruitment of neutrophils by decreasing neutrophil adherence\(^{14}\) and attenuates endotoxin-induced acute lung injury when given before\(^{15}\) or early after\(^{26}\) endotoxin injection. In human studies, volunteers given PTX followed by IV endotoxin developed significantly decreased serum levels of TNF-\(\alpha\).\(^{16}\) whereas in another study, PTX had no effect on endotoxin-induced serum concentrations of TNF-\(\alpha\), IL-6, and IL-8.\(^{27}\) Peripheral blood mononuclear cells taken from subjects receiving PTX released less TNF-\(\alpha\) but not IL-1, IL-6, and IL-8 in response to stimulation with lipopolysaccharide ex vivo.\(^{28}\)

There are a number of possible explanations for the negative results of this study. One potential reason for the lack of efficacy of PTX is the dose administered and the serum levels of PTX attained. Our study subjects were given a daily dose of 1,200 mg/d, which was about 12 to 17 mg/kg/d. On the day of exposure, the dose prior to exposure was 4 to 6 mg/kg. In comparison, animal studies demonstrating the beneficial effects of PTX have used doses ranging from 20 to 100 mg/kg bolus given parenterally as a single dose prior to exposure either with or without an additional continuous infusion.\(^{14,15,24}\) It is possible that a much greater daily dose than what was administered in this study was necessary or a larger single preexposure dose may be needed to produce a beneficial effect. Furthermore, in our study, the serum levels of drug were limited by the maximum achievable blood level with oral dosing. Variability in serum levels of PTX were observed in our study subjects, which is likely a reflection in differences in rate of drug absorption and/or metabolism. However, the serum PTX levels achieved in our study subjects are consistent with levels considered to be therapeutic by other investigators.\(^{21,22}\) The possibility exists that higher serum levels of PTX may have reduced the inflammatory response to inhaled CDE.

A second possibility for the lack of efficacy of PTX in altering the inflammatory response to grain dust is that despite adequate serum levels, delivery of drug to the alveolar space was not significant enough to alter alveolar macrophage function. The alveolar macrophage plays a key role in the inflammatory response to grain dust inhalation, as it may be the principal cell responsible for the production of TNF-\(\alpha\), IL-1\(\beta\), and IL-6 following exposure to grain dust (unpublished observations). Without adequate suppression of cytokine release from these cells, it is unlikely that the inflammatory events will be altered. Since PTX is not significantly protein bound and

![Figure 4. Percent baseline peripheral blood total leukocyte and neutrophil concentrations (±SEM) are depicted for subjects pretreated with PTX (hatched bars) or placebo (solid bars) following exposure to CDE.](http://journal.publications.chestnet.org/pdflatex.ashx?url=/data/journals/chest/21747/ on 04/29/2017)
distributes into extracellular water, however, one would expect that it would freely distribute into the alveoli.

While it has been demonstrated previously that the inflammatory events due to grain dust inhalation appear to be mediated primarily by endotoxin, the lack of efficacy of PTX in inhibiting the acute physiologic and inflammatory events following grain dust inhalation could be secondary to other components found in grain dust that may also contribute to the development of airway inflammation and airflow obstruction. Components such as tannins, mycotoxins, lectins and lymphocyte mitogens, and β-glucans may independently promote airway inflammation. Furthermore, grain dust has been shown to activate complement and has the ability to attract neutrophils in vitro. These agents other than endotoxin may cause inflammation through mechanisms that are not inhibited by PTX.

In conclusion, despite previous evidence of its anti-inflammatory properties, PTX, in the doses administered in this study, was not beneficial in reducing the physiologic and inflammatory events in our model of acute grain dust inhalation. Despite these negative findings, further investigation of other agents capable of reducing the inflammatory response to grain dust is warranted in order to prevent the chronic respiratory symptoms and progressive irreversible declines in measures of airflow associated with long-term exposure.

ACKNOWLEDGMENTS: We would like to thank Margaret Allaman of the CRC Core Lab for technical expertise in performing the pentoxifylline assay.

REFERENCES
28 Neuner P, Klosner G, Schauer E, et al. Pentoxifylline in vivo downregulates the release of IL-1 beta, IL-6, IL-8 and tumor necrosis factor-alpha by human peripheral blood mononuclear cells. Immunology 1994; 83:262-67
33 Olenchock SA, Mull JC, Major P. Extracts of airborne grain dusts activate alternative and classical complement pathways. Ann Allergy 1980; 44:23-8