ACUTE RESPONSE OF RATS TO INTRATRACHEAL INSTILLATION OF AIRBORNE DUST COLLECTED AT A NYLON FLOCKING PLANT

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Abstract

Recently several cases of lymphocytic bronchiolitis have been identified in workers at a nylon flocking plant. The objective of this investigation was to characterize the acute pulmonary reaction to exposure to airborne thoracic/respirable dust collected from this factory and to identify a component of the dust which may cause interstitial lung disease. Rats were intratracheally instilled with 2.5 mg of dust and pulmonary reactions monitored 1 day post-exposure. The acute reactions included: increased breathing rate, PMN infiltration, activation of macrophages, and an elevation of the albumin levels of the lavage fluid. These acute reactions dissipated at 29 days post-exposure. They were not associated with endotoxin or other water extractable components but were related to nylon shreds which were small enough to reach the alveolar region of the lung. The data indicate that nylon shreds can be generated during cutting of large nylon fibers and that this material can enter the lungs and result in inflammation and possible disease.

Introduction

Nylon flocking is a process in which short nylon fibers are adhered to surfaces, such as upholstery or cloth, to provide a velvet-like finish. Procedures common to this process include: cutting long nylon strands (tow), separating cut material into individual short nylon fibers, treating cut fibers with surface agents and dyes, and glueing flock to the desired material.

Recently, several cases of chronic interstitial lung disease have been identified among workers at a nylon flocking plant (Kern et al., 1997; 1998). Although individual cases varied in severity and responsiveness to anti-inflammatory treatment and/or removal from work, some features were common enough to allow a syndrome to be characterized (Castellan, 1999). The affected workers reported shortness of breath and dyspnea. Pulmonary function tests often indicated restrictive disease with decreased diffusion capacity and decreased total lung capacity. Chest x-rays were consistent with diffuse interstitial infiltrates and biopsy samples revealed inflammation of the bronchiolar walls with lymphoid hyperplasia. Thus the syndrome was designated as lymphocytic bronchiolitis.

At the time that these cases were associated with a common occupational setting, the etiologic agent was unknown. Therefore, the plant requested that the National Institute for Occupational Safety and Health (NIOSH) conduct a medical and industrial hygiene survey. Air sampling revealed that organic fume and endotoxin levels were unremarkable. However, total and respirable dust levels were high (NIOSH, 1998; Burkhart et al., 1999; Jones et al., 1999).

To investigate the hypothesis that airborne dust may be responsible for this occupational lung disease, thoracic/respirable dust (airborne dust) was collected at the plant and examined in a rat model for its inflammatory potential. In addition, a water extract of this dust (soluble fraction), the washed airborne dust, and nylon dust prepared in the laboratory by cutting nylon strands (tow) in a rotary knife mill were evaluated.

Methods

Test Materials

Airborne dust was collected at the plant using a vertical elutriator which collected particles of thoracic/respirable size. Samples were collected onto polyvinylchloride filters at a flow rate of 7.4 liters/min. After collection, samples were scraped from the filter into vials for further testing. Microscopic analysis indicated the presence of nylon shreds in this airborne dust (Burkhart et al., 1999; Jones et al., 1999).

Washed airborne dust and the soluble fraction were prepared by suspending 40 mg of airborne dust in 5 ml of sterile, endotoxin-free water (BioWhittaker LAL Reagent Water, Walkersville, MD). After rocking for 1 hour, the suspension was centrifuged at 100xg for 10 minutes at 4ºC and the soluble fraction and washed airborne dust obtained from the supernate and pellet, respectively.

Nylon dust was obtained by cutting nylon tow (10-15 µm in diameter and several cm long), which had not been treated with dyes or flock finishes, in a Tekmar A 10 knife mill. After milling, the tangled mass of nylon fibers was discarded and the fine dust on the inner surface of the mill retained. Microscopic analysis of this dust revealed similarities to the nylon shreds observed in the airborne samples from the plant (Jones et al., 1999).

Endotoxin Analysis

The endotoxin content of airborne dust collected at the plant was determined from water extracts (soluble fraction) of the airborne dust sample using the Limulus amebocyte

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lysate test (QCL-1000, BioWhittaker, Walkersville, MD) as described by Olenchock et al. (1984).

Animal Exposure

Pathogen-free male Sprague-Dawley rats (200-300 g) were obtained from Hilltop Labs (Scottsdale, PA) and were exposed by intratracheal instillation to phosphate-buffered saline (control), airborne dust, washed airborne dust, nylon dust, or the soluble fraction. Briefly dusts were suspended in endotoxin-free, sterile phosphate-buffered saline at 10 mg/ml (wt/vol). The soluble fraction was made isotonic with 5M NaCl prepared with endotoxin-free sterile water. Rats were anesthetized with sodium methohexital (30-40 mg/kg bw; IP) and were intratracheally instilled using a 20 gauge 4 inch ball-tipped animal feeding needle (Perfectum, New Hyde Park, NY). Exposed or control rats received either 10 mg/kg bw dust or 1 ml/kg bw sterile saline, respectively.

The dose of soluble fraction instilled (1.3 ml/kg bw) was chosen to result in an equivalent endotoxin exposure as with the airborne dust treatment.

Physiological Parameters Evaluated

Respiratory rates of rats breathing 10% CO_2 were determined using a flow plethysmograph as detailed by Frazer et al. (1997). Breathing rates were measured for all treatments prior to and 1 day after exposure, as well as 29 days post-exposure for a set of control and airborne dust-exposed rats.

Differential counts of bronchoalveolar lavage cells, chemiluminescence from alveolar macrophages, albumin levels in the acellular lavage samples, and histology were determined 1 day post-exposure for all treatment groups as well as 29 days post-exposure for a set of control and airborne dust-exposed rats. Briefly, rats were euthanized with sodium pentobarbital (130 mg/kg bw IP) and exanguinated by cutting the renal artery. The trachea was cannulated and the left main bronchus clamped. The right lung was lavaged, while the left lung was removed for histopathology.

Bronchoalveolar lavage of the right lung was performed using Ca^{2+} - Mg^{2+} -free phosphate-buffered saline (pH=7.4). The first lavage of 3 ml was kept separate from the subsequent nine lavages, which used 4 ml aliquots. The lavage samples were centrifuged at 500xg for 10 minutes at 4ºC. The supernate from the first lavage was collected and stored at -30ºC for later analysis of albumin levels. The cell pellets from each rat were resuspended in HEPES buffer (10 mM HEPES, 145 mM NaCl, 5mM KCl, 1mM $CaCl_2$ and 5.5 mM dextrose; pH=7.4), combined and washed twice by alternate resuspension, centrifugation and decanting. The washed cells were then suspended in 0.5 ml of HEPES buffer for analysis.

Cell counts were determined using an electronic cell counter fitted with a cell sizing attachment (Coulter Electronics, Hialeah, FL). Alveolar macrophages (AM) and polymorphonuclear leukocytes (PMN) were quantitated by their characteristic cell volume (Castranova et al., 1990).

For electron microscopic analysis of cell/fiber interaction, lavage cells were plated onto coverslips for 1 hr at 37ºC before fixing in gluteraldehyde and post-fixing in osmium tetroxide. Then cells were dehydrated, mounted onto aluminum stubs and coated with gold/palladium for imaging on a JEOL 6400 scanning electron microscope.

Cellular chemiluminescence was monitored as an indicator of cellular activation using a luminometer (Berthold Autolumat LB 953, Wallace Inc., Gaithersubrg, MD). Briefly, 5 x 10^5 AM were suspended in 0.5 ml HEPES buffer plus 0.04 mg% (w/v) luminol (Sigma Chemical Company, St. Louis, MO) and pre-incubated at 37ºC for 5 minutes. After pre-incubation, cells were treated with buffer or unopsonized zymosan (1 mg) and chemiluminescence monitored at 37º C as total cpm for 5 minutes. Zymosan-stimulated chemiluminescence was calculated as counts with zymosan minus counts without. Since PMN do not respond to unopsonized zymosan, this assay monitored the oxidant production by AM (Castranova et al., 1990).

Albumin levels in the acellular lavage fluid were determined colorimetrically at 628 nm by measuring the binding of albumin to bromcresol green (albumin BCG diagnostic kit, Sigma Chemical Company, St. Louis, MO).

For histopathology, the left lung was inflated with 6 ml of 10% neutral-buffered formalin. Lung tissue was processed, embedded in paraffin, sectioned at 4-5 μm, and stained with hematoxylin eosin and Gamori's trichrome. Slides were examined by a board-certified veterinary pathologist under bright field and polarized light microscopy.

Results

Microscopic analysis of airborne dust collected at the nylon flocking plant revealed the presence of fibrous material. These particles had similarities to the nylon tow under polarized light and exhibited the same melting point. Therefore, they appeared to be nylon shreds generated during the cutting of nylon tow. Indeed respirable fibers of similar appearance were generated when long, thick tow material was cut with a rotary knife mill in the lab.

Airborne dust samples were found to have a relatively low endotoxin content (11.2 EU/mg dust). In our experience, such endotoxin levels would not be sufficient to cause substantial pulmonary inflammation under the intratracheal instillation conditions (112 EU/kg bw) used in the present study.

Intratracheal instillation of airborne dust (10 mg/kg bw) resulted in an increase in breathing rate, pulmonary inflammation, and lung damage at 1 day post-exposure (Table 1). Pulmonary inflammation was noted as an increase in lavagable PMN and zymosan-stimulated chemiluminescence by AM, while pulmonary damage was noted as in increase in acellular lavage fluid albumin levels. After 29 days post-exposure, these pulmonary parameters had returned to the control levels (Table 1). At 1 day post-exposure mild to moderate, multifocal, suppurative pneumonia (PMN dominated), usually centered around bronchioles, was observed. Microscopic analysis of bronchioalveolar lavage material 1 day post-exposure revealed nylon shreds with several attached phagocytic cells. At 29 days post-exposure diffuse inflammation was absent. However, small foci of histiocytic alveolitis (AM dominated) was observed in association with thin birefringent fibers sometimes as long as 15 μm.

In general, rats were also responsive 1 day post-exposure to washed airborne dust and nylon dust, i.e., exhibiting significant increases in breathing rate, PMN, chemiluminescence and acellular albumin (Table 2). However, these responses tended to be somewhat smaller than with airborne dust. The soluble fraction, which contained low amounts of endotoxin plus any water extractable dyes or surface agents, was the least potent treatment, i.e., failing to increase either breathing rate or lavagable PMN (Table 2). Similarly, histological results from washed airborne dust and nylon dust exposed rats showed inflammation, while lungs exposed to soluble fraction were indistinguishable from control.

Discussion

The working hypothesis of nylon plant operators was that worker illness was not related to dust levels because nylon flock was too large (15 μm in thickness) to be respirable and nylon was viewed to be inert. Although the majority (by mass) of total airborne dust in the plant was thick flock fibers, thin fiber shreds were found (Burkhurt et al., 1999). These shreds were more prominent in the thoracic/respirable fraction of plant airborne dust. Optimal features along with melting point determination suggest that these are shreds of nylon. Evidence indicates that these nylon shreds are thin enough to reach the alveolar regions of the lung. Indeed, bronchoalveolar lavage material collected 1 day after instillation of airborne dust contained thin fibers with numerous attached alveolar macrophages attempting to phagocytize them. In addition, histopathology demonstrated birefringent fibers in the alveoli 1 day post-exposure. At least some of the nylon shreds are durable enough to be retained in the alveolar spaces 29 day after instillation.

Airborne dust was highly inflammatory 1 day post-exposure. This inflammation was not due to endotoxin contamination, since (1) the endotoxin content was low, (2) the water extract of this dust was not very inflammatory, and (3) the washed airborne dust was inflammatory. In fact, it appears that the nylon itself is inflammatory, since nylon dust generated in the lab from tow samples which did not contain flock surface agents and dyes induced inflammation.

The degree of pulmonary inflammation caused by instillation of airborne dust was compared to that for other materials tested in our laboratory (Blackford et al., 1997). Airborne dust was 3 times more potent than silica in causing PMN infiltration and nearly 20 times more potent than coal mine dust, titanium dioxide, or carbonyl iron. Washed airborne dust and nylon dust also were more potent than these mineral dusts. Therefore, nylon can not be considered to be inert.

In summary, nylon shreds of respirable size are generated in the nylon cutting process in flocking operations. These shreds can reach the alveoli and are very inflammatory. Some nylon shreds are retained in the lung one month after exposure and are associated with foci of inflammation. As a result of these findings, NIOSH has suggested to the flocking plant operators that it would be prudent to control dust levels (NIOSH, 1998).

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Table 1. Pulmonary Responses to Intratracheal Instillation of Airborne Dust

Treatment	**Breathing Rate**	**PMN**	**Chemilumine-scence**	**Albumin**
1 day post				
control	143 ± 4.5	0.56 ± 0.06	2.5 ± 0.4	0.18 ± 0.01
exposed	177 ± 9.2*	8.89 ± 1.48*	260.0 ± 24.4 *	0.35 ± 0.04 *
29 days post				
control	190 ± 9.4	0.66 ± 0.04	2.0 ± 0.4	0.20 ± 0.02
exposed	194 ± 8.5	0.80 ± 0.05	1.2 ± 0.2	0.20 ± 0.01

Values are means ± standard errors of n $\geq$ 5 separate rats. Breathing rate (breaths/min); PMN (10^6 cells/rat); chemiluminescence (cpm/5 x 10^5 AM/15 min); albumin (mg/ml).
* Indicates a significant elevation from control ($p < 0.05$).

Table 2. Pulmonary Responses to Intratracheal Instillation of Airborne Dust, Washed Airborne Dust, Soluble Fraction and Nylon Dust 1 Day Post-exposure

Treatment	**Breathing Rate**	**PMN**	**Chemilumine-scence**	**Albumin**
Control	140.9 ± 3.0	0.75 ± 0.11	2.3 ± 0.5	0.20 ± 0.01
Airborne Dust	177.3 ± 9.2*	8.89 ± 1.48*	260.0 ± 24.4*	0.35 ± 0.04*
Washed Dust	173.0 ± 7.5*	3.94 ± 0.55*	66.8 ± 12.3*	0.34 ± 0.02*
Soluble Fraction	149.6 ± 10.6	1.43 ± 0.19	7.7 ± 3.1*	0.33 ± 0.05*
Nylon Dust	167.0 ± 6.2*	5.59 ± 1.32*	21.0 ± 6.2*	0.37 ± 0.03*

Values are means ± standard errors of n $\geq$ 5 separate rats. Breathing rate (breaths/min); PMN (10^6 cells/rat); chemiluminescence (cpm/5 x 10^5 AM/15 min); albumin (mg/ml).
* Indicates a significant elevation from control ($p < 0.05$).