PULMONARY INFLAMMATION OF GUINEA PIGS IN RESPONSE TO INHALATION OF COTTON DUST: EFFECT OF EXTENDED EXPOSURE DAY

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Abstract

Exposure to cotton dust can result in acute pulmonary inflammation marked by elevation of neutrophils in nasal and bronchoalveolar lavage fluids and by activation of alveolar macrophage. Such exposure may also result in chest tightness and airway constriction. For this reason, OSHA has established a permissible exposure limit (PEL) of 0.2 mg/m^3 for yarn manufacturing and cotton washing. This PEL is for an 8 hr workday. Questions concerning an appropriate standard for extended workdays (longer than 8 hrs) have been raised. As an initial attempt to address this issue, we employed a guinea pig model exposed to 1.5 mg/m^3 of cotton dust for 4-16 hrs and monitored pulmonary inflammation 0 and 18 hrs post-exposure. Total bronchoalveolar lavage cells, leukocytes, and red blood cells increased linearily with total exposure (concentration x exposure time) both immediately and 18 hours post-exposure. Macrophage activity, measured as zymosan-stimulated chemiluminescence, exhibited a similar linear relationship to total exposure. These animal results suggest that the concentration-time product may be an appropriate exposure metric when evaluating the risk of acute inflammation in response to cotton dust.

Introduction

The byssinotic syndrome in cotton mill workers was first characterized by Schilling et al. (1955) as chest tightness, which is most pronounced on the first day of the workweek (Monday accentuation). In addition to the classic symptom of chest tightness, inhalation of cotton dust results in a number of other pulmonary responses (Rylander et al., 1987). Cotton mill workers may experience increased airway resistance which results in a cross-shift decline in forced expiratory volume in 1 second (FEV_1) (McKerrow et al., 1958). As with chest tightness, this cross-shift decline in FEV_1 exhibits Monday accentuation (Merchant et al., 1972). Cotton dust exposure is also associated with an inflammatory response characterized by elevations of neutrophils in the blood (Bouhuys et al., 1961), nasal fluids (Merchant et al, 1975) and bronchoalveolar lavage fluid (Baur et al., 1993).

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NIOSH has published a recommended exposure limit (REL) of <0.2 mg/m^3 for lint-free cotton dust (NIOSH, 1994). OSHA's current permissible exposure limit (PEL) is 0.2 mg/m^3 for yarn manufacturing and cotton washing; 0.5 mg/m^3 for textile mill house operations or for yarn manufacturing with washed cotton; 0.75 mg/m^3 for textile slashing and weaving; and 1 mg/m^3 for cotton waste processing and garnetting. All these PEL's are for an 8-hour workday (OSHA, 1984).

Recently, the issue of longer than 8-hour workdays has been raised in regard to the cotton dust PEL. For example, an 8-hour exposure to 0.2 mg/m^3 of cotton dust would result in a total daily exposure of 1.6 mg.hr/m^3, i.e., the concentration-time product. If a worker were exposed at 0.18 mg/m^3 for 10 hours (1.8 mg.hr/m^3 daily exposure), would the individual be at risk of acute respiratory inflammation? To date, no information is available to address this issue. The simplest approach would be that pulmonary reactions are directly related to the time-concentration product. However, it is known that pulmonary responses to cotton dust are not as great on subsequent exposure days as they are after the initial exposure (Monday accentuation). This indicates that negative feedback mechanisms are in place to limit the reaction to cotton dust. The question is: would these limiting factors occur within an extended workday? If so, the exposure-response relationship would deviate from linearity and a 10-hour exposure to 0.18 mg/m^3 might not be more inflammatory than an 8 hour exposure to 0.2 mg/m^3.

Extensive effort from a number of laboratories has resulted in the development of a guinea pig animal model for cotton dust exposure (Castranova et al., 1996). This model exhibits concentration-dependent increases in breathing rate (Ellakkani et al., 1985), airway constriction (Smith et al., 1993), neutrophil infiltration (Robinson et al., 1988), and alveolar macrophage activation (Robinson et al., 1988). As in workers, these responses exhibit Monday accentuation (Castranova et al., 1990) and a dependence on the endotoxin content of the cotton dust (Castellan et al., 1987; Robinson et al., 1995). The objective of the present investigation was to employ this animal model to investigate whether a linear relationship exists between the concentration-time product for cotton dust inhalation and pulmonary response in guinea pigs. This was accomplished by exposing guinea pigs to an average of 1.5 mg/m^3 cotton dust for 4-16 hours and monitoring pulmonary reactions immediately, as well as, 18 hrs post-exposure.

Materials & Methods

Experimental Design

The objective of this study was to determine the role that exposure time plays in the inflammatory response observed following cotton dust exposure in guinea pigs. Guinea pigs were housed in the exposure chambers for the duration of the exposure, during which time, they were exposed to a

computer controlled aerosol of approximately 1.5 mg/m^3 cotton dust. At set intervals throughout the exposure, ten animals were removed from the chamber. The breathing rates of all ten animals were measured in 10% CO_2 in air. Half of each group, i.e., five animals, were sacrificed immediately (0 hours post exposure) for bronchoalveolar lavage and cellular measurements. The remaining five guinea pigs of each group were sacrificed at 18 hours post-exposure.

Forty animals were housed in the exposure chamber at the beginning of each of 3 exposure runs. The durations of exposure for the different groups was 4, 5, 6, 7, 8, 10, 12, 14 and 16 hours. Three separate runs were made in order to expose all of the groups. The mean concentration of the exposures was 1.47 ± 0.10 mg/m^3 (mean ± standard error), measured gravimetrically with Gelman VM-1 filters.

Cotton Dust and Animals

Bulk cotton dust (DB 5/89) was obtained from the National Cotton Council. Male Dunkin Hartley guinea pigs were purchased from Sprague Dawley (Indianapolis, IN). The animals were specific pathogen free and acclimated for one week at the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) approved NIOSH animal facility prior to the cotton dust exposures. The body weight of the guinea pigs was 305.1 ± 2.6 grams (mean ± SE) at the time of exposure.

Cotton Dust Generator and Exposure System

The system used to expose guinea pigs to cotton dust is shown in fig. 1. Two modified Pitt-3 acoustical generators similar in design to the one described by Weyel et al. (1984) were used in parallel to generate a cotton dust aerosol. Details of the generator design have been described previously (Frazer et al., 1991). Filtered air from a compressed air source passed through each generator at a constant rate of 15 l/min. During an exposure period, animals were housed in individual cages within a large stainless steel chamber (Hazlton 2000) that was comparable in design to the one described by Moss et al. (1982). The mass concentration of cotton dust inside the chamber was held constant by adjusting the amount of diluent air added to the elutriated aerosol produced by the generation system. The diluent air was conditioned with an air handling system that continuously monitored and recorded the temperature and humidity of the room air being pulled through a pre-filter and HEPA filtering system. Four minirams (Miniature Real Time Aerosol Monitor, MIE, model PDM-3) were spaced at equal intervals near the top of the chamber. Analog outputs of the minirams were sampled, digitized (DATAQ Model DI220) and averaged with a computer to estimate the average mass concentration of the cotton dust aerosol at 5 second intervals. The estimated mass concentration was used by a computer controlled geared stepper motor to regulate the amount of diluent air that was mixed with the aerosol. Exhaust aerosols and gases were removed from the exposure chamber and cleaned by HEPA filtration. In addition, the mass concentration of the respirable dust in the exposure chamber was measured gravimetrically by pulling aerosol samples through a VM-1 filter at a flow rate of 2 liter/min. Filters were changed at one-half hour intervals.

Bronchoalveolar Lavage Cell Assays

Lung cells were obtained by bronchoalveolar lavage after the method of Castranova et al (1990). In short, guinea pigs were anesthetized with sodium pentobarbital and exsanguinated. Calcium and magnesium-free phosphate buffered saline (145 mM NaCl, 5mM KCl, 1.9 mM NaH_2PO_4, 9.35 mM Na_2HPO_4, and 5.5 mM dextrose; pH=7.4) was instilled into the cannulated trachea of the guinea pigs. This solution was then aspirated from the lung and collected. A 6 ml aliquot of buffer was used for the initial instillation and 8 ml thereafter, until 80 ml of lavage fluid were collected per guinea pig. The harvested cells were concentrated by centrifugation at 1500 x G, washed in HEPES buffered medium, (145 mM NaCI, 5 mM KCl, 10 mM Na HEPES, 10 mM $CaCl_2$, and 5.5 mM dextrose; pH=7.4) and reconcentrated. The final cell pellet was resuspended in HEPES buffered medium.

Total and differential cell counts were determined with an electronic cell counter equipped with a cell sizing attachment. Red blood cells, alveolar macrophage and leukocytes (lymphocytes and polymorphonuclear leukocytes) were identified by their characteristic cell volumes (Castranova et al, 1990).

Macrophage activation was determined by the measurement of luminol-enhanced chemiluminescence. Alveolar macrophages (5 X 10^5/0.5 ml of HEPES buffered medium) were preincubated at 37°C for 15 min. After preincubation, luminol was added (0.08 µg/ml) along with an aliquot of medium without or with unopsonized zymosan (2 mg/ml) for resting and zymosan samples, respectively. Chemiluminescence, an indicator of production of reactive species, was then measured at 37°C for 10 min using a Berthold luminometer. Chemiluminescence was determined as cpm/5 X 10^5 AM/10 min. Zymosan-stimulated chemiluminescence was determined by the subtraction of the resting rate (without zymosan) from the rate with zymosan. Evidence indicates that unopsonized zymosan stimulates alveolar macrophages but not polymorphonuclear leukocytes (Castranova et al, 1990); therefore, zymosan-stimulated chemiluminescence is an indicator of alveolar macrophage activity.

Breathing Rate Measurements

Guinea pigs were placed in a flow plethysmograph that has been previously described in detail (Frazer et al., 1997). The plethysmograph chamber was constructed of an acrylic tube enclosed at each end. One end of the chamber had a circular port that contained three 400 mesh stainless steel screens. Pressure variations across the screen generated by flow into and out of the chamber were measured with a

pressure transducer (Setra, model# 239). A digital oscilloscope (Tektronix, model# 222) was used to record flow signals that were transferred to a digital computer for analysis. After the guinea pigs were equilibrated with 10% CO_2 in air for 3 minutes, their average breathing rates were calculated based on the time between zero crossings of the flow signal at the beginning and end of an inhalation-exhalation cycle.

Results

Breathing Frequency

Although at 0 hrs post-exposure there was a tendency for the breathing rate of guinea pigs in 10% CO_2 to increase as exposure duration increased, these breathing frequencies were not significantly different from the control (unexposed) rate (Fig. 2). At 18 hrs post-exposure, even these slight elevations were absent.

Bronchoalveolar Cell Yield

There was a significant increase in the total number of cells harvested by bronchoalveolar lavage from exposed guinea pigs compared to controls (Fig.3). This increase was linearly related to the total exposure, i.e., the concentration-time product, over the exposure duration range of 4-16 hrs. This linear elevation in total lavaged cells was noted both at 0 and 18 hrs post-exposure and was due to significant, total exposure-dependent increases in both leukocytes (Fig. 4) and red blood cell counts (Fig. 5). Alveolar macrophage yield did not increase as a result of this cotton dust exposure (Fig. 6).

Macrophage Activation

Zymosan-stimulated chemiluminescence was significantly increased both 0 and 18 hrs after cotton dust exposure. This increase was linearly related to the concentration-time product of exposure (Fig. 7). Chemiluminescence, i.e., the production of reactive species by alveolar macrophages, tended to be higher 18 hrs post-exposure than 0 hrs post-exposure.

Discussion

To date, little or no information is available concerning the health effects of a longer than 8 hr workday in cotton textile mills. Is it true that a 12 hr exposure to a given level of cotton dust is 50% worse than an 8 hr exposure? Although a linear relationship between pulmonary response and total exposure (concentration x exposure duration) is the simplest hypothesis, it need not be correct. Indeed, it is well documented in workers and in animal models that the magnitude of pulmonary responses to cotton dust declines on successive exposure days (Schilling et al., 1955; Castranova et al., 1990). These results indicate that negative feedback or down regulation of responsiveness to cotton dust can occur. The question is, will such down regulation occur within a given workday? If it does, a 12 hr exposure to cotton dust may result in pulmonary responses which would be less than 150% of those for an 8 hr exposure.

Merchant et al. (1975) have reported responses of human volunteers to inhalation of cotton dust for the exposure times of 2, 4, and 6 hrs. In general, as exposure time to a given level of cotton dust increased, blood white cell counts increased and FEV_1 values decreased. Although these data exhibit a response-exposure duration relationship, they do not address the issue of down regulation occurring within an extended workday. In the present investigation, we employed the guinea pig animal model exposed to 1.5 mg/m^3 of cotton dust for 4-16 hrs to evaluate this question. Our results indicate that total cells (Fig.3), leukocytes (Fig. 4) and red blood cells (Fig. 5) harvested by bronchoalveolar lavage either 0 or 18 hrs post exposure increased in a linear fashion with total exposure determined as the product of concentration and exposure duration. A similar linear relationship was demonstrated between alveolar macrophage activity and total exposure (Fig.7). Therefore, over the course of a 16 hr exposure to cotton dust, no evidence of down regulation of the inflammatory response was discernible in this guinea pig model.

In previous studies, an increase in breathing rate has been viewed as a hallmark of the guinea pig animal model (Ellakkani et al., 1985; Castranova et al., 1996). However, breathing rate was not significantly elevated in response to cotton dust inhalation in the present study (Fig. 2). This lack of response is due to the relatively low exposure concentration used (1.5 mg/m^3) in our study. Indeed, Robinson et al. (1988) have reported the concentration dependence of pulmonary responses to cotton dust and have demonstrated that the breathing rate response was not discernible at doses below 2 mg/m^3 while cellular changes (leukocyte infiltration and macrophage activation) were more sensitive responses.

The present study found no increase in lavageable alveolar macrophages in response to cotton dust inhalation (Fig. 6). This agrees with the previous studies where decreases in macrophage yield have been reported (Castranova et al., 1987; Ryan and Karol, 1991). This decreased yield may reflect an increase in the adherence of alveolar macrophage within cotton dust-exposed lungs rather than an actual decrease in pulmonary macrophage number. Hirano (1996) has shown that LPS increases the adherence of alveolar macrophages to alveolar epithelial cells in co-culture. Furthermore, activation of alveolar macrophage following inhalation of cotton dust has been demonstrated by enhanced TNF release, superoxide production, and chemiluminescence (Ryan and Karol, 1991; Castranova et al., 1987; Fig. 4). In fact, histological evaluation of lungs from dust-exposed guinea pigs demonstrates substantial recruitment of alveolar macrophage into the alveolar air spaces (Castranova et al., 1987).

In conclusion, data presented in this investigation using the guinea pig animal model suggest that at a relatively low exposure concentration (1.5 mg/m^3) pulmonary inflammation increases with exposure duration. It should be noted the results in animals may not always be directly relevant to humans. However, the data are consistent with the simple linear extrapolation of the concentration-time product as a reasonable estimation of total exposure over extended workdays. The data do not support the hypothesis that down regulation of responsiveness occurs within an extended workday.

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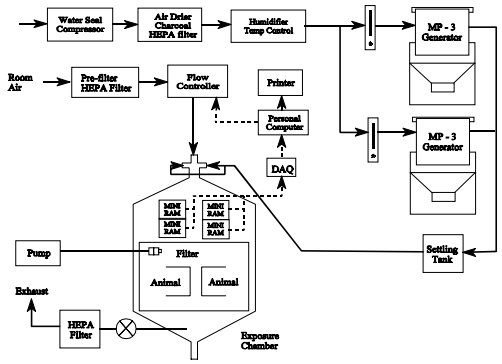

Figure 1. Schematic of the cotton dust generator and exposure system.

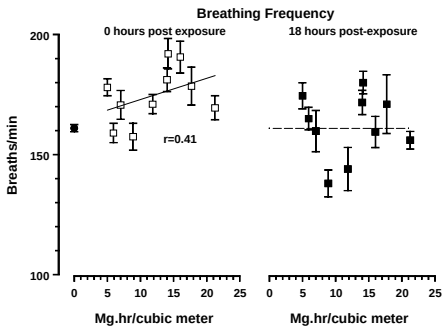

Figure 2. Breathing frequencies of guinea pigs in 10% CO_2 immediately following and 18 hours following inhalation of cotton dust. Exposure is given as the time-concentration product. Data points are means +/- standard errors. The control group is noted as ● and by the dashed line.

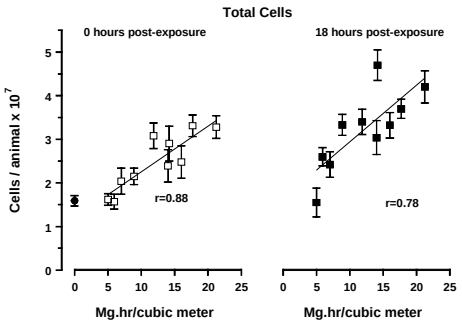

Figure 3. Total cells harvested by bronchoalveolar lavage immediately following and 18 hours following inhalation of cotton dust. Exposure is given as the concentraion-time product. Data points are means +/- standard errors, and control values are represented by ●.

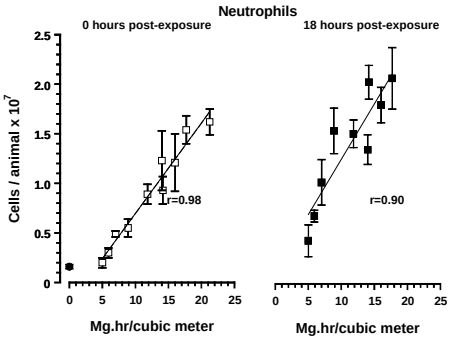

Figure 4. Leukocytes (lymphocytes and polymorphonuclear leukocytes) harvested by bronchoalveolar lavage of guinea pigs immediately following and 18 hours following inhalation of cotton dust. Exposure is expressed as the concentration-time product. Data points are means +/- standard errors, and the control group is represented as ●.

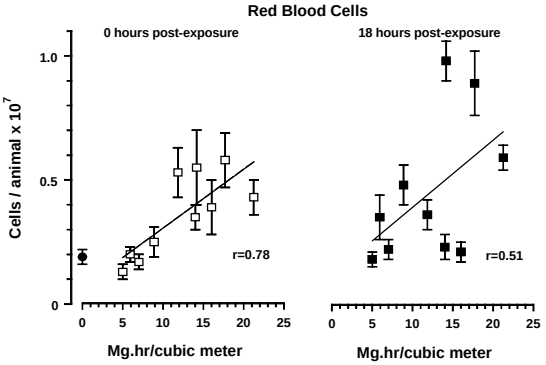

Figure 5. Red blood cells harvested by bronchoalveolar lavage of guinea pigs immediately following and 18 hours following inhalation of cotton dust. Exposure is expressed as the concentraion-time product. Data points are means +/- standard errors, and the control values are represented by ●.

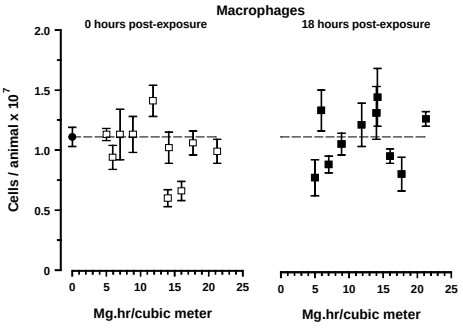

Figure 6. Macrophages harvested by bronchoalveolar lavage of guinea pigs immediately following and 18 hours following cotton dust inhalation. Exposure is expressed as the concentraion-time product. Data points are means +/- standard errors, and the contol values are represented by ●.

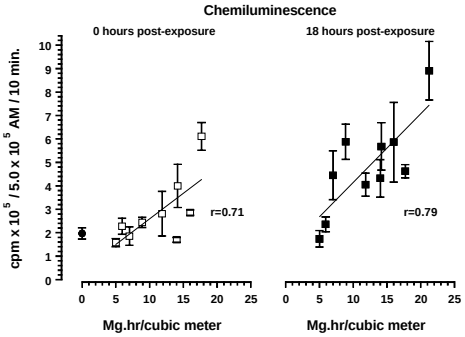

Figure 7. Zymosan-stimulated chemiluminscence from alveolar macrophages harvested by bronchoalveolar lavage of guinea pigs immediately following and 18 hours following cotton dust inhalation. The exposure is expressed as the concentration-time product. Data points are means +/- standard errors, and the control values are represented by ●.