EFFECT OF INHALED COTTON DUST AQUEOUS EXTRACT ON CELLULAR STRUCTURE OF THE AIRWAY EPITHELIUM OF THE GUINEA PIG

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Abstract

The effect of daily inhalation of aqueous extracts of cotton dusts were examined in the respiratory ciliated epithelium in guinea pig by electron microscopy. Following five days exposure, there was a significant loss of cilia, cellular disruption, detachment from basal membrane and swollen mitochondria compared to control. Extrusion of cellular components were also observed. This indicates that exposure of the guinea pig to cotton dust extract causes marked cellular damage to the airway epithelium. This toxic reaction may have an important role in the development of byssinosis.

Introduction

The inhalation of cotton dust can lead to the development of an occupational respiratory disease byssinosis. The symptoms of this condition are commonly chest tightness, dyspnea, coughing, wheezing and reduction in the forced expiratory volume (Rylander 1987; Jiang et.al., 1995). This disease is prevalent in cotton mills workers and the symptoms increase with increasing exposure time particularly in workers with reactivity to cotton dust extract (Li et. Al., 1995). In the study of lung diseases, the airway epithelium has gained importance in recent years. Increased epithelial permeability to ^{99m}-Technetium-diethylenetriamine penta-acetate (Tc-DTPA) has been demonstrated in guinea-pigs chronically exposed to cotton dust extract (Bates et.al., 1990). Epithelial permeability is of interest in lung diseases because it is a sensitive indicator of epithelial cell damage which may be the primary stage before further changes and symptoms appear. Other studies have provided evidence using in-vitro techniques that cotton dust causes damaging effects to lung epithelium (Ayeres et.al., 1986).

It was proposed that the increase in epithelial permeability arose from a breakdown in the normally tight, occluding junctions between pulmonary epithelial cells, allowing easier passage of molecules from airway to bloodstream. The damaging effects of cotton dust on pulmonary epithelium have also been suggested from the results of other studies which have demonstrated lysis and detachment of pneumocytes from human and rat trachea exposed to dust extracts (Ayeres et.al., 1986).

Supportive evidence has been obtained from observations with canine tracheal epithelium that cotton bract extract interferes with the electrophysiological properties of the paracellular pathways impairing its barrier function (Cloutier et.al., 1984).

The aim of the present study was to examine the effects of aqueous extracts of cotton dusts on the respiratory ciliated epithelium in guinea-pig using electron microscopy.

Methods

Preparation of Dust Extracts

The cotton dust was from a batch of airborne material supplied by Professor P.J. Nicholls, Welsh School of Pharmacy, UWCC, Cardiff, U.K. and previously used in other studies (Bates et.al., 1992).

Dust extracts were prepared by grinding 6 gm of dust with 50 ml of double distilled and deionised water in a mortar for 15 min. The mixture was allowed to macerate overnight at 4°C, and the liquid removed by straining through cotton gauze, and centrifuged at 3000 g for 15 min. The extracts were passed through a No. 1 Whatman filter paper and the resulting liquid extracts were then freeze dried until a crumbly freeze dried powder formed. The freeze dried powder was stored at -10°C and reconstituted immediately prior to use by the addition of sterile vehicle.

Animal Exposure

The extraction yields were approximately 10% of freeze-dried material from the original dusts. Doses given to the guinea-pigs were 60 mg freeze-dried aqueous extracts in 5 ml sterile saline, equivalent to 6 g of original dust in 50 ml. An aerosol of this concentration given for 6 minutes to a guinea-pig has been adopted as an equivalent to the dust exposure suffered by a cotton worker in one day in a dusty cotton mill where the airborne dust concentration is 5 mg/m^3. This exposure period was used in previous studies and shown to induce bronchoconstriction in guinea-pigs when assessed by a plethysmograplic technique (Ayeres et.al., 1986; Bates et.al.,1990).

Male guinea-pigs (Dunkin-Hartley strain, weight range 400-600 g) inhaled an aerosol of the aqueous extracts of cotton dust (AECD) or saline vehicle for a period of 6 minutes daily for five consecutive days. The aerosol was generated by a nebuliser (Kit Rekord, ARTSANA, Italy) operated with medical quality air at 20 p.s.i. (138 kPa). The aerosol output was calculated as 0.2 ml.min and a laser light diffraction technique described the aerosol product as having 75% of droplets below 5 microns in diameter with a mass median aerodynamic diameter (MMAD) of 3.5

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microns. Restrained guinea-pigs inhaled the aerosol from a glass container via a mask permitting nose-only exposure. On the fifth day at 1h post-exposure, animals were given a lethal peritoneal injection of pentobarbitone 60 mg/ml.

Transmission Electron Microscopy

Sections of trachea and major bronchi were cut into 0.5 cm cubes and fixed with 2% gluteraldehyde in 0.1 M phosphate buffer pH 7.4 for 5 min. They were then rinsed in the same buffer for 45 minutes to remove free aldehyde groups and post-fixed in 1% v/v osmium tetroxide (in phosphate buffer) for 90 minutes at 4°C. The tissues were mashed in 0.05 M maleic acid (pH 7.0) for 1 h to remove excess osmium and stained in 1% v/v aqueous solution of uranyl acetate (in the dark) for 30 minutes.

The tissues were then dehydrated in a graded series of ethanol solutions (30, 50, 70, 80 and 95% v/v, 10 minutes each at 4°C) and rinsed in absolute alcohol and Spurr's resin for 16 h. They were then transferred into flat moulds, containing pure resin. The resin with the tissues were cured at 70°C for 24 h.

The resulting polymerised block was cut into 90 nm thick sections using an ultracut microtome and thin sections collected on carbon-coated copper grids. The sections were examined on a Phillips EM 400T electron microscopy with magnifications of x6,000.

Results

Following 5 days saline exposure tracheal cells are seen in Figure 1. The epithelial cells appear normal with lightly stained cytoplasm. There are few electron dense granules present and the cilia appear normal. Figs. 2 and 3 show tracheal cells following 5 days AECD exposure. The organelles of the secretory epithelial cells appeared swollen. The cilia on these cells were more electron dense than normal. Cellular protrusions could be observed on the secretory cells and some of these cells had ruptured, thus releasing their cytoplasmic material into the lumen. Some erythrocytes were also noted in the lumen. Further, granulocytes with electron dense granules could be seen moving between two ciliated cells towards the luminal surface. There appeared to be a thick layer of mucus covering the epithelium and the cilia appeared matted.

Figure 4 shows bronchial tissue following 5 days saline exposure. The epithelial cells appear normal with lightly stained cytoplasm. There are few electron dense granules present and the cilia also appear normal. Bronchial tissue exposed for 5 days to AECD could be seen in Figs. 5 and 6. There was damage to the luminal surface of some ciliated cells. There were dark granules accumulated in the vicinity of the damaged area. The cilia in the damaged area appeared thicker and more electron dense than normal. Vacuoles and cellular protrusions were also observed. Further, there were numerous granulocytes seen in the region of the damaged epithelium, with granules and cellular debris being observed in the lumen, attached to the cilia.

Discussion

Daily inhalation of aqueous extracts of cotton dusts for five consecutive days caused changes and damage in the tracheal and bronchial epithelial surfaces in guinea pigs. The extent of damage in the epithelium observed correlates closely with other studies (Davey and Nicholls 1994).

The damage induced by the aqueous extracts of cotton dust on the airway epithelium could be the result of many mechanisms involved. Impairment of ciliary function, increase in epithelial permeability, bacterial endotoxins and release of cellular mediators have all been implicated as possible causes.

Ciliary function is the first line of defense of the respiratory tract against inhaled insults. The cilia beat in a co-ordinated fashion in the periciliary fluid layer beneath the overlying mucus. Their movement is characterized by a stiff downstroke which propels the mucus forward. They are then withdrawn in a curved manner within the periciliary fluid in such a way that the mucus layer is not disturbed. Studies have shown that aqueous extracts of cotton dusts slowed ciliary beat frequency and caused epithelial disruption in a dose-dependent manner (Wilson et.al., 1990). Our results also showed changes in the ciliary appearance on the tracheal and bronchial cells in which the cilia looked thicker and more electron dense. In some damaged epithelium where there was a thick layer of mucus covering the cells, the cilia appeared matted. This could possibly affect ciliary function, and plays a role in the respiratory symptoms of byssinosis.

Bacterial endotoxins have been implicated as potential causative agents for epithelial changes and damages. Their presence in a wide range of occupational environments where adverse pulmonary effects have been shown including cotton mills, and their ability to alter lung function makes them prime candidates for a role in the pathogenesis of organic dust toxicity syndrome, including byssinosis and chronic bronchitis (Gordon and Harkema 1995). Inhaled endotoxin has been shown to cause significant damage to the pulmonary epithelium and endothelium (Davey and Nicholls 1994).

Other possible causes could be the release of cellular cytokines, such as tumor necrosis factor (TNF) or Thromboxane $A_{2,}$ and/or neutrophil chemotactic factor (NCF). Studies with animal model have reproduced the neutrophilic inflammation characteristic of the alveolitis, and have shown the presence of TNF in the bronchoalveolar lavage fluid induced by cotton dust inhalation (Shvedova et. al., 1994). Specks et.al., 1995 have also shown NCF secretion from human and rabbit alveolar macrophages by

cotton dusts. The NCF secreted was not leukotrienes B4 or platelet-activating factor.

In conclusion, inhalation of aqueous extracts of cotton dusts induce inflammation and damage to the airway epithelium, which could be a combination of some of the above mechanisms. This toxic effect may have an important role in the development of byssinosis.

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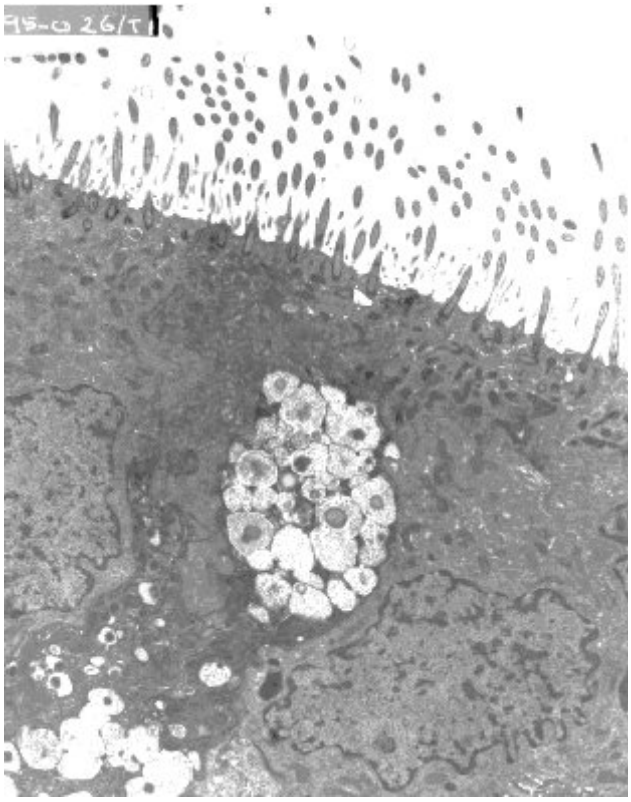
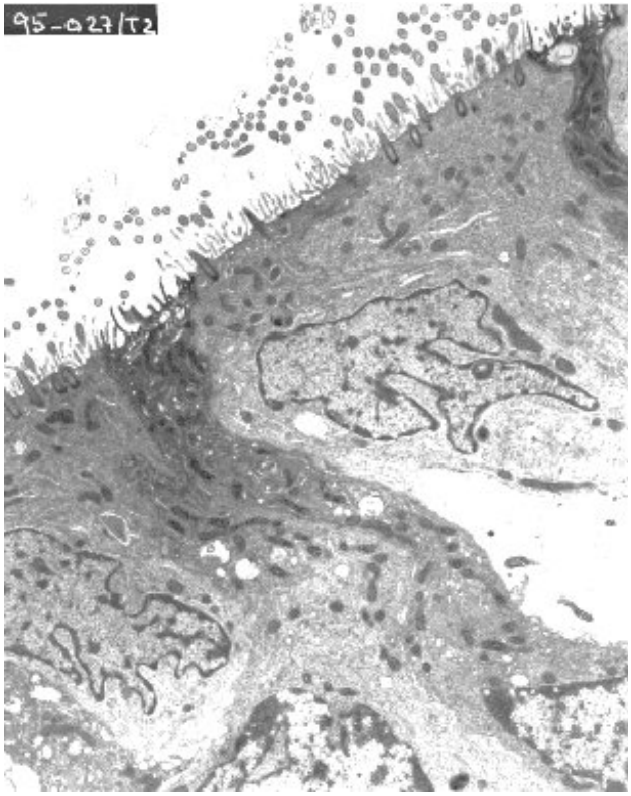
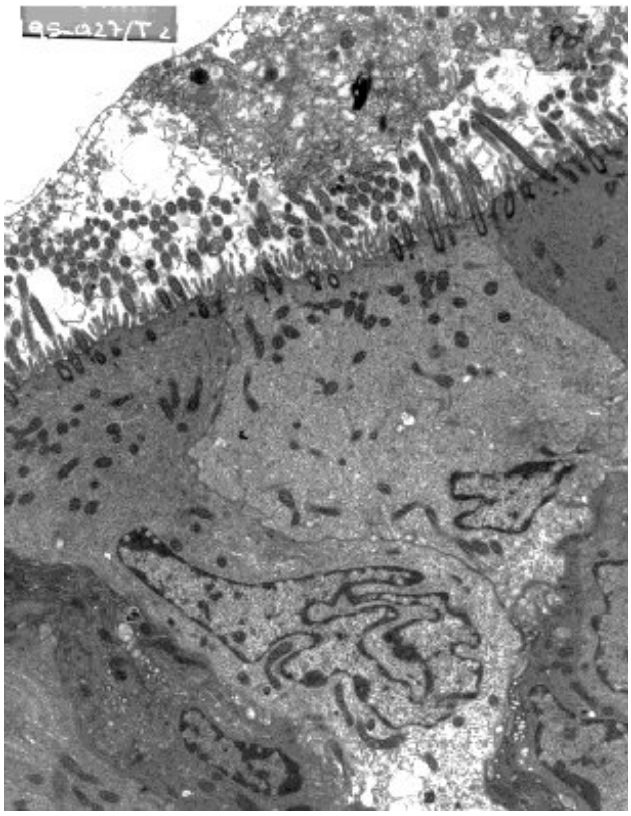


Fig. 1. Trachea following saline exposure for 5 days.
This electron micrograph is typical of that seen for saline exposed guinea pigs. The epithelial cells appear normal with lightly stained cytoplasm. There are few electron dense granules present and the cilia also appear normal. Magnification: 6,000 X.





Figs. 2 & 3. Trachea following aqueous extract of cotton dust exposure for 5 days. There is rupture of an epithelial secretory cell with consequent release of cytoplasmic material into the lumen. Dense granules can be seen. The cilia appear more electron dense than normal. A thick layer of mucus is seen coating the epithelial surface. Magnification: 6,000 X.

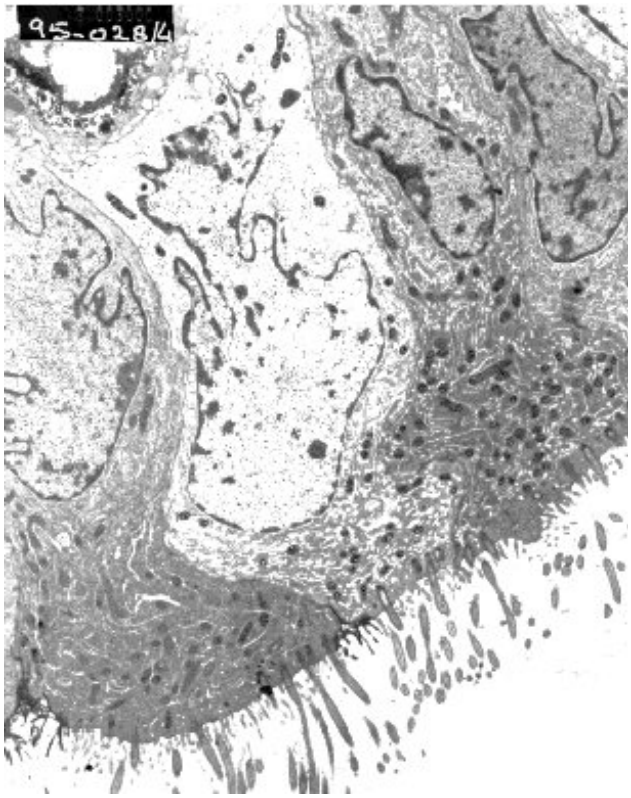
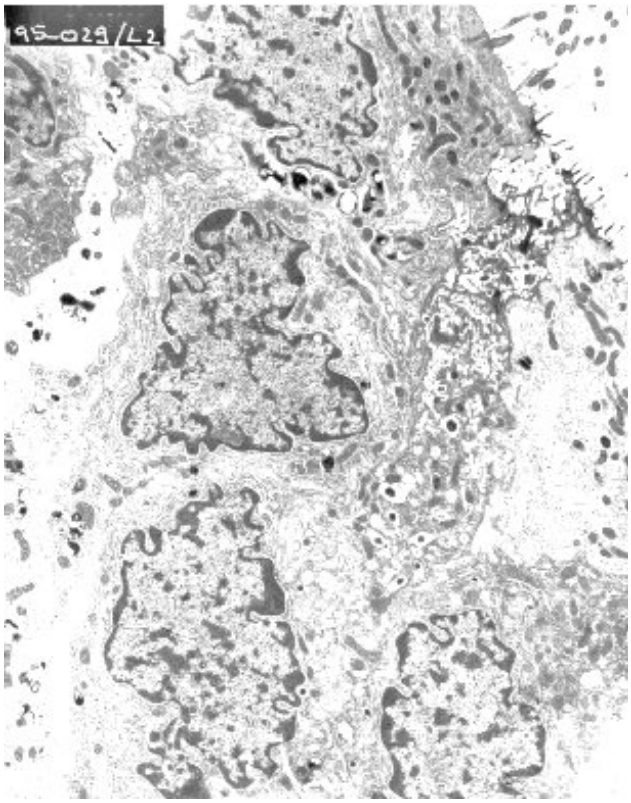
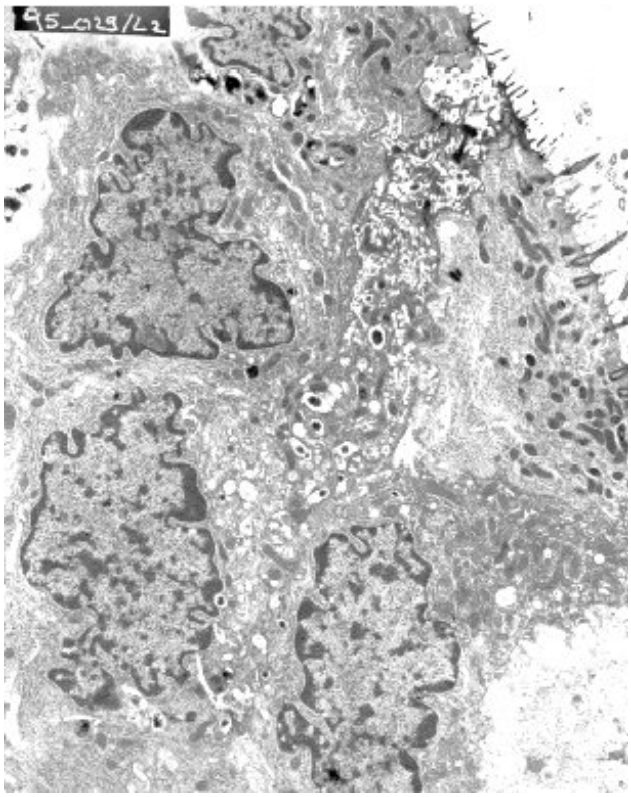


Fig. 4. Bronchial tissue following saline exposure for 5 days. This electronmicrograph is typical of that seen for saline exposed guinea pigs. The epithelial cells appear normal with lightly stained cytoplasma. There are few electron dense granules present and the cilia also appear normal. Magnification: 6,000 X.





Figs. 5 & 6. Bronchial tissue following aqueous extract of cotton dust exposure for 5 days. The epithelial surface appears distorted and the luminal surface of some epithelial cells appears damaged. Cellular protrusions can be seen. Cellular membranes and electron dense granules have been released into the lumen, and are also attached to the cilia. Magnification: 6.000 X.