IN VITRO STUDIES OF TOBACCO DUST EXTRACT ON AIRWAY SMOOTH MUSCLE: ROLE OF SENSITIZATION

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Abstract

We have previously used guinea pig tracheal rings to analyze the effect of cotton and other organic dusts on airway smooth muscle (ASM) contraction. In a recent epidemiologic study we documented that workers exposed to dust in the tobacco industry developed a high frequency of respiratory symptoms and ventilatory changes similar to those of textile workers. Recent studies with pharmacologic agents using a water soluble extract of tobacco dust (TDE) demonstrated that TDE causes ASM contraction by non-immunological mechanisms involving mediators. Clinical studies suggest that the airways of allergic individuals may be particularly prone to the effects of environmental agents. In order to study this question we used our guinea pig model and TDE. Dose related contractions of nonsensitized and sensitized (ovalbumin injected) guinea pig tracheas (GPT) were demonstrated. Tracheal tissues were studied with and without epithelium. Using this model we demonstrated that sensitization of GPT enhances the response to TDE. Removal of the epithelial layer significantly diminishes the TDE response in both sensitized and nonsenstized GPT. Removal of the epithelium from senstized GPT reduces its response to that seen in nonsensitized GPT with the epithelium intact. We suggest that the enhanced effect in sensitized GPT is mediated primarily through the inflamed epithelium.

Introduction

Tobacco processing workers are exposed to aerosols of organic compounds. Previous experience with workers in this industry indicate that they are at risk for developing respiratory problems. Respiratory disorders among tobacco workers have been reported by many authors. As early as 1948, McCormick et al (1) reported that because of the character of the manufacturing operations in the tobacco industry, certain potential health hazards exist for tobacco workers. Several investigators (2-5) found significant decreases in FVC and FEV1 as well as constriction of smaller airways. The duration of exposure to dust in the tobacco industry did not appear to be a factor.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:250-252 (2000)
National Cotton Council, Memphis TN

York et al (6) demonstrated that cigarette tobacco extract affected the O2 consumption of pulmonary macrophages. Tobacco extract produced a biphasic effect on macrophage respiration: a stimulation at low concentrations and an inhibition at high concentrations. Bernal-Madrazo et al (7) studied leukocyte migration inhibition factor in healthy individuals using an antigen of tobacco dust and found that this immunogen is capable of sensitizing a high proportion of the healthy population both smokers and non-smokers. Gleich and Welsh (8) showed that extracts of green tobacco leaf and cured tobacco leaf contain antigens that stimulate antibody formation in laboratory animals. The antigens in these extracts were acid proteins. Experimental studies with tobacco extract demonstrated that application of the extract on rat lip mucosa caused irritational hyperplasia which was related to the level of irritation. It was greatest in those rats subjected to the longest period of application of the tobacco extract with the greatest concentration of tar (9). The purpose of the current report is to further characterize acute respiratory effects of tobacco dust in an in vitro system examining in particular, the effects of sensitization on tracheal response to TDE.

Methods

The contractile response to tobacco dust extracts was studied in isolated trachea from male Hartley-Albino guinea pigs. Both sensitized and non-sensitized animals were studied. Young male Hartley guinea pigs (300-400gms) were housed in the animal facility of Mount Sinai School of Medicine. Guinea pigs were sensitized with three intraperitoneal injections of crude ovalbumin suspended in 0.9% saline. These were administered on days 1, 3 and 5. Control animals were injected with saline. On day 21 both sensitized and nonsensitized guinea pigs were challenged with an aerosol of double distilled water. The aerosol of the sensitized animals contained 2,5% ovalbumin.

On the following day, guinea pigs were sacrificed by CO2 narcosis. Tracheas were trimmed of fat and connective tissue. Four 4 to 6 mm rings were cut and suspended between two L-shaped stainless steel hooks mounted in 20 ml organ baths containing Kreb's buffer. Half of the rings had the epithelium removed by passing a cytology brush through the airway lumen. The buffer in each bath was maintained at 37°C and continuously aerated with 5% CO2 in oxygen. Tracheal rings were initially set at 2 grams tension and were allowed to relax for about 2 hours before experimentation. During this time, the tissue was washed with Kreb's buffer every 30 minutes. Isometric contractions were measured with Grass FT103C force displacement transducers attached to a Grass polygraph recorder. A total of 12 organ baths were connected by transducers to a 12 channel recorder.

Tobacco dust extracts were prepared from dust and leaves collected on machines in a tobacco processing industry located in Zagreb, Croatia. This plant had been previously surveyed for respiratory findings in workers.

Tobacco dust extract was prepared in a weight to volume ratio of 1:10 by the standard method of Sheldon to the preparation of antigens.

Dose dependent contraction of tracheal smooth muscle was consistently shown for tobacco dust extracts (TDE). TDE was added in amounts of 10,30,100,300,1000 ul to the organ bath. The tension developed by the smooth muscle was not normalized in this set of experiments to the baseline maximal contraction of these rings by carbachol 10^{-5} molar because we determined that sensitization and removal of epithelium had an independent effect on the contractile response of GPT to carbachol.

Results

A total of 18 guinea pigs (9 sensitized and 9 control) underwent dose response studies with progressively increasing doses of TDE (10,30,100,300, 1000ul). The response characteristics of the dose response curve in the sensitized animals included an Emax of 5.3 $\pm$ 1.2 gms and an EC50 of 96 $\pm$ 21 ul. For the unsensitized animals, the Emax was 2.97 $\pm$ 0.5 gms and the EC50 was 79.1$\pm$ 17.6 ul. Comparisons of EMAX and EC50 for sensitized and nonsentized tissue with and without epithelium are detailed in Table 1 and 2.

Discussion

These studies of TDE on sensitized and unsensitized guinea pig tracheal smooth muscle imply a complex effect of this airway irritant. Sensitization of guinea pig trachea leading to airway inflammation enhances the effect of TDE on contractile responses. The removal of the epithelial layer eliminates this enhancement. These findings suggest that inflammatory cells in the epithelial layer modulate the effect of airway irritants such as tobacco dust.

Conclusions

1. Tobacco dust extract causes dose dependant constriction of guinea pig trachea.
2. Sensitization of intact GPT enhances the response to TDE
3. Removal of the epithelial layer significantly diminishes the TDE response in both sensitized and nonsensitized GPT.
4. There was no difference between the TDE response in sensitized GPT without epithelium and unsensitized GPT with epithelium.

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Table 1. Summary data of Emax and EC50 values obtained for Tobacco Dust Extract in GPT which have been sensitized in the presence (+) and absence (-) of epithelium.

TISSUE	EPITHELIUM	EMAX (GMS)	EC50 (ul)
SENSITIZED	+	5.3 $\pm$ 1.1	96 $\pm$ 21.2
	-	2.9 $\pm$ 0.5	107 $\pm$ 15

Table 2. Summary data of Emax and EC50 values obtained for Tobacco Dust Extract in GPT which have not been sensitized in the presence (+) and absence (-) of epithelium.

TISSUE	EPITHELIUM	EMAX (GMS)	EC50 (ul)
NONSENSITIZED	+	2.97 $\pm$ 0.46	79 $\pm$ 17.5
	-	1.8 $\pm$ 0.4	66 $\pm$ 7.7