CYTOTOXIC EFFECT OF PATULIN ON HUMAN PNEUMOCYTES, *IN VITRO*.

V. Roepstorff and T. Sigsgaard
Steno Institute of Public Health, Dept of Environmental & Occupational Medicine, University of Aarhus
Denmark

Abstract

We have previous presented a study of the cytotoxic effect of untreated and heat-treated cotton dust. The lipopolysaccharide (LPS) content in heat-treated cotton dust was reduced by 73% compared to untreated cotton dust. We found a slightly higher cytotoxic effect of the heat-treated cotton dust compared to untreated cotton dust. We investigated the content of mold spores in the two dust samples and found nearly the same species in both samples. The aim of this project was to test if components of the molds still remaining in the heat-treated cotton dust might cause the cytotoxic effect. We have tested the cytotoxic effect of β 1-3-D-glucan and Patulin, a mycotoxin produced by several *Aspergillus* and *Penicillium* species. Patulin showed a cytotoxic effect after 24 hours of incubation with concentrations in the range of 1-100μg/ml. The effect of the mycotoxin was significantly higher than the effect of cotton dust. Hence, if Patulin is present on cotton it might explain the cytotoxic potentiale of the dust. But we don't know if these mycotoxins are present in concentrations high enough to induce these effects.

Introduction

It has been known for years that byssinosis is a common disease among cotton workers (1). Several investigations (2,3) have shown an association between number of cases of byssinosis and the quantity of respirable organic dust and LPS. Some investigations (4,5) have shown that heat-treatment of the cotton could decrease the content of LPS in the dust. Last year we received dust from a heat-treated and an untreated bale of cotton from Marie-Alice Rousselle, Southern Regional Research Center USDA-ARS. We tested the cytotoxic effect of extracts from these two dust samples in our assay. We found (6) contrary to what we expected that the cytotoxic effect was slightly higher with extract of the heat-treated cotton dust than with extract of the untreated dust. We knew that the LPS content in the heat-treated cotton dust was approximately 27% of the content in the untreated cotton dust. Hence, it could be excluded that the LPS was the cause of the cytotoxic effect. This was in accordance with earlier studies, where we have tested pure LPS (7) and found no cytotoxic effect. We tested the two dust samples for their content of mold spores, and found nearly the same species in both the untreated and the heat-treated cotton dust.

The aim with this project was to test if components of the molds could cause the cytotoxic effect. We tested a cell-wall component from mold spores β 1-3-D-glucan (Curdlan) and a mycotoxin produced from some of the most common species of molds found in organic dust, *Aspergillus* and *Penicillium*. We have tested two cell lines against dilutions from 1pg/ml to 100μg/ml of the mycotoxin Patulin.

Materials and methods

Cell cultures:
A) Cultures of monkey kidney cells (VERO from American Type Culture Collection ATCC no: CCL 81 pass: 125-130) were grown in Earle's medium 199 (95%) with fetal bovine serum (5%, LPS content < 0.02 ng/ml; Gibco-BRL) and penicillin/streptomycin (100IU/100μg pr ml; JRH-Biosciences) at 37°C, 5% CO_2. For the assay $5x10^3$ cells/well were seeded.

B) Cultures of human lung carcinoma cells (A 549 from American Type Culture Collection ATCC no: CCL 185 pass: 98-103) were grown in HAM's F 12K medium (90%) with fetal bovine serum (10%, LPS content < 0.02 ng/ml; Gibco-BRL) and penicillin/streptomycin (100IU/100μg pr ml; JRH-Biosciences) at 37°C, 5% CO_2. For the assay $4x10^3$ cells/well were seeded.

Cytotoxic assay:
CellTiter 96™ Non-Radioactive Cell Proliferation/Cytotoxicity assay (Promega) were used. Cells were grown in microtiter plates for cell cultures (Greiner) for two days. On day 3 the medium was exchanged with extracts of Patulin (Sigma Chemical CO., St Louis, P-1639) and a β 1-3-D-glucan (Curdlan, kindly received from J. Douwes, Wageningen) in conc. 1pg - 100μg/ml suspended in medium and incubated 2-24 hours. At this time the cells were nearly confluent and the medium was exchanged once again with medium containing dye (15% tetrazolium-salt). After another 4 hours of incubation solubilizer was added into the dye-medium and the suspension was left overnight at room temperature in a dark humid container. The results were read with a 570 nm messenger and 630 nm reference filter in an ELISA microplate reader (BIO-RAD 450), and plotted as percent of non exposed cells.

Statistics
For significance testing Mann-Whitney and Wilcoxons Signed ranks tests (SPSS statistical Package for the Social Science/IBM) were used. The measurement of 555pg/ml was the mean of 16 wells expressed as percent of untreated control cells (8 readings at concentration 100pg/ml and 8 readings at concentration 1μg/ml). The measurement of 55μg/ml was the mean of 16 wells expressed as percent of untreated control cells (8 readings at concentration 10μg/ml and 8 readings at concentration 100μg/ml).

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:331-333 (1996)
National Cotton Council, Memphis TN

Results

The two cell lines showed the same pattern (Table 1). The β 1-3-D-glucan showed no cytotoxic effect neither after 2 hours and after 24 hours of incubation (Figure 2).

Patulin showed no cytotoxic effect after 2 hours of incubation. However, after 24 hours of incubation a significant cytotoxic effect was seen after exposure to Patulin in concentrations in the range of 1-100μg/ml (Table 1, Figure 2 and 3).

Discussion

In earlier studies we have tested untreated cotton dust and heat-treated cotton dust, the latter with a known LPS reduction of 73%, the cytotoxic effect was nearly the same (6). We have also tested pure LPS from *Enterobacter agglomerance* and found no cytotoxic effect (7). We have now tested β 1-3-D-glucan and we still found no cytotoxic effect. The mycotoxin Patulin is so far the only pure component, we have tested, which exerts a cytotoxic effect on the cell lines in our assay. When we looked at the cytotoxic effect of high concentrations of the cotton dust extracts and the pure components (Figure 3) we saw nearly no effect of LPS and β 1-3-D-glucan, a significant cytotoxic effect of the untreated and the heat-treated cotton dust and a significant higher cytotoxic effect of the mycotoxin.

Mycotoxins are organic compounds produced naturally by numerous fungi. The most common classes of mycotoxins isolated from organic dust samples contained aflatoxins and trichothecenes (8). Several studies (9-11) have investigated the toxicity of the trichothecenes, including Patulin produced by some *Aspergillus* and *penicillium* species. The cytotoxicity of Patulin has been studied in rat alveolar macrophages (12). These studies showed that Patulin inhibit protein as well as the RNA-synthesis. The inhibition of protein synthesis was the most sensitive parameter, with 50% inhibition after 1 hours at 0.002mM Patulin. The cytotoxic effect of Patulin in our assay was seen after 24 hours of incubation with concentrations in the range of 1-100μg/ml. The cotton dust exerts a cytotoxic effect at concentrations from 52 to 208 μg dust/ml.

The question is if Patulin is the only mycotoxin or if there will be other mycotoxins exerting cytotoxic effect? We don't know if such mycotoxins occur in cotton dust in concentrations high enough to exert such effects. Furthermore we don't know if the concurrent exposure to more toxins might elicit an adjuvant effect, and hence decrease the critical concentration. These questions needs further investigations.

Acknowledgements

This study has been possible because of grants from the Danish Work Environment Fund.

References

1. Haglind, P., M. Lundholm, and R. Rylander. 1981. Prevalence of byssinosis in Swedish cotton mills. Br J Industr. Med. 38:138-143.

2. Sigsgaard, T., O. F. Pedersen, S. Juul, and S. Gravesen. 1992. Respiratory disorders and atopy in cotton, wool, and other textile mill workers in Denmark. Am J Ind Med. 22:163-184.

3. Rylander, R., P. Haglind, and M. Lundholm. 1985. Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. Am Rev Respir Dis. 131:209-213.

4. Rousselle, M-A. and L. N. Domelsmith. 1992. Endotoxin reduction in cotton fiber: comparison of heat sources for detoxification. Proc. sixteenth cotton dust res. conf. R. R. Jacobs, P. J. Wakelyn and L. N. Domelsmith eds. National Cotton Council, Memphis, TN. 243-245.

5. Rousselle, M-A. 1994. Endotoxin content of dust from heat-detoxified cotton fiber. Proc. eighteenth cotton dust res. conf. R. R. Jacobs, P. J. Wakelyn, L. N. Domelsmith eds. National Cotton Council, Memphis, TN. 362-364.

6. Roepstorff, V. and T. Sigsgaard. 1995. Cytotoxic effect of extracts from tannin, treated and untreated cotton on human pneumocytes. Proc. nineteenth cotton dust res. conf. P. J. Wakelyn, R. R. Jacobs, and R. Rylander, edt. National Cotton Council, Memphis, TN. 272-275.

7. Sigsgaard, T., V. Roepstorff, and A. F. Tuxford. 1994. Cytotoxicity of cotton dust and dust components to A549 and VERO cells. Proc. eighteenth cotton dust res. conf. R. R. Jacobs, P. J. Wakelyn, L. N. Domelsmith eds. National Cotton Council, Memphis, TN. 322-325.

8. Hendry, K. M. and E. C. Cole. 1993. A review of mycotoxins in indoor air. J Toxicol Environ Health. 38(2):183-198.

9. Sorenson, W. G., G. F. Gerberick, D. M. Lewis, and V. Castranova. 1986. Toxicity of mycotoxins for the rat pulmonary macrophage in vitro. Environmental Health Perspectives. 66:45-53.

10. Gerberick, G. F., W. G. Sorenson, and D. M. Lewis. 1984. The effect of T-2 toxin on alveolar macrophage function in vitro. Environ. Research. 33:246-260.

11. Gerberick, G. F. and W. G. Sorenson. 1983. Toxicity of T-2 toxin, a Fusarium mycotoxin, to alveolar macrophages in vitro. Environ. Research. 32:269-285.

12. Sorenson, W. G., J. Simpson, and V. Castranova. 1985. Toxicity of the mycotoxin Patulin for rat alveolar macrophages. Environ. Research. 38:407-416.

Table 1. Cytotoxic effect on both cell lines after 2 and 24 hours of incubation.

		A 549		VERO	
		2 hours	**24 hours**	**2 hours**	**24 hours**
LPS	**550ng**	104#	91*	99#	80
	55μg	113	91	110	95
Curdlan	**550ng**	100#	96#*	88	97#
	55μg	99#	95#*	98#	103#*
Patulin	**550ng**	93	96#*	93	83
	55μg	97	35	91	1

not significant different from 2 hours of incubation P<0.05
not significant different from unexposed cells P<0.05

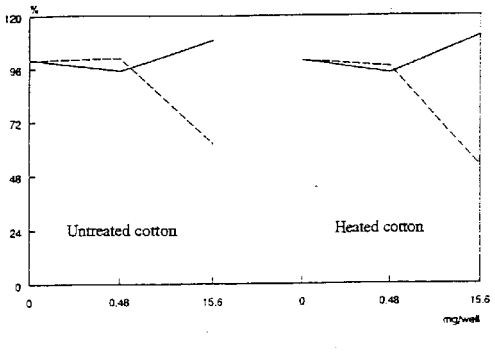

Figure 1. Cytotoxic effect of heat-treated and untreated cotton dust, expressed as percent of unexposed cells. Straight line 2 hours of incubation, dotted line 24 hours of incubation. These results have been presented at the conference 1995 (6).

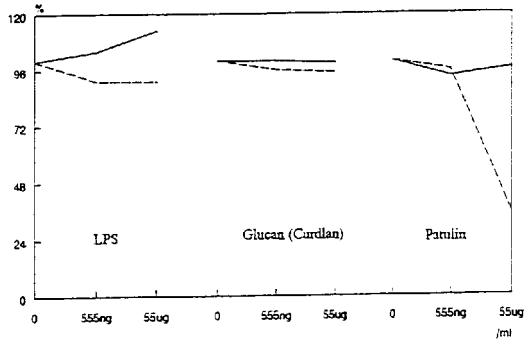

Figure 2. Cytotoxic effect expressed as percent of unexposed cells, after exposure of LPS and mold-components. Straight line 2 hours of incubation, dotted line 24 hours of incubation.

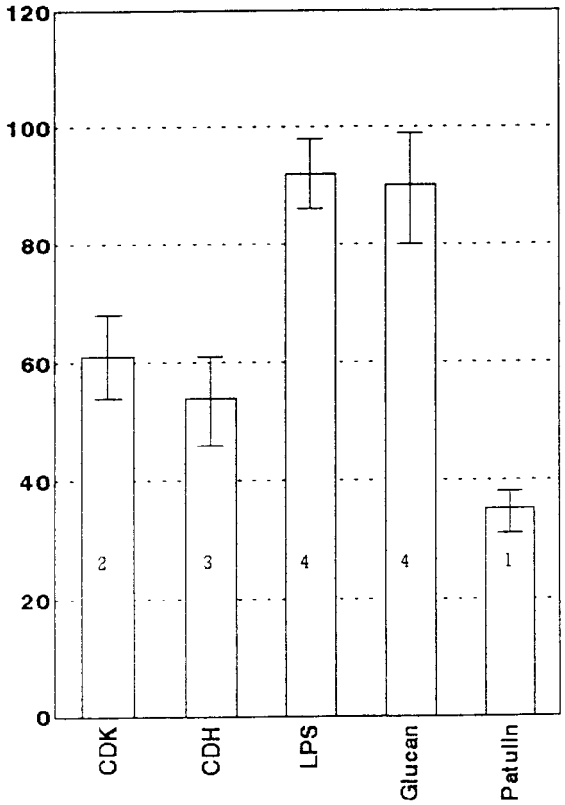

Figure 3. Cytotoxic effect of dust and dust components (CDK untreated cotton dust, CDH heat-treated cotton dust). Bars with the same number are not significantly different.