INFLUENCE OF CHEMICAL/THERMAL PRETREATMENT ON THE CYTOTOXICITY OF COALMINE DUSTS

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SUMMARY

Investigations of mine dusts have partly yielded great discrepancies between the effects in humans and experimental animals on the one hand and cytotoxicity in vitro on the other hand. With the aid of chemical and thermal pretreatment it was attempted to simulate the demasking and metabolic processes occurring in vivo. Furthermore it is demonstrated that an increased information can be obtained by using a wider spectrum of concentrations and modifications of culture conditions.

INTRODUCTION

The results of earlier studies and the related observations that

- Great discrepancies exist in the evaluation of the cytotoxicity on the one hand, and of the animal experiments on the other [1,2,3,4,5,6, 7] and that
- The cell toxicities determined for a series of dusts depend very heavily on the conditions in the cell cultures and the dust dose[8,9,10,11]

induced us to try to characterize the cytotoxicity of high-rank and low-rank mine dusts from the Saar and Ruhr coalfields more accurately via their dose dependence and the effect of chemical and thermal pretreatment.

MATERIALS AND METHODS

Two groups of dusts were selected for the chemical and thermal treatment:

Group A: Dusts which caused higher reactions for one parameter in the animal experiments than for two parameters in the in vitro test, and

Group B: Dusts which, in comparison to Group A, caused a lesser reaction for the one parameter in the animal experiment than in the corresponding two parameters of the in vitro test, which exhibited either the same or a stronger reaction.

The parameter for the animal experiment was the measure of the quartz-typical dust deposition area in the lymph node test as described by Hilscher.[14] The parameter in the in vitro test was the TTC test, i.e., the influence on the reduction activity of the cells to 2, 3, 5-triphenyl-tetrazole chloride (TTC) and the pO_2 test, i.e., the polarographic measurement of the oxygen consumption (Table I,[12,15]).

The dusts were subjected to ultrasonic treatment for a total of 70 hours at a temperature of 38°C in each of 30% H_2O_2, 100% acetone, 5 n HCl, 5 n NaOH, 5% RBS (detergent) and in double distilled water. Finally they were washed out four times at 3500 rpm (g = 1500 m/sec^2) in double distilled water + 0.01 RBS and dried at 100°C.

As cytotoxic parameter we used the release of cytoplasmatic lactic dehydrogenase (LDH) of guinea pig alveolar macrophages into the medium. Toxicity was expressed as enzyme activity/10^6 cells or as percent toxicity between control and the 100 µg/10^6 cells concentration of Doerentruper quartz No. 12 (DQ_{12}).

RESULTS

The following results were obtained from the in vitro tests:

- The toxicity of DQ_{12} is reduced by acetone, but is slightly increased by HCl and NaOH (Table II).
- Treatment with HCl and NaOH causes a sharp increase in the toxicity of the Gambach quartz (Table III).
- The toxicity of the Gambach quartz and DQ_{12} after chemical treatment was changed by the addition of small quantities of fetal calf serum (Table IV): The toxicity of HCl-treated Gambach quartz cannot be inhibited by 0.1% of fetal calf serum (FKS), as is the case for the untreated dust.[10]

The toxicity of acetone-treated DQ_{12}, on the other hand, can be slightly inhibited by FKS (Table IV).

- Tables V and VI show the LDH release after incubation with treated and untreated mine dusts:
- For the 3 dusts from Group A, an increase in toxicity is to be observed after the various types of treatment: The toxicity of mine dust U 120 is particularly increased by treatment with acetone, dust V 521 by treatment with H_2O_2 and with acetone, H 520 by treatment with HCl and particularly hy treatment with NaOH.
- In Group B, only the acetone treatment caused an increase in the toxicity of mine dust Y 320, whilst the H_2O_2 treatment resulted in an increase in the toxicity of dust T 124.

Analysis of the change in toxicity after chemical treatment

Table I

Characterization of the Tested Mine Dusts

Group	Dust	QTA/LKA	100-TTC-RA	pO_2	Quartz (%)
high-rank:					
A	U 120	14,16	36,0	0,240	7,3
A	V 521	-,-	37,5	0,229	7,5
A	H 520	8,68	29,0	0,200	1,0
low-rank:					
B	Y 320	0,87	41,0	0,268	7,5
B	N 220	0,62	54,5	0,348	9,3
B	T 122	0,60	40,0	0,200	2,8

Control dusts: Corundum, DQ_{12}, Gambach quartz

Table II

LDH-Release (mU/10^6 Cells) of Guinea Pig—Lung Macrophages 20 Hours After Exposure with Untreated and Pretreated Doerentruper Quartz

	µg/10^6 cells		
DQ_{12}	25	50	100
1 untreated	31	68	87
2 H_2O_2 (30%)	22	40	67
3 Aceton	16	23	65
4 HCl 5 n	43	77	85
5 NaOH 5 n	56	81	87
6 RBS 5%	31	59	85
7 Aqua bidest.	47	72	94

Table III

LDH-Release of Guinea Pig—Lung Macrophages 20 Hours After Exposure with Pretreated Gambach Quartz in Percent Toxicity (100 µg Corundum/10^6 Cells = 0%; 100µ DQ_{12}/10^6 Cells = 100% Toxicity)

	µg/10^6 cells		
Gambach quartz	100,0	200,0	300,0
1 untreated	14,1	53,5	80,3
2 H_2O_2 (30%)	14,1	54,9	83,1
3 Aceton	11,3	57,7	90,1
4 HCl 5 n	85,9	87,3	100,0
5 NaOH 5 n	100,0	100,0	100,0
6 RBS 5%	21,5	57,7	77,5
7 Aqua bidest.	15,5	47,9	77,5

Table IV

Influence of Fetal Calf Serum (FKS) on the Cytotoxicity of Untreated and Treated Gambach Quartz and DQ_{12} in the Guinea Pig—Lung Macrophages Culture Measured by the LDH-Release (mU/ml)

	without Serum	+ 0,1% FKS
Gambach quartz		
200 µg/10^6 cells		
1 untreated	69	29
2 H2O2 (30%)	82	40
3 Acetone	45	20
4 HCl 5 n	114	112
5 NaOH 5 n	-	-
6 RBS 5 %	63	38
7 Aqua bidest.	64	27
DQ_{12}		
100 µg/10^6 cells		
1 untreated	138	136
3 Acetone	89	36
Corundum 1 untreated		
100 µg/10^6 cells	21	20
200 µg/10^6 cells	32	30

of mine dust N 220 is difficult. At a dust concentration of 100 µg/10^6 cells, the cytotoxicity drops after treatment with HCl, RBS and double distilled water. This effect can no longer be observed, however, with bigher dust concentrations.

DISCUSSION

The chemical and thermal treatments resulted in a change in the toxicity of a number of the mine dusts, and generally towards an increase in toxicity; in view of the altered dust surfaces, however, these changes do not permit a direct assessment of the membrane toxicity. It can be assumed that the dust surfaces are altered in such a way by the treatment with the organic and inorganic compounds, either by the leaching out of surface constituents or by absorption, that it is very difficult to draw conclusions with regard to the original dust, even if the cytotxicity is increased as a result. The treatment can result in the removal of impurities from the dust surface; with quartz, the treatment can also result in a change in the basic crystal structure, and therefore in the electron structure. A notable feature is that in Table I, the mine dusts from Group A differ more widely than those of Group B with regard to their quartz-typical deposition areas in the lymph node test than the quartz contents of the dusts. During the in vitro test, this difference is not apparent in the untreated dusts. Only the cbemical and thermal treatment of the dust surfaces caused an increase in the toxicity in the mine dusts from Group A, but less so for the dusts from Group B. Since the quartz contents of the dusts in the

Table V

Influence of the Chemical-Thermal Treatment
Group A: Reaction in Animal—High;
Cytotoxicity —Low
LDH Release of Guinea Pig—Lung Macrophages 20 Hours After Dust-Exposure in Percent Toxicity
(Corundum = 0%; DQ_{12} = 100% Toxicity)

		100	200	300		
		µg/10^6 cells				
Corundum		0	0	0		
DQ_{12}		100	100	100		
U 120	- 1	26	53	73	untreated	
	- 2	22	58	77	H_2O_2	30%
	- 3	33	89	105	Acetone	100%
	- 4	32	76	80	HCl	5 n
	- 5	38	68	80	NaOH	5 n
	- 6	21	58	65	RBS	5%
	- 7	16	51	73	Aqua bidest.	
V 521	- 1	23	47	73	untreated	
	- 2	36	82	87	H_2O_2	30%
	- 3	34	76	90	Acetone	100%
	- 4	22	50	68	HCl	5 n
	- 5	18	43	62	NaOH	5 n
	- 6	17	43	58	RBS	5%
	- 7	20	36	45	Aqua bidest.	
H 520	- 1	11	17	30	untreated	
	- 2	9	11	33	H_2O_2	30%
	- 3	6	15	42	Acetone	100%
	- 4	13	35	57	HCl	5 n
	- 5	41	89	105	NaOH	5 n
	- 6	7	3	5	RBS	5%
	- 7	8	22	40	Aqua bidest.	

Table VI

Influence of the Chemical-Thermal Treatment
Group B: Reaction in Animal—Lower;
Cytotoxicity —Similar or Higher than in Group A
LDH Release of Guinea Pig—Lung Macrophages 20 Hours After Dust-Exposure in Percent Toxicity
(Corundum = 0%; DQ_{12} = 100% Toxicity)

		100	200	300		
		µg/10^6 cells				
Corundum		0	0	0		
DQ_{12}		100	100	100		
Y 320	- 1	23	46	74	untreated	
	- 2	45	74	65	H_2O_2	30%
	- 3	63	84	84	Acetone	100%
	- 4	36	73	79	HCl	5 n
	- 5	40	73	85	NaOH	5 n
	- 6	29	80	80	RBS	5%
	- 7	35	69	76	Aqua bidest.	
N 220	- 1	42	91	89	untreated	
	- 2	69	92	92	H_2O_2	30%
	- 3	63	83	81	Acetone	100%
	- 4	22	77	82	HCl	5 n
	- 5	55	89	88	NaOH	5 n
	- 6	18	62	67	RBS	5%
	- 7	22	72	81	Aqua bidest.	
H 520	- 1	14	42	56	untreated	
	- 2	48	75	79	H_2O_2	30%
	- 3	20	63	72	Acetone	100%
	- 4	15	48	56	HCl	5 n
	- 5	15	52	67	NaOH	5 n
	- 6	11	28	47	RBS	5%
	- 7	46	46	56	Aqua bidest.	

two groups are less than 10% and differ widely from one another in only two cases, other factors must be responsible for these differences in the size of the quartz-typical dust deposition areas in the lymph node test and in the level of the toxicity. In the lymph node test, a shift in the mean particle size of the quartz content could play a role, since the filtering of the dust particles in the lymphatic system results in a trend to smaller particle sizes and higher toxicities. More likely, however, is that the reactions and toxicities depend on the rank of the dusts from the Saar and Ruhr coalfields. An interesting fact is that the mine dusts in Group A originated from high-rank seams, whilst those in Group B originated from low-rank seams, i.e., from younger seams.

Conclusion correlates with the investigations into the specific noxiousness of the respirable dusts from the Ruhr coalfields by Reisner and Robock,[16] Robock et al.[12] and the more recent studies by us[17] which revealed that for dusts originating from different collieries and different stratigraphic horizons hut with comparable mineral content, the cytotoxicity increases with the age and rank of the seams.

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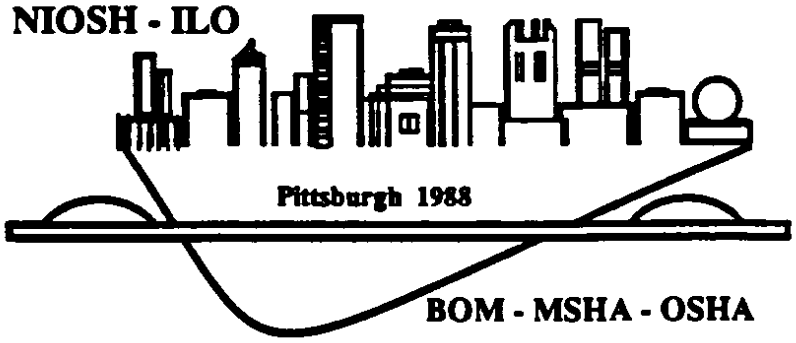

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