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Respiratory Effect of Selected Asbestos Substitute Mineral Fibres - Using as Insulation Materials in Building Industry

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Abstract

The effects of industrial fibrous dusts on the respiratory system represents a potencial environmental and occupational health hazard for humans. Long time of asbestos exposure can cause pleural plaques, asbestosis and oncological diseases. From these facts follow important tasks on the deep and broad research activities aiming at the study of the effects of fiber substitutes.

The aim of the present work was to find out the influence of asbestos and asbestos substitute mineral fibres (ASMF) on selected bronchoalveolar lavage fluid (BALF) inflammatory and cytotoxic parameters as well as dose and time dependence of studied fibrous dust effect.

The 2 types of substitutes (rock wool, glass fibers) as well as amosite asbestos were intratracheal instilled at 2 doses (2 and 8 mg/animal). Following parameters in bronchoalveolar lavage fluid after 4 and 16 week exposure were investigated: Inflammatory response parameters: The number of leukocytes / ml, the number of alveolar macrophages (AM) / ml, the differential number of cells (% AM; Gr; Ly). Cytotoxic parameters: Phagocytic activity of AM, viability of AM, the lactate dehydrogenase activity, the acid phosphatase activity, the cathepsin D activity (in the cell-free lavage fluid and in the BAL suspension).

Sequential arrangement of examined fibrous dust according to their harmfulness from the point of view of inflammatory and cytotoxic parameters after intra tracheal instillation:

Amosite > Rockwool > Glass fibers.

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Introduction

The study of the effects of industrial fiber dusts on respiratory system has been a long lasting urgent problem. Typical example of industrial fiber dusts are asbestos fibers which, after a long time exposure, can cause many of lung diseases [1,2].

Owing to these findings there are trends to substitute the fibers with adverse effects by fibers technologically and qualitatively similar to asbestos, yet with lower biological effects. From these facts follow important tasks on the deep and broad research activities aiming at the study of the effects of fiber substitutes. These activities are in line with World Health Organization, International Labour Office and International Agency For Research On Cancer guidelines.

The problem of the adverse effects of asbestos fibres on the respiratory tract remains topical, because it is still used in many developing countries. After long - term exposure of men and experimental animals to asbestos, besides asbestoses and pleural hyalinoses, neoplasms were also observed, e. g. bronchial carcinoma, pleural and peritoneal mesothelioma and mesotheliomas of other organs. A lot of cases have shown that years of exposure to high concentrations of asbestos are necessary to induce lung cancer and mesothelioma, while others cases report that only brief occupational, household, or neighborhood exposure is necessary. Concern over low exposure to substances that are designated as carcinogens is based on the concept that even the smallest exposure to such substances can increase cancer risk. The expression "one asbestos fiber can possibly produce a tumor" is repeated over and over until it is accepted as a truth. However, the fact that asbestos causes cancer among heavily exposed workers, coupled whit the hypothesis that there is no known threshold for the induction of tumor, were used to initiate program of asbestos removal in schools, homes and commercial buildings in a lot of countries. Over 1400 air samples taken in 219 North American school buildings show the average fiber level to be 0,00022 fibers per ml of air. But after building removal there are cca 50 000 asbestos fibers /m^3 in the ambient air, which remain there for a long months [3].

Table 1: Design of intratracheal instillation study (Fisher 344 rats).

Intratracheal instillation.	Exposure:	
Dose:	4 weeks	16 weeks
2 mg/ animal	8	8
8 mg/animal	8	8
Control 2 mg/animal	8	8
Control 8 mg/animal	8	8

Control - (saline solution – negative control)
Amosite - (positive control)
Rock wool - RW1 (ASMF)
Glass fibres - MMVF10 (ASMF)

Table 2: Amosite - i. t. instillation, Inflammatory response parameters in BAL.

Time post last istillation:	4 weeks	16 weeks	4 weeks	16 weeks
dose:	2 mg	2 mg	8 mg	8 mg
BALF cell count	↑*			↑**
Lymphocytes % of BAL cells	↑**	↑**	↑**	
AM % of BAL cells	↓**	↓***	↓**	↓*
PMN % of BAL cells		↑***	↑*	↑**
Total amount of protein (g)				↑*

Comparison of exposed group to corresponding control group
*p< 0.05, **p< 0.01, ***p< 0.001
↑: increase against compared group; ↓ : decrease against compared group
abbreviations: AM: alveolar macrophages; PMN : polymorphonuclear cells ; BAL: bronchoalveolar lavage

Table 3: Rockwool - i. t. instillation, Inflammatory response parameters in BAL.

Time post last instillation:	4 weeks	16 weeks	4 weeks	16 weeks
dose:	2 mg	2 mg	8 mg	8 mg
BALF cell count 10^3.ml^{-1}				↑*
AM count 10^3 ml^{-1}				↑*
Lymphocytes % of BAL cells			↑**	
AM % of BAL cells	↓*	↓*	↓**	↓*
PMN % of BAL cells		↑*	↑**	↑**
Immature forms of AM(%)	↑**			
Total amount of protein (g)			↑**	↑***

Comparison of exposed group to corresponding control group
*p< 0.05, **p< 0.01, ***p< 0.001
↑: increase against compared group; ↓: decrease against compared group
abbreviations: AM: alveolar macrophages; PMN: polymorphonuclear cells
BAL: bronchoalveolar lavage

There is thus a necessity to substitute asbestos by fibres technologically similar, but with less biological effects. From these facts follow important tasks on the deep and broad research activities aiming at the study of the effects of fiber substitutes. The mechanism of action of asbestos and other fibrous structures - substitutes- are still not clearly understood. Many industrial dusts (asbestos substitutes) are used as insulating materials for thermal insulating of buildings.

The aim of the present work was to find out the influence of asbestos and asbestos substitute mineral fibres (ASMF) on selected bronchoalveolar lavage fluid inflammatory and cytotoxic parameters as well as dose and time dependence of studied fibrous dust effect.

Further on, our results are used for legislative purposes to amend the MAC (maximum allowable concentration on the workplace and environmental) of fiber dusts in the workplace air as well as to find out the suitability of the particular fiber substitute for asbestos. Our results provided and will also provide a basis for manufacturers and makers to select for production and processing biologically acceptable materials. In addition to it, our results can be used for better understanding of environmental health in given specific field and of health protection of the inhabitants in the respective area [4-8].

Methods

The 2 types of ASMF (rockwool, glass fibers) as well as amosite asbestos were instilled at 2 doses (2 and 8 mg/animal). Animals (number: 8 per group) were intratracheally instilled (noninvasively) with fibrous suspension (2 mg suspended in 0,2 ml of saline solution per animal) or only with 0,2 ml saline per animal (control group). Dose 8 mg was divided and instilled 4 times (weekly 2 mg/0,2 ml saline solution). After sacrifice (4 or 16 weeks after last intratracheal instillation the animals were anesthetised with thiopental –150 mg/kg of animal) cells were harvested by bronchoalveolar lavage (BALF). The trachea was cannulated, and the lungs were washed 5 times with 4 ml of saline solution (in situ). Following parameters of BALF were investigated: a) Inflammatory response biomarkers: the number of BALF cells / ml, the number of alveolar macrophages (AM) / ml, the differential number of cells (% AM and neutrophils). b) Cytotoxic parameters: phagocytic activity of AM, viability of AM, lactate dehydrogenase activity (in the cell-free lavage fluid), acid phosphatase activity (in the cell-free lavage fluid and in the BAL suspension), alkaline phosphatase activity (in the cell-free lavage fluid), cathepsin D activity (in the cell-free lavage fluid and in the BAL suspension). The methods are described in more detail in the paper [9].

The length (L) and diameter (d) of fibres in our intratracheal instillation study are following:

Amosite:

length (μm)	% fibres	mean diameter (μm)
< 20	5	0.71
20-30	75	
>30	20	

Rockwool:

L: 16.5 μm (SD: 2.51)

d: 1. 8 μm (SD: 2.32)

Glass fibers: (MMVF 10):

L: 22.6 μm (SD: 13.6)

d: 1.31 μm (SD: 0.85)

The results were statistically evaluated using Mann Whitney`s test.

Discussion

At present, the question of the toxicity of asbestos substitutes is an area of extensive research. However, there is only limited knowledge concerning the biological effects of these fibres. The processes which lead to morphological changes of the lungs after long-term exposure to some industrial mineral fibrous dusts are still not clear, they involve many molecular and cellular reactions, including an inflammatory response and cytotoxicity. Fiber length has been found to be the major predictor of the ability of industrial fibers of different types to

Table 4: Glass fibers - i. t. instilation, Inflammatory response parameters in BAL.

Time post last instillation:	4 weeks	16 weeks	4 weeks	16 weeks
dose:	2 mg	2 mg	8 mg	8 mg
BALF cell count $10^3.ml^{-1}$	↑*			
Numer of AM 10^3 ml^{-1}	↑*			
Immature forms of AM (%)	↑*			
Total amount of protein (g)	↑**	↑**	↑***	

Comparison of exposed group to corresponding control group
*p< 0.05, **p< 0.01, ***p< 0.001
↑: increase against compared group
abbreviations: AM: alveolar macrophages; BAL: bronchoalveolar lavage

Table 5: Amosite - i. t. instillation, Cytotoxic parameters in BAL.

Time post last instillation:	4 weeks	16 weeks	4 weeks	16 weeks
dose:	2 mg	2 mg	8 mg	8 mg
n	7	8	7	8
Phagocytic activity of AM (%)		↓**	↓**	↓**
Viabilty % of living cells	↓**	↓**	↓**	↓**
LDH µkat.g $prot^{-1}$	↑*	↑*	↑**	↑**
ACP nkat.g $prot^{-1}$				↑**
Cathepsin U_{tyr}.mg $prot^{-1}$			↑**	↑**
Cathepsin D $U_{tyr}.10^{-6}$ cells			↑**	↑**
AP			↑***	↑**

Comparison of exposed group to corresponding control group;
*p< 0.05, **p< 0.01, ***p< 0.001;
↑: increase against compared group., ↓: decrease against compared group
abbreviations: LDH: lactate dehydrogenase; ACP: acid posphatase; U_{tyr} µg of thyrosine released in an hour time; AP: alkaline phosphatase; prot : protein; AM: alveolar macrophages

cause lung pathology [10,11,5,12].

It is usually assumed that particles with a diameter greater than 1 µm do not penetrate the alveolar area of the rat. Based on animal experiments with asbestos, erionite, and MMMF, also Jaurand as well as other researchers, has suggested that while fiber size is an important factor of carcinogenicity, other factors such as fibre type, geometry and quantity also play a significant role [13]. In addition, physico-chemical properties such as surface chemistry, chemical composition, surface area and biopersistence are also important factors.

Many papers indicate that inflammation is linked to neoplasia through several mechanisms after exposure to asbestos. Partial ingestion of long fibers by macrophages activates these cells to release substances such as lymphokines, growth factors, reactive oxygen intermediates and proteases. Some of these may be genotoxic and others may cause cell proliferation. The number and type of the cells obtained by lavage fluid as well as their viability, phagocytic activity and state of AM activation enable to understand potential injurious effect of inhaled noxious substances. Increase in the number of leukocytes as a result of inflammatory response was documented by numerous researchers [14,11,6,15].

Decrease in macrophage number or phagocytic capacity may result in a reduction in clearance of inhaled materials and thus an increase in the effective dose of the potentially injurious agent [14]. BALF cell count in our study was increased after exposure to: amosite (4 weeks/2 mg and 16 weeks/8 mg), rockwool (16weeks/8 mg) and glass fibers (4 weeks/2mg). As regards the phagocytic activity of AM

Table 6: Rockwool - i. t. instillation, Cytotoxic parameters in BAL.

Tme post last instillation:	4 weeks	16 weks	4 weeks	16 weeks
dose:	2 mg	2 mg	8 mg	8 mg
Viabilty % of living				↓**
LDH µkat.g $prot^{-1}$	↑**		↑**	
ACP nkat.10^{-6} cells	↑*		↑**	
Cathepsin D U_{tyr}.mg $prot^{-1}$		↑**		↑*
Cathepsin D $U_{tyr}.10^{-6}$ cells	↑*	↑*	↑**	
AP	↑*			

Comparison of exposed group to corresponding control group
*p< 0.05, **p< 0.01, ***p< 0.001
↑: increase against compared group; ↓: decrease against compared group1)
LDH: lactate dehydrogenase; ACP: acid posphatase;
U_{tyr} µg of thyrosine released in an hour time; AP: alkaline phosphatase; prot: proteins

Table 7: Glass fibers - i. t. instillation, cytotoxic parameters in BAL.

Time post last instillation:	4 weeks	16 weeks	4 weeks	16 weeks
dose:	2 mg	2 mg	8 mg	8 mg
ACP nkat.g prot. $^{-1}$			↓**	
ACP nkat.10^{-6} cells		↑*		
Cathepsin D $U_{tyr}.10^{-6}$ cells		↑**	↑*	↑*
AP		↑*	↑**	

Comparison of exposed group to corresponding control group
*p< 0.05, **p< 0.01, ***p< 0.001
↑: increase against compared group; ↓: decrease against compared group
abbreviations : ACP: acid posphatase; U_{tyr} µg AP: alkaline phosphatase; prot: proteins

– that was decreased: amosite (16 weeks/2 mg and 4 and 16 weeks/8 mg) only. Viability of AM was supressed: amosite (4 and16 weeks/2 mg and 4 and 16 weeks/8 mg) and rockwool (16 weeks/8 mg). PMNs represent an important part of the acute response to fibrous dust exposure in animal models. Numerous studies report increased numbers of neutrophils in BAL-fluid after exposure to asbestos and other fibrous dusts in the acute phase [16,17].

In our study a significant increase of granulocytes was recorded 16 weeks/2 mg and 4 and 16 weeks/8 mg after exposure to asbestos and rockwool only. These fibers can persist as extracellular fibers in tissue due to their hight biopersistence and can thus be the cause of inflammatory response. Long-term persistence of granulocytes after exposure to noxious substances may be also an important predictor of development of metaplastic processes in the lungs.

The LDH enzyme is an important indicator of lung injury. The fibers longer than 8 µm cannot be engulfed completely by the cells and during "frustrated phagocytosis" they damage cytoplasmatic cell membranes resulting in lysosomal and cytoplasmatic enzyme release and subsequent damage of surrounding lung tissue. In our study, activities of LDH were increased: amosite (after both times and doses), rockwool (4 weeks/ 2 and 8 mg). The activities of AcP in cell free BALF were increased after amosite fiber exposure (16weeks/ 8mg) only. AcP level in BALF cells was increased after rockwool treatment (4weeks/2mg) and 4weeks/8mg) and glass fibres (16 weeks/2mg). Cathepsin D levels in cell free BALF increased: amosite (4 and 16 weeks/8mg)and rockwool (16weeks/2 and 8mg). Cathepsin D level in BALF cells was increased: amosite (4and 16 weeks/8mg), rockwool (4 and 16 weeks/2 mg and 4 weeks/8 mg) and glass fibers (16 weeks/2 mg and 4 and 16 weeks/8 mg). AP level was incresed: amosite

(4 and 16weeks/8mg), rockwool (4weeks/2mg) and glass fibers (16weeks/2mg and 4 weeks/8mg.The results of our study indicate that the mechanisms of action of amosite and ASMF are different and may result from differences in biopersistence. Biopersistence is a function of different parameters: low solubility of the vitreous phase in physiological media, good mechanical properties of altered fibres, limited ability of residual fragments to digestion through phagocytosis [18].

Because industrial fibrous dusts are used in many industrial branches – and by reason of their harmful effect on the respiratory tract they can induce a lot of respiratory diseases – it is necessary to test their biological impact and to choose and use only these which are the less dangerous for people and which have the less negative influence on environment.

Conclusions

Inflammatory parameters

The time and dose dependence in inflammatory biomarkers after amosite exposure (Dose: 2 and 8 mg; Time of exposure: 4 and 16 weeks) were not explicit. Significant changes (in similar large extent) of inflammatory parameters were recorded after amosite instillation in both doses and time of exposure. The results point out that also the low dose of amosite can cause inflammatory processes endurig till 16 weeks after the last instillation and suggest that in the future experiments we have to study much more lower doses with the aim to find amosite dose „treshold". The number of significantly changed inflammatory parameters in groups instilled with rockwool (mainly after higher dose) seems to be comparable with amosite exposure. Dose and time dependence (but only after higher dose) after rockwool instillation in inflammatory parameters were observed.

The inflammatory parameters after glass fiber exposure were the most significantly changed after 2mg/ 4 week exposure. Because after 16 week exposure we found out much less significantly changed inflammatory parameters we suggest that it my be caused with the change of the mechanical strenght of residual fibres in later phase by desquamation, the surface leached layer or to break in fragments of amenable size for phagocytosis. Time or dose dependence in this case was not found.

Cytotoxic parameters

Cytotoxic parameters in amosite treated groups were the most statistically changed after dose 8 mg in both times of exposure. Dose dependence was in this case evident. The time dependence was not explicit (the extent of changes was similar). Despite the fact, that the dose dependence was in this case evident, it would be suitable to pay attention to lower doses to find dose "threshold".

Cytotoxic parameters after rockwool instillation were more significantly changed after 4 week exposure independently on the dose. After 16 week exposure are the cytotoxic parameters much less influenced.

Dose dependence was evident only after 4 week exposure to glass fibers and time dependence was observed only in 2 mg dose (because only a few cytotoxic parameters were changed, we consider these dependences to be rather occasional).

Sequential arrangement of examined fibrous dust according to their harmfulness from the point of view of inflammatory and cytotoxic parameters after intratracheal instillation:
AMOSITE > ROCKWOOL > GLASS FIBERS

• Biopersistence of asbestos fibres is the highest from the our examined fibrous dusts,

• Follows Rockwool and Glass fibers,

• In chronic phase (more then 6 months after instillation) asbestos fibres persist in the lung tissue,

• Rockwool and Glass fibers are in this time eliminated,

• That means asbestos subtitutes are not so dangerous as asbestos fibres),

• But users (mainly workers in building industry) have to be also very careful and adhere to all safety ordinance using asbestos substitutes.

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