INDUSTRIAL FIBROUS DUSTS - BIOLOGICAL EFFECT TESTING

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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 3 types of substitutes (wollastonite, rock wool, glass fibers) as well as amosite asbestos were instilled at 2 doses (2 and 8 mg/animal). Following parameters in BAL fluid were investigated: Inflammatory response biomarkers: The number of leukocytes / ml, the number of alveolar macrophages (AM) / ml, the differential number of cells (% AM; Gr; Ly), phagocytic activity of AM, the levels of cytokines (TNF-alpha, IL-1, INF- gamma), the total amount of protein. 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). We found out:

| 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 > WOLLASTONITE |

**Introduction**
The effects of industrial fibrous dusts on the respiratory system represent a potential environmental and occupational health hazard for humans. Typical examples of industrial fibrous dusts are asbestos fibers which, after long-time exposure, can cause pleural plaques, asbestosis and oncological diseases. 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. These activities are in line with World Health Organization, International Labour Office and International Agency For Research On Cancer guidelines. The mechanism of action of asbestos and other fibrous structures - substitutes- are still not clearly understood.
It is necessary to study how the tissue reactions are related to the duration of the exposure, the concentration, the biopersistence, the individual disposition and the fiber parameters (nature, dimension, surface properties etc.). It is usualy believed that various cytokines, growth factors and reactive oxygen intermediates are involved, among other proinflammatory mediators, in pathological processes such as fibrosis of the lungs and pleura, bronchogenic carcinoma and malignant mesothelioma. However, fiber dimensions play also an important role in lung tissue injury after asbestos exposure. According to Stanton, the long and thin particles (length greater than $>8$ $\mu$m and diameter less then $< 0.25$ $\mu$m are considered to be especially pathogenic. In our present work, we have aimed to study the effects of asbestos (amosite) and 3 substitute fibrous dusts (wollastonite, rock wool, glass fibers) on major inflammatory and cytotoxic parameters of the respiratory system as a function of the fiber length, duration of exposure and quantity of fibrous dust dose.

**Aim**

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 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.

**Methods**

The 3 types of ASMF (wollastonite, 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) BALF cells were harvested by bronchoalveolar lavage. 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), the levels of cytokines (TNF-alpha, IL-1alpha), the total amount of protein. **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 results were statistically evaluated using Mann Whitney`s test.

**Hypothetical mechanisms of lung tissue injury according to Tarkowski and** Górski (15)



HYPOTHETICAL MECHANISMS OF CELLULAR INTERACTIONS LEADING TO LUNG TISSUE INJURY
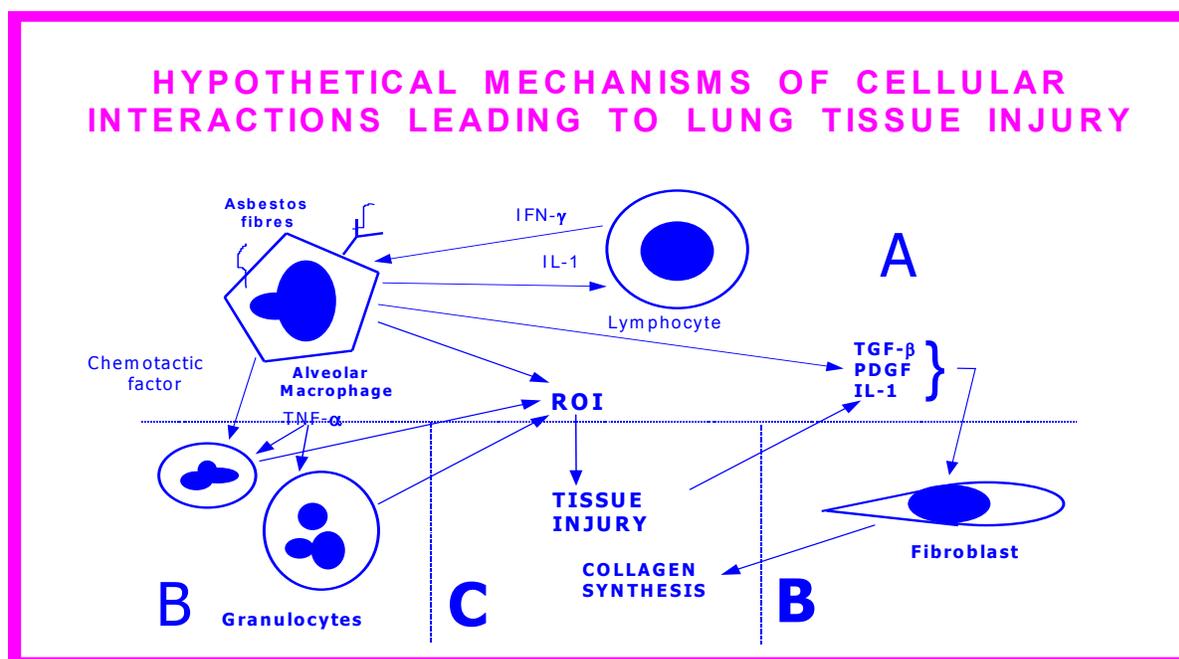
*According to this hypothesis alveolar macrophages can´t completely digest (phagocyte) long fibres - and come to "frustrated phagocytosis" and alveolar macrophages are activated.*

*These aktivated AM release many proinflamatory mediators, cytokines, fibroblast growth factors and reactive oxigen intermediates.*

*Activated AM release IL-1 and this cytokine influences lymphocytes to produce INF- gamma , which in turn activates AM again.*

*Chemotactic factor (which is also released from AM) attracts granulocytes to fibre deposition.*

ROI from granulocytes  are produced (originated) under the  influence of TNF- alpha.  ROI cause lung tissue injury. Injured tissue facilitates the transport of IL-1 which together with grow factors TGF-alpha and PDGF stimulates fibroblasts to their proliferation and collagen production and deposition.

A . In the first stage, macrophages which do not phagocyte completely long asbestos fibers,  release in an uncontrolled way an increased level of  proinflammatory mediators, radical oxygen intermediates and fibroblast growth factors. Macrophages may be additionaly  stimulated to release these factors by interferon (IFN) originating from activated lymphocytes

B. In the second stage there is an increased accumulation of chemo-attracted granulocytes which stimulated by the tumour necrosis factor-$\alpha$ (TNF$\alpha$) release an increased level of ROI. There is also concomitant accumulation of fibroblasts which proliferate in response to a transforming growth factor-$\beta$ (TGF-$\beta$), and platelet derived growth factor (PDGF),  IL-1 which results in increased collagen deposition.

C. A highly increased level of ROI, originating from macrophages and granulocytes, may cause lung tissue injury. Oxidative lung tissue injury may in turn additionally elevate release of IL-1 by macrophages and since the proliferation of fibroblasts and collagen deposition   (15 ).

**Intratracheal instilation,  Fisher 344 rats**

Design of intratracheal instillation study

| 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)
Wollastonite (ASMF)
Rock wool - RW1 (ASMF)
Glass fibres -  MMVF10 (ASMF)

**The  length and diameter 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 | |

**Wollastonite:**

| length (µm) | % fibres | diameter (µm) | % fibres |
|---|---|---|---|
| 0 -  8 | 38.5 | 0.8 | 70 |
| 9 - 15 | 14.85 | < 2.0 | 30 |
| 16 - 25 | 23.2 | | |
| 26 - 35 | 15.58 | | |
| > 35 | 7.84 | | |

**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)

**Results and 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 cause lung pathology (1,2,3). It is usually assumed that particles with a diameter greater than 1 $\mu$m do not penetrate the alveolar area of the rat. Based on animal experiments with asbestos, erionite, and MMMF, also Jaurand (4) 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. In addition, physico-chemical properties such as surface chemistry, chemical composition, surface area and biopersistence are also important factors (5).

Recent investigations 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 proliferatin (6,7) 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 (8, 9). 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 (10). 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 – that was decreased: amosite (16 weeks/2 mg and 4 and 16 weeks/8 mg) and increased after 4 weeks/8 mg in wollastonite group. Viability of AM was supressed: amosite (4 and16 weeks/2 mg and 4 and 16 weeks/8 mg),wollastonite (16 weeks/2 mg) and rockwool (16 weeks/8 mg). PMNs represent an important part of the acute response to fibrous dust exposure in animal models (5). Numerous studies report increased numbers of neutrophils in BAL-fluid after exposure to asbestos and other fibrous dusts in the acute phase (11, 12). 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 the fibers 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 $\mu$m 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 (13). In our study, activities of LDH were increased: amosite (after both times and doses), wollastonite (16 weeks/2 mg), rockwool (4 weeks/ 2 and 8 mg). The activities of AcP in cell free BALF were increased after amosite fiber exposure (16weeks/ 8mg), wollastonite (4 weeks/2mg and 16 weeks/ 8mg). 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). Levels of cytokines TNF-$\alpha$ a IL-1$\alpha$were significantly increased after all fibrous dust exposures. Normally, processes of inflammation and response to injury are associated with augmented release of TNF-$\alpha$ a IL-1$\alpha$ (14,15,16,17).

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".
- Wollastonite fibers caused  nearly no changes in inflammatory parameters (the least of all ASMF
  examined  by us). Time or dose dependence in this case was not recorded.
- 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".
- Results of cytotoxic parameter measurement point at mild tissue destruction after wollastonite
  treatment.
  The time and dose dependence of inflammatory and cytotoxic parameters
  after wollastonite exposure was not explicit.
- 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).

### DOSE  AND  TIME  DEPENDENCE  AFTER  EXPOSURE TO  FIBROUS  DUSTS
**(Dose: 2 and 8 mg;  Time of exposure: 4 and 16 weeks)**

| FIBRES | INFLAMMATORY PARAMETERS | | CYTOTOXIC PARAMETERS | |
|---|---|---|---|---|
| | Dose dependence | Time dependence | Dose dependence | Time dependence |
| AMOSITE | - * | - * | + | ± |
| WOLLASTONITE | - | - | - | - |
| ROCKWOOL | + | - * | - | - |
| GLASS FIBRES | - | - | ± | ± |

- no dependence
+ dependence
* similarity of extent of parameter changes after 2 and 8 mg of dose  or  4 and 16 weeks of
   exposure

**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  >  WOLLASTONITE**

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