Lung effects of conventional and biosoluble glass fibers as asbestos substitutes. An experimental study

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After having recognised the lung damaging, fibrogenic and tumorigenic effects of asbestos, different types of fibrous substitutes were produced world-wide.

Experimental studies of the possible adverse physiological and morphological effects of them (glass wool, basalt wool, ceramic fiber, mineral wool) are justified by the fact that the lung damages caused by asbestos fibers appear mostly 20–40 years after the exposition. The damaging effects depend above all on the geometry, composition and biopersistence of the fibers.

According to these characteristics, conventional and biosoluble fibers can be distinguished among the glass wools. Upon the effect of the more and more rigorous international recommendations, production of the latter had started at the end of July, 1998 in Hungary. 80 Wistar rats received an instillation of 2 mg conventional or biosoluble glass wool dust as a suspension in saline into the lower lobe of the right lung. The animals were sacrificed in groups after 3, 7 and 30 days, and 6 months. Histological, histochemical, immunohistochemical and electron microscopical studies were performed. The element composition was detected by Oxford ISIS and LINK energy dispersive X-ray spectrometer. The dust of glass fibers induced non-specific macrophage alveolitis and mild perivasculitis, peribronchitis. Diffuse pronounced fibrosis and adenomatoid bronchial proliferations, characteristic for the inhalation of natural mineral dusts, did not develop till the end of the experiments.

The composition and geometry of the conventional glass fibers showed no significant changes, while the samples of biosoluble glass fibers were almost completely dissolved by the 30th day of the experiments. As a result of quick solubility, local intoxication, tuberculoid granulomas and intensive vasculitis developed in one fifth of the animals at the end of the 6th month.

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Introduction

The silicium and silicates amount to about 87% of the earth’s crust. Although these compounds are ubiquitous in our world, they have produced notable problems only along with the expansion of industrialisation.1,2

The risk of occupational dust exposition has been recognised since the ancient times. The first fibrous silicate dust proved to be fibrogenic and carcinogenic in the lung was the asbestos. Many substitutes of asbestos induced similar occupational lesions.

Military service seems to be free of the inhalation risk of fibrous dusts. It should be considered, however, that army exercises in the densely populated area European territories might happen on contaminated areas and not only the fighting activity but also the transport on isolated vehicles, machinery creation of bunkers, explosions in isolated buildings are connected with a considerable exposure to dusts and many of them are silicates and of fibrous character. It is also important in relation to national defence that the insulation materials of the buildings demolished in war-time (also at rubble clearance) as well as the hearth and noise defence in the military vehicles may create intensive outdoor and indoor environmental pollution and threaten both the civil inhabitants and military troops.3 This type of mixed dust pollution may cause different lung diseases. Exposition to fibrous-, especially asbestos-dusts, can lead to fibrosis, lung cancer and malignant mesotheliomas.

For substitution of asbestos many types of man made mineral fibers have been introduced.

To the group of the so called man-made mineral fibers (MMMF) belong the slagwool, mineralwool, basaltwool, glass wool and microfibers, like the thermostesistent ceramic wool. The toxicity of the above mentioned fibers is determined by many factors: diameter (<3 µm), length (<100 µm), physicochemical characteristics, intensity of exposition and biopersistency. The latter is much shorter in case of MMMFs than asbestos.4

The chemical composition of the different MMMFs depends directly on the raw material used for production.5 Natural mineral fibers reveal mostly crystalline structure, while MMMFs deriving from amorph silicates need different metal oxides and complementary substances. The silicates are of particular significance since the International Agency for Research on Cancer (IARC) ranged the danger of exposition to crystalline silicates into group I of human carcinogens in 1997. The glass industry is one of the most intensive consumers of natural silicates. Besides the optical- table- and bottle glass the production of glass wool is gaining more and more significance, as a substitute to the evidently carcinogenic asbestos. The use of this product was introduced only a few decades ago, so there is not enough experience yet at hand in respect to its
human lung effects. Biosoluble glass wool was developed a few years back in the western countries, because of the strict hygienic regulations. This material dissolves fully at the pH values of the pulmonary interstitium within a few months. Comparing the dissolution of mineral and glass fibers, the removal of the latter was quicker at pH 4.5 of the alveoli, than in the interstitium at pH 7.4,6,7

The demands of the market have also changed the domestic prescriptions as a consequence of which the Salgótarján Therwoolin Glassworks has developed the Hungarian variant of biosoluble glasswool. This product differs from the conventional one by its aluminium and boron content. The aluminium oxide content is one quarter, the boron content the double of the former product (Table 1).

Table 1. The different composition of the two types of Hungarian glasswool

<table>
<thead>
<tr>
<th>Composition of glasswool (%)</th>
<th>Conventional glasswool (was made before 1998)</th>
<th>Biosoluble glasswool (was made after 1998)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al₂O₃</td>
<td>4.0</td>
<td>1.0</td>
</tr>
<tr>
<td>B₂O₃</td>
<td>6.0</td>
<td>12.0</td>
</tr>
<tr>
<td>CaO</td>
<td>7.7</td>
<td>7.5</td>
</tr>
<tr>
<td>MgO</td>
<td>2.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Na₂O</td>
<td>16.8</td>
<td>17.8</td>
</tr>
<tr>
<td>K₂O</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>SiO₂</td>
<td>62.6</td>
<td>59.0</td>
</tr>
<tr>
<td>KI</td>
<td>25.3</td>
<td>38.0</td>
</tr>
</tbody>
</table>

KI=CaO+MgO+NaN₂O₄+K₂O+B₂O₃+2×(Al₂O₃)

**Material and methods**

In our experiments we compared the lung and connective tissue effects of phenol-formaldehyde coated conventional (SCG ), and biosoluble (SBG) glasswool products of the Salgótarján Therwoolin Glassworks as well as the German made B0901 biosoluble glasswool (GBG) (Figures 1 and 2).

The wools were either pulverised or as dust samples collected in the Salgótarján factory. The size and elementary composition of the particles was measured by a DSM 950 Zeiss scanning electron microscope and by connected LINK or Oxford Instruments EDS ISIS energy dispersive X-ray spectrometers.
Dust samples were suspended in physiological saline (10 mg/ml) and 2 mgs were instilled by a tracheostoma into the right lower lobe of three months old (250–300 g) Wistar rats. As the exact localisation of glass fibers is not possible macroscopically and they are almost invisible by light microscope, 0.5% India ink was mixed into the suspension. Control groups received India ink stained saline instilled into the lung.

Animals were sacrificed in groups 3, 7, 30 days and 6 months after the treatment. Bronchoalveolar lavage was performed on three animals from each group. Following intrabronchial and immersion formalin fixation, paraffin embedding, haematoxylin-
eosin staining of the sections served the purpose of general orientation. Collagen fibers were stained by Sirius-red, the secretion activity by Alcian blue. The integrity of the connective tissue matrix was examined by immuno-histochemical demonstration of type 1 collagen. Alveolar macrophages from bronchoalveolar lavages were stained by Giemsa. The ultrastructural changes of selected representative specimens from the lung tissue were examined after glutaraldehyde-osmium fixation and epoxy resin embedding with uranyl acetate-lead citrate staining. Neutron activating analysis was performed on the native glassfibers and on the lung tissue one month following the instillation.

Results

The pathogenicity of three types of glasswool were compared. The geometry was characteristic, resulting from the production. Numerous fibres possessed club-like thickened ends, which thus prevented direct, membrane-damaging entry into the cell. The rest of the fibres had straight or showed fractured ends. The geometry of the particles allowed the access of dust into the alveoli or the impactation of fibers in the bronchial mucosa (Table 2). All of the glass fibres revealed varying fibrelengths and wide-ranging thickness parameters. The analyses displayed slight quantitative deviations between the elementary composition of the thinner and thicker fibres. Based on our former experiences, this is significant in relation to penetration, thus tumour-causing irritative effect (Figure 3).

![Optical microscopic image of a biosoluble fiber glass sample](image-url)
The occurrence of all glass fibers was quickly reduced with time in the bronchoalveolar lavages. The fibers were incorporated or surrounded by macrophages, depending on their 2 to 25 µm lengths (Figure 4).

![Image of incorporated glass fiber in the cytoplasm of macrophage from bronchoalveolar lavage on the third days of the instillation](image)

**Figure 4.** Incorporated glass fiber in the cytoplasm of macrophage from bronchoalveolar lavage on the third days of the instillation

Intensive, non specific macrophage alveolitis developed in all three animal groups already on the third day after instillation (Figure 5).

The alveolitis revealed a decreasing tendency with time. The peak of acute peribronchial and perivascular neutrophilic infiltration was observed on the seventh day and was least pronounced in the group treated with GBG fiber dust. The secretory activity of the mucosa was increased in all groups at any examined interval. The fibers on the mucosal surface were embedded in mucus. In contrast to the asbestos-instilled animals of former experiments, bronchial adenomatoid proliferations or fibrosis failed to develop during the relatively short time of the experiment.

Electron microscopic examinations revealed a slight increase in the number of type II pneumocytes.
Alveolar macrophages of all groups contained a number of phagosomes. Fibers of the biosoluble glass wool were found only scattered in the alveolar and interstitial macrophages. Occasional giant cells containing intra- or subepithelial glass fiber granulomas were found in the animals of the two weeks group (Figure 6). After one month the macrophage alveolitis decreased in all groups. Neutron activating analysis found $228\pm15 \text{ ppm arsenic and } 1800\pm\text{ppm stibium in the SBG fibers}$. After one month the concentration of arsenic was $8.6\pm0.6 \text{ ppm in the analysed lung tissue}$. This value was $1.9\pm0.2 \text{ ppm in the control group’s lung}$.

At the end of the experimental period the chronic inflammatory reaction was very slight, and we did not detect fibrosis and adenomatoid proliferation like in case of asbestos installation in earlier. In some cases instilled with SBG, tuberculoid like granulomas were developed at the end of 6th month.
Discussion

The lung damaging effects of fibrous dusts depend on several factors, among which the biosolubility and elementary composition were studied in this experiments. The concentration in the lung tissue was mainly related to geometry. In the first part of the experiments the long fibres escaped the lungs owning to the cleaning mechanism of the respiratory cilia. The approx. 5 µm long fibres became decomposed in the macrophages, while a few longer ones reached the interstitium.

Transmission electron microscopic examinations revealed the numeric increase of type II pneumocytes, while it was not recognisable by conventional histology in form of cuboidal alveolar epithel metaplasia. This should be an early discrete defence reaction. The count of the biosoluble glass fibers decreased much quicker than the conventional ones.

Based on the experiments, the conventional and biosoluble glass fibers have no severe tissue damaging effects and induce neither fibrosing processes nor the development of benign or malignant cell proliferation in the lungs. The local dissolution of biosoluble glass fibers might have later consequences caused by the arsenic and antimonial compounds. The tuberculoid reaction of the lung tissue reflect type IV immunological process with uncertain immunological background.

In spite of the slight tissue changes, observed optimistic conclusions can’t be drawn. Owning to the chronic inhalation, and other environmental factors the glass fibers might have further unfavourable influence on human lung.

Perhaps the most important of them is smoking, which can used as tranquilliser during the military operations. Dust exposure is inevitable, can’t be controlled and not at all expected during certain military activities. It is by all means higher than in the civilian life. On the other hand, rats are quite resistant to carcinogenic effects. Therefore, despite the present favourable results, further studies should be conducted since flow cytometric studies on cells taking part in defence and the biochemical evaluation of free radical production might be helpful in weighing the risk of glass dust inhalation.

References


