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EFFECT OF INTRATRACHEAL FIBRES EXPOSURE ON THE RAT LUNG

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The changes in antioxidant status of rat lung after intratracheal instillation of stone-wool and glass fibres were studied. The animals were exposed to 2 or 8 mg of fibres for 4 or 16 weeks, the bronchoalveolar lavage was performed and the activity of superoxide dismutase, glutathione peroxidase and the total amount of glutathione was estimated both in tissue and in cell free fraction of bronchoalveolar lavage and the ascorbic acid was determined in lung tissue. The results showed the higher burden by stone-wool. Most changes were detected in groups exposed to higher dose of fibres for shorter time period, the most sensitive parameter was superoxide dismutase. The lung tissue was studied also by light microscopy and transmission electron microscopy.

INTRODUCTION

Because the inhalation of asbestos, a naturally occurring inorganic fibrous material, is associated with lung fibrosis and thoracic cancer, consensus has been raised about the possible health effects of synthetic vitreous fibres[1]. Those are traditionally divided into three subcategories based on the composition: fibreglass, mineral wool and refractory ceramic fibres. Toxicity of several fibrous and nonfibrous particulates has been associated with the production of reactive oxygen species generated either from the particles themselves or by inflammatory cells activated by particles[2]. The present study is focused on the effect of fibreglass and mineral wool (stone-wool) on the antioxidant status of lung in experimental rats.

MATERIAL AND METHODS

Animals. Male Fischer rats 344 (Anlab, Prague, Czech Republic) weighing 180-200g were used. They were housed under standard laboratory conditions and were given a conventional laboratory diet ST1 (TOP-Dovo, Horné Dubové, Slovak Republic) and tap water *ad libitum.*

The study was conducted with the approval of the Animal Ethics Committee of Research Base of Slovak Medical University, Bratislava and in accordance with the guideliness of European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes.

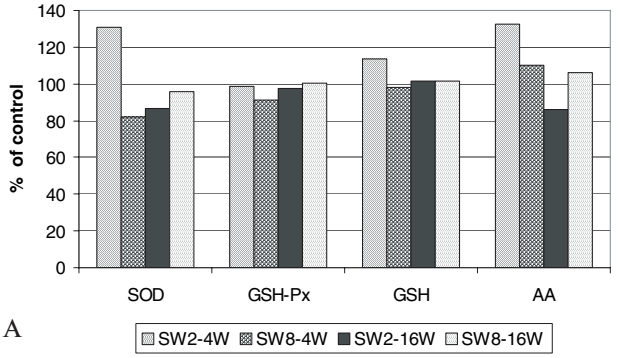
Materials. Superoxide dismutase was estimated using the Randox kit (Randox laboratories Ltd., UK). All other chemicals were supplied by Sigma.

Intratracheal exposure. There were done two independent experiments. In the first one the animals were exposed to stone-wool (SW) and in the second one to glass fibres MMVF 10 (GF). In every experiment the animals were randomly divided into 8 groups per 6 and they were exposed to two different doses (2 mg and 8 mg) of fibres and for two different lengths of exposure (4 and 16 weeks from the last instillation). The 8 mg dose was instilled in 4 doses per 2 mg in 0.2 ml saline in week intervals. Each exposed group has a corresponding control group instilled with the same volume of saline. After finishing the exposure animals were exsanguinated in anaesthesia and the bronchoalveolar lavage was performed[3]. The bronchoalveolar lavage fluid (BALF) was centrifuged and the cell-free fraction was separated and analysed. The lung tissue was homogenised in PBS and the 10% homogenate was centrifuged (30 min, 10 000 rpm). Supernatant was used for analysis. All samples were stored in aliquots at –80 °C till analysis.

Biochemical analysis. Superoxide dismutase (SOD) activity was estimated using RANDOX kit; the method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye; the activity is measured by the degree of inhibition of this reaction. Glutathione peroxidase activity (GSH-Px) was estimated using cumene hydroperoxide as a substrate[4]. One unit of GSH-Px activity was defined as μmol NADPH oxidized per minute and the results were expressed as U/mg protein. Total glutathione (GSH) was determined using the GSH reductase method[5]. Ascorbic acid (AA) was estimated spectrophotometrically by 2,4-dinitrophenylhydrazine method[6] and protein according Lowry et al[7].

Morphology. The detail methods were described in previous paper[8].

Statistical analysis. The results were evalueted by Wilcoxon's test.



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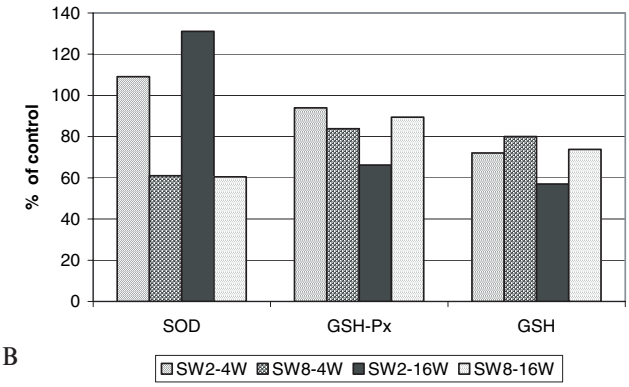

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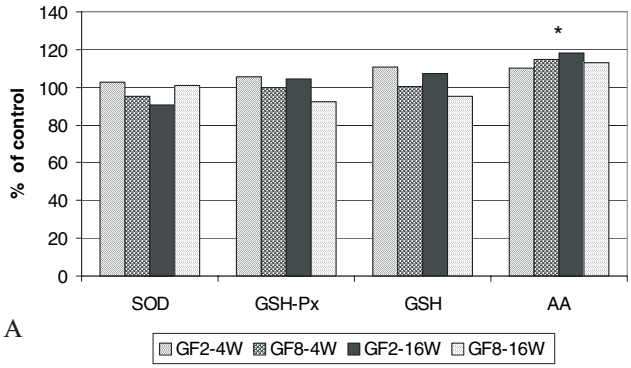
Fig. 1. Effect of intratracheal stone-wool exposure on the antioxidant status in lung tissue (A) and BALF (B). Values are given as percentage of control group. SOD – superoxide dismutase, GSH-Px – glutathione peroxidase, GSH – glutathione, AA – ascorbic acid, SW2-4W exposure to 2 mg of stone wool for 4 weeks, SW8-4W to 8 mg for 4 weeks, SW2-16W to 2 mg for 16 weeks, SW8-16W to 8 mg for 16 weeks, * $P < 0.05$ – versus control.



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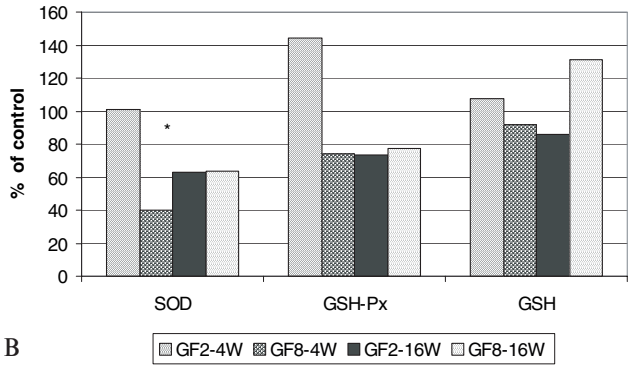

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Fig. 2. Effect of intratracheal glass fibres exposure on the antioxidant status in lung tissue (A) and BALF (B). Values are given as percentage of control group. SOD – superoxide dismutase, GSH-Px – glutathione peroxidase, GSH – glutathione, AA – ascorbic acid, GF2-4W exposure to 2 mg of glass fibres for 4 weeks, GF8-4W to 8 mg for 4 weeks, GF2-16W to 2 mg for 16 weeks, GF8-16W to 8 mg for 16 weeks, * $P < 0.05$ – versus control.

RESULTS AND DISCUSSION

The antioxidant status of the lung tissue after intratracheal exposure to stone-wool is shown in Fig. 1A and the status of BALF in Fig. 1B. All parameters in the exposed groups are compared to the corresponding control. The activity of GSH-Px was not significantly changed either in lung tissue nor in BALF. The level of total GSH was not changed in lung tissue but it was lowered in BALF in all exposed groups, with statistical significance in group SW2-4W and SW2-16W. SOD was statistically significantly lower in lung tissue in the goup SW8-4W and in BALF in both group exposed to the higher dose of stone wool. AA was enhanced in lung tissue in both group exposed to the fibres for shorter period.

Figures 2A and 2B show the antioxidant status after intratracheal exposure to glass fibres in lung tissue and BALF, respectively. There were no statistically significant differences between exposed and control groups in lung tissue, only the AA was enhanced in group exposed for 4 weeks to 8 mg of glass fibres (GF8-4W). In BALF the only significant decrease was seen in the SOD in the group exposed to 8 mg for 4 weeks.

The histological study revealed very weak fibrogenic effect of stone-wool. The fibrosis showed a minimal tendency for progression. In lung exposed to glass fibres only mild dose-dependent histological alterations were seen. The ultrastructural studies justified the results of light microscopy.

The purpose of the present work was to investigate and compare the changes in the level of some antioxidants evoked by intratracheal instillation of two types of fibres: stone-wool (SW) and glass fibres (GF). Two doses and two time intervals were compared either. In animals exposed to stone-wool most changes were seen in group exposed to higher dose for shorter period (SW8-4W) and no significant changes in group exposed to lower dose for longer time (SW2-16W). The most sensitive parameter was SOD which was significantly decreased in BALF in groups exposed to higher dose for both

time intervals (SW8-4W, SW8-16W) and in lung tissue in group exposed to higher dose for shorter time (SW8-4W). In animals exposed to glass fibres, only in group exposed to high dose for shorter time (GF8-4W) significant changes were seen: SOD in BALF was decreased and AA in tissue was increased. By comparing the effect of these two fibres we can conclude that exposure to stone wool brought more changes than exposure to glass fibres, the most critical was the exposure to higher dose for shorter time interval and the most sensitive parameter was SOD, predominantly in BALF.

To factors influencing the toxicity of various fibres belongs also biopersistence which involve the effect of chemical composition and structure, dissolution characteristics and fibre size[9]. This can explain the different results of exposure to fibres with similar size and the highest toxicity in groups exposed to shorter time to higher fiber dose. The longer biopersistance of stone-wool compared to glass fibres was confirmed also by above cited author. The changes of oxidative potential of stone-wool in time was proved also in *in vitro* system[10].

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