

INTRATRACHEAL FIBER GLASS INSTILLATION IN RATS: IL8 AND LYMPHOCYTES LEVELS IN BRONCHOALVEOLAR LAVAGE, CORRELATION WITH THE HISTOPATHOLOGICAL FINDINGS

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Abstract

Introduction. Fiberglass (FG) is the largest category of man-made mineral fibers. Many types of FG are manufactured for specific uses building insulation, air handling, and sound absorption. Because of increasing use and potential for widespread human exposure, a chronic toxicity instillation study was conducted in Wistar rats, which were found to be sensitive to the induction of mesotheliomas with another MMVF.

Aim. The present study is focused on the effect of fiber glass on lung through intratracheal exposure, the analysis of bronchoalveolar lavage and measurement of IL8 levels, lymphocytes number and histopathological finding after the exposure period.

Material and method. Four groups of 8 female Wistar rats were included in the study. The animals were divided into three groups of 8 each, exposed to different doses of FG and one control group. The first group (1-8) was exposed to 6 mg dose/0.2 ml saline 5 days/week for 10 weeks, the second (9-16) group was exposed to 10 mg/0.2 ml saline 5 days/week 10 weeks, the third group (17-24) was exposed to 12 mg FG/0.2 ml saline solution 5 days/week 10 weeks and the control group (25-32) was exposed to the same volume of saline. The fibers had been size selected to be rat respirable. At the end of the exposure period of 10 weeks the rats were killed one week after the last exposure. Following preparation of the lungs, they were lavaged with 2x5 ml saline without massage. The lavage fluid was collected in calibrated tubes and harvested volume was recorded. Supernatant was obtained after centrifugation at 1,500 r.p.m for 5 minutes and IL8 levels and lymphocytes number were measured.

Results. The IL8 levels were found to be dose related; the first group had values ranging from 10 to 19.8 pg/ml and the total lymphocytes number in the bronchoalveolar lavage fluid ranging from 1,500-1,900 and minimal/slight inflammatory lesions. The second group had the IL8 levels ranging between 60.4-80.4 pg/ml, lymphocytes number between 680-881 and moderate to marked inflammatory lesions. For the third group the IL8 values ranged between 88.3-113.2, the lymphocytes number ranged between 241-342 and the histopathological findings were marked and severe including emphysema, lung and pleural fibrosis. The control group had IL8 values between 10-19.4, there were no lymphocytes in the bronchoalveolar lavage and no histopathological findings.

Conclusion. These findings indicate that IL8 levels were dose related and IL8 levels have an inverse correlation with lymphocytes count in BAL, also correlated with the histopathological findings for the studied groups.

Keywords: fiber glass, IL8, lymphocytes, bronchoalveolar lavage, histopathology.

Aims

In the last decades there have been significant changes regarding the use of materials in different areas, changes claimed by the requirements regarding

the production of the consumer goods and also by the ecological requirements.

Fiberglass is the largest category of man made mineral fibers. Many types of fiber glass are manufactured for specific uses such as building insulation, air handling, filtration and sound absorption, aeronautic industry etc. The continuous development of different types of synthetic

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fibers is a challenge for toxicologists. The International Agency for Cancer Research (IARC) has classified fiber glass as possibly carcinogenic fiber [1,2,3]. Respirable fiber glass is a class of material that was anticipated as possibly carcinogenic due to a large range of animal studies and the obtained results [4,5].

Because of its increasing use and potential for widespread human exposure, a chronic toxicity instillation study was conducted in Wistar rats, which were found sensitive to the induction of mesotheliomas by another man made vitreous fibers [6,7,8].

Exposure to fiber glass and the carcinogenicity was demonstrated do be influenced by several parameters: inhaled dose, fiber length, chemical composition and the fibers bio-persistence in lung [1].

The present study is focused on the effect of fiber glass on the lung through intratracheal exposure, the analysis of bronchoalveolar lavage and measurement of IL8 levels, lymphocyte count and histopathological findings after the exposure period.

Material and method

For this study female Wistar rats weighting 150-250 g were used. The animals were housed under standard laboratory conditions and were given a standard laboratory diet and tap water *ad libitum* [9,10,11,12,13]. The age of the animals at the start of the study was 9-10 weeks. When not being exposed, the rats were housed in groups of eight in polycarbonated cages, the rooms were air conditioned and had a monitored environment with a temperature of 22+/-2°C. A period of 12 h of artificial light and 12 h of darkness were used [14,15,16].

The study was conducted with the approval of the Ethics Committee Of Research of Iuliu Hațieganu Medical University Cluj Napoca with the guidelines of European Convention for the protection of Vertebrate animals used for experimental purposes [10].

Four groups of eight female Wistar rats, randomly selected, were included in this study. Wistar rats were recommended by an EPA workshop (Vu et al., 1996) [17] for use in subchronic and chronic toxicity studies of fibers. The animals were divided into three groups of eight each, exposed to different doses of fiber glass and one control group. In the first group the rats were noted from 1-8 and they were exposed through intratracheal instillation to 6 mg fiber glass/0.2 ml saline solution 5 days/week for 10 weeks. In the second group the rats were noted 9-16 and were exposed to 10 mg/0.2 ml saline solution 5 days/week 10 weeks.

The third group (17-24) was exposed to the highest dose: 12 mg fiber glass/0.2 ml saline solution for the same period of time and the control group (25-32) was exposed to the same volume of saline.

The fiber size was selected to be rat respirable to a length of 20 µm and diameter of 1 µm. The exposure period was 10 weeks and the rats were killed one week after the

last exposure.

Observations, examinations and measurements

The rats were examined daily for clinical signs, they were individually examined outside the cage once a week. The body weight was recorded once every week for ten weeks. Necropsy was performed on all animals. At the scheduled kills the lungs were removed *in toto*, weighed without the trachea and carefully examined.

The preparation of the lungs: they were washed 2 times with 5 ml of saline solution without massage using the method of Henderson et al. (1987) [18]. The lavage fluid was collected in calibrated tubes and harvested volume was recorded. One milliliter of the cell free supernatant was obtained after centrifugation at 1,500 rpm for 5 minutes and was stored at -70°C and used for the determination of IL8 concentration. Bronchoalveolar lavage cytology, bacteriological exam and IL8 concentrations were measured for evaluation of the inflammatory response [19].

The assessment also included the histological examination of multilevel biopsies from the trachea, main bronchia, right upper lobe, cardial lobe, diaphragmatic lobe. The slides were stained with hematoxilin-eosin and red sirius was used as a specific staining for collagen. For the histopathological exam the tissue sample was placed into fixing solution: buffered formalin 10%, pH=7 for 24 hours. Sections were made at 4-5 microns using Leica Microtome RM 2125 RT. After staining the slides were examined with a Olympus microscope and the images were obtained with a digital camera (Olympus DP25). Hematoxilin-eosin staining (HE) was used in order to obtain a better differentiation of the cell types. The Sirius Red stain (SR) was used for the observation of the collagen fibers in a colour selective way.

Results

The obtained values of IL8 and the lymphocytes number in the bronchoalveolar lavage for the studied groups are presented in Table I.

Table I. BAL IL8 levels and Ly number in BAL.

Subject	S1	S2	S3	S4	S5	S6	S7	S8
IL8 (pg/ml)	20.2	26.0	38.4	36	15.8	38.4	17.5	33.4
Ly (no/mm ³)	1500	1500	1800	1700	1900	1500	1800	1900
Subject	S9	S10	S11	S12	S13	S14	S15	S16
IL8 (pg/ml)	70.6	76.3	80.4	68.5	56.6	60.4	76.6	63.2
Ly (no/mm ³)	804	916	840	680	719	881	780	802
Subject	S17	S18	S19	S20	S21	S22	S23	S24
IL8 (pg/ml)	113	110.3	101.6	113.2	90.6	88.3	106.5	90.7
Ly (no/mm ³)	301	324	241	289	255	311	342	275
Subject	S25	S26	S27	S28	S29	S30	S31	S32
IL8 (pg/ml)	12.3	10	19.4	12.6	18.8	10	12.3	18.9

The histopathological examination of the trachea for the subjects in the first groups shows tracheitis with mononuclear cells infiltrate extending from mucosa to the muscular layer (Figure 1). The biopsies taken from the

main bronchia highlights a proliferative bronchitis (Figure 2) and from the upper right lobe a interstitial pneumonia with inflammatory infiltrate with mononucler cells and PMN (Figure 3).

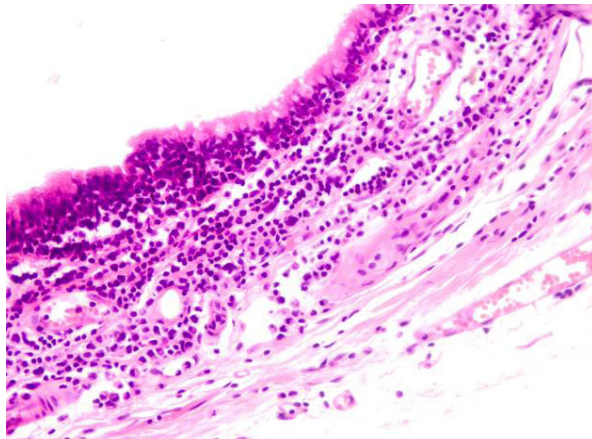


Figure 1. Tracheitis first group (HE, x200).

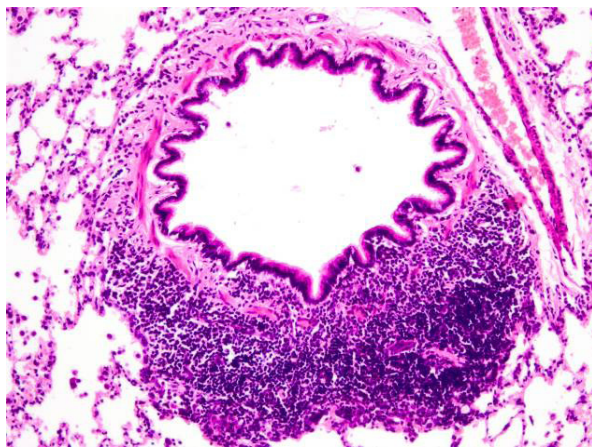


Figure 2. Lympho-proliferative bronchitis (HE, x200).

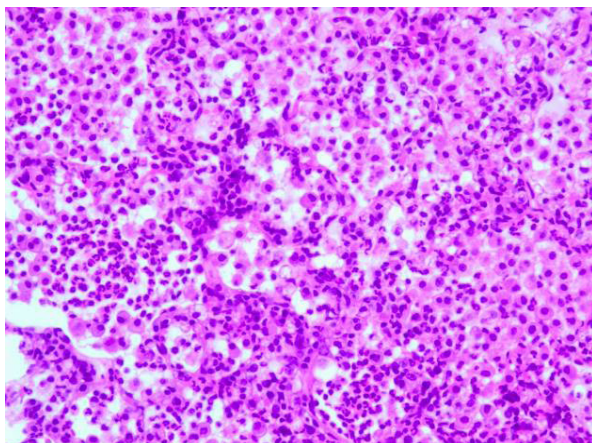


Figure 3. Interstitial pneumonia (HE, x200).

For the second groups exposed to a higher dose of fiber glass (10 mg) the histopathological exam evidenced tracheitis with mononucler cell infiltrate and macrophages (Figure 4), chronic bronchitis (Figure 5) and chronic interstitial pneumonia with proliferation of peribronchial lymph tissue and discrete fibrosis (Figure 6).

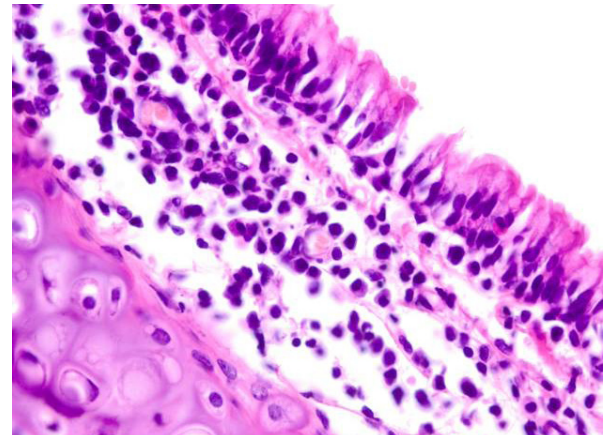


Figure 4. Chronic tracheitis, 2 (HE, x200).

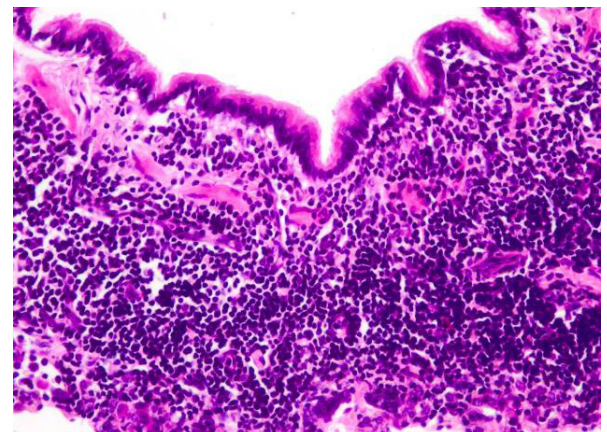


Figure 5. Lung, cardial lobe, pneumonia, proliferation of the lymph tissue (HE, x200).

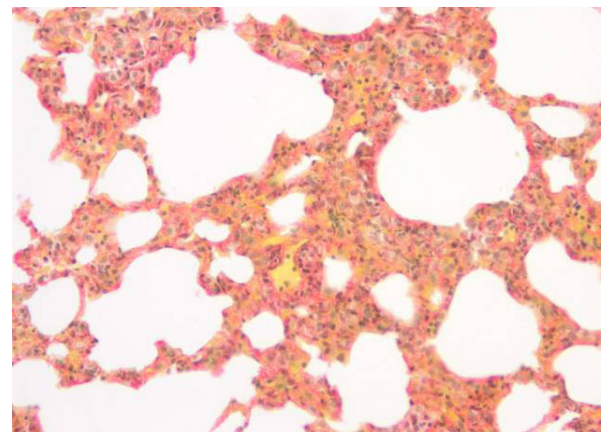


Figure 6. Interstitial chronic pneumonitis. Discrete fibrosis (SR, x100).

The histopathological examination of the trachea, main bronchia and lung of the subjects from the third group show chronic tracheitis and bronchitis, desquamating alveolitis (Figure 7) and extended fibrosis (Figure 8).

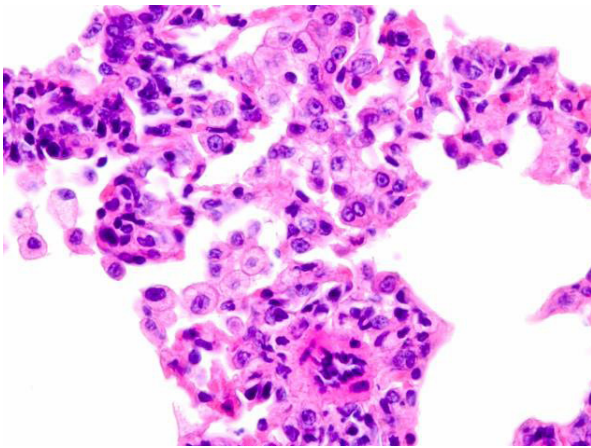


Figure 7. Lung, desquamating alveolitis, alveolar epithelization. Case 24 (HE, x200).

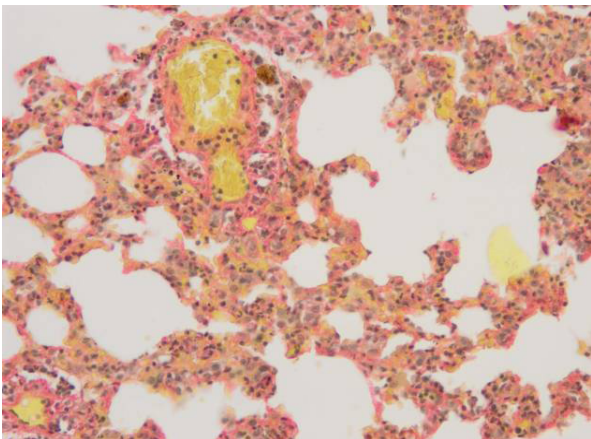


Figure 8. Chronic interstitial pneumonia. Fibrosis (SR, x100).

Study of IL8 and Ly number in BAL

For the statistical analysis Sigma Plot 12 was used. The Kruskal-Wallis one way analysis of variance on ranks was used to determine the average values of the IL 8 and lymphocytes number in the studied groups and to compare the groups. The average values of IL 8 levels per group were higher then the average values obtained fort the control group: 29.7 for the first group, 69.5 second group, 103.8 for the third group vs 14 for the control group which shows a direct correlation with the instilled dose (Figure 9).

The average values of the lymphocytes count was also determined using the Kruskal-Wallis one way analysis test for the test groups taking into consideration that the control group had no lymphocytes present in the bronchoalveolar lavage, the results showing an inverse relationship with the administered dose (Figure 10).

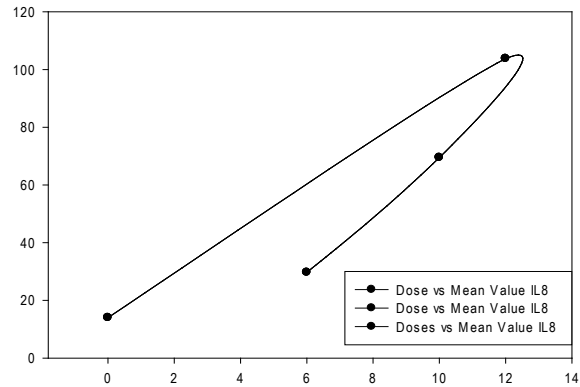


Figure 9.

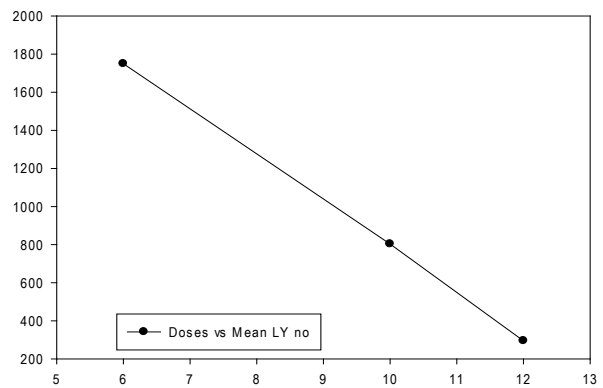


Figure 10.

The correlation between IL8 levels and the lymphocyte count was determined using the Spearman rank order correlation which showed that the IL8 values were increasing while the lymphocytes number were decreasing in the study groups.

The histological results for the study groups were graded according to alveolar broncholization, microgranulomas and collagen deposition at the bronchiolar-alveolar junction, pleural collagen deposition, parenchymal collagen deposition and by the presence of the macrophages in the alveolar lumina and graded according to distribution, severity and morphological character and scored from 0→5. There were no changes in the control group.

For the test groups an Anovo on ranks test was performed and the Tukey test was used for the comparison of the results (Figure 11). The mean values of the histopathological changes are also related to the administered dose. For the third group (highest dose 12 mg) a grater extent of lesion was found than in the other groups, with extended fibrosis and lung emphysema.

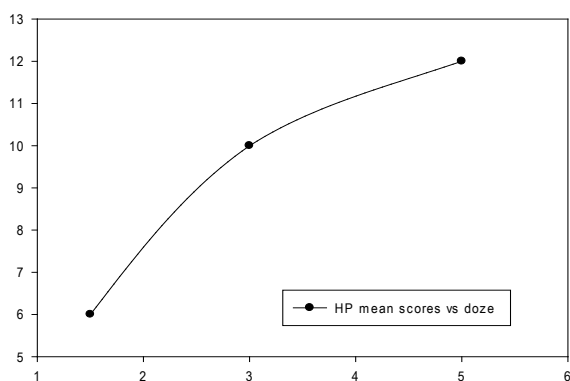


Figura 11.

Discussion and conclusions

The primary objective of this study was to assess the pathological effects of fiber glass administered in three different doses through intratracheal instillation. In the present study we wanted to assess one biochemical parameter: IL8 in the bronchoalveolar lavage at the end of the exposure period, the lymphocytes count in BAL and the histopathological lesions.

In the control group the IL8 level in BALF had a mean of 14, there were no significant number of lymphocytes in the bronchoalveolar fluid and there were no histopathological changes. For the First group a significant effect on the IL8 was found at the end of the study, an increased number of lymphocytes in the BAL and minimal or slight inflammatory lesion (grades 1-2). In the second group a high concentration of IL8 was also found in the bronchoalveolar lavage, a decreased number of lymphocytes and moderate to marked inflammatory lesions with discrete fibrosis. The third group which was exposed to the highest dose showed the highest IL8 levels in the bronchoalveolar lavage with the lowest lymphocytes count and marked to massive histopathological lesions (grades 4, 5) with collagen proliferation, lung fibrosis, pleural fibrosis, emphysema. The statistical analysis of the obtained results showed a correlation of the IL8 levels with the dose, an inverse relationship of the Ly number in BAL with the dose. These data are sustained by the histopathological changes also found to be dose related.

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