EVALUATION OF BRONCHOALVEOLAR LAVAGE FLUID CYTOTOXIC PARAMETERS AFTER INHALATION EXPOSURE TO AMOSITE AND WOLLASTONITE FIBROUS DUSTS COMBINED WITH CIGARETTE SMOKE

Černá S.[1], Beňo M.[1], Hurbánková M.[1], Kováčiková Z.[1], Bobek P.[1], Kyrtopoulos S. A.[2]

[1] Slovak Medical University, Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic

[2] Institute of Biological Research and Biotechnology, National Hellenic Research, Foundation, Athens, Greece

SUMMARY

The aim of this work was to compare the influence of amosite-asbestos and wollastonite fibrous dusts combined with cigarette smoke on chosen cytotoxic parameters of bronchoalveolar lavage fluid (BALF) in rats. Fisher 344 rats inhaled wollastonite or amosite fibrous dusts (60 or 30 mg.m^{-3} air) one hour every two days combined with daily breathing of diluted mainstream tobacco smoke (30 mg of TPM.m^{-3} air). The experiment lasted 6 months. After sacrifying the animals bronchoalveolar lavage (BAL) was performed and the viability and phagocytic activity of alveolar macrophages (AM), lactate dehydrogenase (LDH) and alkaline phopshatase activity (in the cell-free BALF), acid phosphatase (ACP) and cathepsin D activity (in cell-free BALF and BAL cell suspension) were examined. Exposure to amosite without tobacco smoke significantly decreased the viability of AM and increased the cathepsin D activity in BAL cells. Exposure to wollastonite significantly increased only the cathepsin D activity in BAL cells. Smoking significantly depressed the phagocytic activity of AM and amplified the amosite-induced increase of lysosomal enzyme activities - especially the activity of cathepsin D in BAL cells.

Key words: smoking, inhalation exposure, amosite, wollastonite, phagocytic activity, viability, lysosomal enzymes, lactate dehydrogenase, alkaline phosphatase

Address for correspondence: S. Černá, Slovak Medical University, Institute of Preventive and Clinical Medicine, Limbová 12, 833 03 Bratislava, Slovak Republic. E-mail: cerna@upkm.sk

INTRODUCTION

The pathogenic effect of asbestos has been known since the beginning of the 20th century, but the interest in it increased especially after the Second World War, when the pathogenicity of asbestos became a world problem (1). Epidemiological studies on people exposed to asbestos together with animal experiments and studies in vitro confirmed the adverse effect of asbestos including its

genotoxicity and carcinogenity (2, 3). The great health risk of asbestos materials led to the ban of its usage in most of countries and started searching for other materials with comparable technical parameters but with lower health risk. Various types of man-made mineral fibers have been developed and together with some naturally ocurring mineral fibres they are used as asbestos substitutes (4). Their biological effects are still unclear and have to be evaluated. In this experiment amosite-asbestos was used as a positive control for the evaluation of effects of wollastonite – an asbestos substitute.

Wollastonite is a naturally occurring acicular metasilicate. Wollastonite fibers are rather similar in form, length, and diameter to amphibole asbestos fibers but mineralogically they are different (5). Wollastonite is considered to have lower negative effects on the respiratory tract than asbestos (6, 7). The hypothesis explaining these differences is based on the more effective elimination (lower biopersistence) of wollastonite fibres (8). Despite of this fact, the impact of wollastonite on the respiratory tract is not quite negligible (5,6) and might be amplified by another noxae, e.g. cigarette smoke.

MATERIAL AND METHODS

Details for animals, animal maintenace, exposure devices and schemes and amosite fibre size distribution are described in the work of Beňo et. al published in this journal issue (9). Wollastonite fibre size distribution is shown in Table 1.

BAL performance and cell separation. After the 6 month exposure the animals were anesthetised by thiopental (150 mg/kg of animal), exsanguinated by cutting the vena cava caudalis and BAL was performed by modified method of Myrvik described in work of Hurbánková and Kaiglová (6). The BAL fluid was centrifuged at 450 *g* for 10 minutes at 4 °C, the cell free bronchoalveolar lavage fluid (BALF) was transferred into the clean glass tubes and the cell sediment was resuspended and adjusted to 1×10^6 cells/ml by adding sterile saline solution

Examined parameters of BAL: phagocytic activity and viability of AM (6), activity of LDH (LD 105 UV Lachema Brno, Czech Republic) and AP (10) in cell-free BALF, activity of ACP (Acid phosphatase EC 3.1.3.2. Randox Laboratories, Antrim, U.K.) and cathepsin D (11) in cell-free BALF and BAL cell sediment. Protein was measured by the method of Lowry. Activity of LDH was measured immediately after the BAL cell separation. Samples of cell-free BALF and BAL cell suspension for measuring other enzyme activities were stored at –70 °C until the time of analysis.

Statistical methods. Mann-Whitney test was used for the comparison of values from chosen groups.

RESULTS

Significant decrease of AM viability was detected only after exposure to higher dose (60 mg.m^{-3} air) of amosite. Inhalation of lower dose of amosite and inhalation of wollastonite did not change this parameter (Table 2).

Exposure to wollastonite or amosite without tobacco smoke did not change the phagocytic activity of alveolar macrophages.

Table 1. *Length and diameter of used wollastonite fibres*

Length (μm)	Proportion (%)	Diameter (μm)	Proportion (%)
1 - 10	48	=1	47
11 -30	40	<1	22
>30	12	<3	21
		=3	6
		>3	4

Table 2. *Significance of changes in cytotoxic parameters after inhalation exposure*

	Fibrous dust alone	Tobbaco smoke alone	Tobbaco smoke + fibrous dust	
Dose (mg.m^{-3})	60		30	60
amosite inhalation				
Viabilty	↓*			↓*
wollastonite inhalation				
Phagocytic activity		↓*		
ACP (cell-free BALF)			↑*	
Cathepsin D (BAL cells)	↑*		↑*	↑*

Comparison of exposed groups with the correponding control group:
* p<0.05; ** p<0.01; ↑- increase; ↓ - decrease

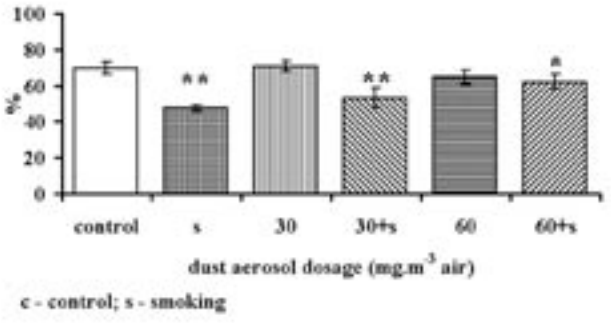

Fig. 1. *Phagocytic activity of alveolar macrophages after amosite inhalation. Error bars represent standard error of mean. Comparison with the control group: *p < 0.05; **p <0.01.*

Significant decrease of phagocytic activity was found in groups exposed to smoking without fibres (Table 2) and after combined exposure to amosite and tobacco smoke (Fig. 1).

The differences of LDH activity in cell-free BALF between the exposed groups and their corresponding controls were not significant. Similarly no significant changes were found in the activity of alkaline phosphatase.

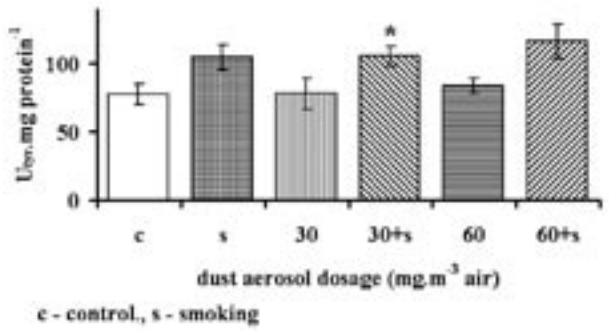

Fig. 2. *Activity of cathepsin D in cell-free bronchoalveolar lavage fluid after amosite inhalation. U_{tyr}: µg of tyrosine released in an hour time. Error bars represent standard error of mean. Comparison with the control group: *p < 0.05.*

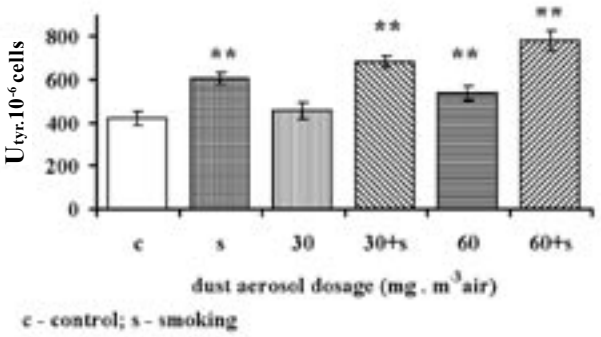

Fig. 3. *Activity of cathepsin D in bronchoalveolar lavage cells after amosite inhalation. U_{tyr}: µg of tyrosine released in an hour time. Error bars represent standard error of mean. Comparison with the control group: *p < 0.05; **p <0.01.*

The activity of acid phosphatase in cell free BALF significantly increased in the group exposed to 30 mg of wollastonite combined with tobacco smoke (Table 2). Changes in other groups were not significant .

Activity of cathepsin D in cell-free BALF did not change in any group after wollastonite exposure and in groups exposed to amosite without tobacco smoke. Significant increase of activity was detected in combined exposure - amosite (30 mg.m^{-3} air) with smoking (Fig. 2).

The ACP activity in BAL cell sediment of groups involved into wollastonite exposure experiment remained unchanged. No changes were found also in groups exposed to smoking without amosite fibres and to lower dose of amosite without cigarette smoke. A visible rising trend after combined exposure (amosite and tobacco smoke) and exposure to higher dose of amosite without cigarette smoke was not significant.

Cathepsin D activity in BAL cells of animals exposed to wollastonite was significaty higher than in the control group, but the influence of smoking was not detectable (Table 2). Cathepsin D activity in BAL cells of animals after amosite exposures was significantly influenced both by amosite (60 mg.m^{-3} air) and smoking. In combined exposure with higher dose of amosite might be the effect of these two substances considered more than additive (Fig. 3).

DISCUSION AND CONCLUSIONS

Strongly dose dependent decrease of AM viability after amosite inhalation ($p<0.01$) found in this experiment is in accordance with previously described effect of asbestos (6).

The expected influence of amosite exposure on the phagocytic activity of AM (6, 12) was not found. This absence might be explained by short time of inhalation exposure (only one hour) and by relatively long time between two consecutive exposures in comparison with other authors (13).

Increase of LDH and AP activity in extracellular fluids are generally accepted as a good marker of cell or tissue injury and used for evaluation of the cytotoxic effect of fibrous dust (14, 12, 14, 15). We did not find significant changes in activities of LDH and AP as well as in the activities of measured lysosomal enzymes in BALF after amosite inhalation. We suppose that the reason might be similar as we described in the case of phagocytic activity, i.e. short exposure time and long inter-exposure recovery time with consequent lower degree of lung tissue damage. Great variability of values might contribute to the latter and also to absence of significant changes in ACP activity in BAL cells. Cathepsin D activity in BAL cells was after amosite inhalation significantly changed. These results are in good accordance with the work of Sjörstrand et al.(20)

We can conclude that:

- wollastonite inhalation confirmed the low cytotoxicity of wollastonite. Significant changes were found only by measurement of cathepsin D activity in BAL cells;
- smoking significantly depressed the phagocytic activity and increased the activity of cathepsin D in the cell-free BALF and in BAL cells;
- influence of combined exposure was found only in groups exposed to amosite and cigarette smoke (phagocytic activity of AM, cathepsin D activity). The most expressive changes after combined exposure were found in the case of cathepsin D activity in BAL cells;
- measurement and evaluation of lysosomal enzymes activity in BAL cells is more relevant than measurement in cell-free BALF;
- cathepsin D activity (especially in BAL cells) can be considered as the most sensitive parameter for detection of cytotoxic influence of studied noxious substances.

Acknowledgement

The work was supported by an E.U. grant, contract No. QLK4 - CT-1999 – 01629 (FIBRETOX project).We wish to thank RNDr. S, Wimmerová for many advices at the evaluation of the results, Ing. J. Navarová, Ph.D. for help and advices at the estimation of lysosomal enzyme activities, Mrs. H. Bobeková, Mrs. D. Čepcová, Mrs. G. Dragúňová, Mrs. M. Valentová and Mr. D. Klamo for skilful technical assistence.

REFERENCES

1. **Navrátil M:** Lung asbestosis and its complications. Praha, Avicenum, 1982. (In Czech.)
2. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans.14. Asbestos. Lyon, IARC, 1977.
3. **Brody AR:** Asbestos induced lung diseases. Environ. Health. Perspect. 1993; 100: 21-30.
4. **Kohyama N, Tanaka I, Tomita M, Kudo M, Shinohara Y:** Preparation and characteristics of standard reference samples of fibrous minerals for biological experiments. Ind Health 1997; 35: 415- 432.

5. **Huuskonen MS, Tossavainen A, Koskinen H, Zitting A, Korhonen O, Nickels J, Korhonen K, Vaaranen V:** Wollastonite exposure and lung fibrosis. Environ Res 1983; 30: 291-304.
6. **Hurbánková M, Kaiglová A:** Compared effect of asbestos and wollastonite fibrous dusts on various biological parameters measured in bronchoalveolar lavage fluid. J Trace Microprobe Tech 1999; 17: 233-243.
7. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. 68. Silica, some silikates, coal dust and para-aramid fibrils.. IARC , Lyon, 1997.
8. **Bellmann B, Muhle H:** Investigation of the biodurability of wollastonite and xonolite. Environ Health Pespect 1995; 102, Suppl. 5: 191-195.
9. **Beňo M, Hurbánková M, Dušinská M, Volkovová K, Staruchová M, Černá S, Barančoková M, Kažimírová A, Kováčiková Z, Bobek P, Horecký M, Mikulecký M, Kyrtopoulos SA:** Some lung cellular parameters reflecting inflammation after combined inhalation of amosite dust with cigarette smoke by rats . This issue of CEJPH.
10. **Kováčiková Z:** Effect of enviromental pollution from nickel smelter waste dump on the lung of rabbits. Two-generation study. Biológia 1990; 45: 477- 482.
11. **Navarová J, Nosáľová V:** Effect o H2-receptor antagonists on indomethacin-induced lysosomal enzyme release from rat gastric mucosa. Meth Fin Exp Clin Pharmacol 1994; 16: 119-124.
12. **Hurbánková M, Kaiglová A:** Some bronchoalveolar lavage parameters and leukoucyte cytokine release in response to intratracheal instillation of short and long asbestos amosite fibres in rats. Physiol Res 1998; 46: 459-466.
13. **Hesterberg TW, Hart GA, Chev J, Müller WC, Hamilton RD, Bauer J, Thevenaz P:** The importance of fiber biopersistence and lung dose in determining the chronic inhalation effects of X607, RCF1, and chrysotile asbestos in rats. Toxicol Appl Pharmacol 1999; 153: 68-82.
14. **Drent M, Cobben NAM, Henderson RF, Wounters EFM, Van Dieijen--Visser MP:** Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. Eur Respir J 1996; 9: 1736-1742.
15. **Sjöstrand M, Rylander R, Bergström R:** Lung cell reactions in guinea pigs after inhalation of asbestos (amosite). Toxicology 1989; 57: 1-14.