Physiol. Res. 46: 41–45, 1997

The Chamber Exposure of Laboratory Rats to Metal Oxides Originating From Metal Producing Industry

Z. KOVÁČIKOVÁ, D. CHORVATOVIČOVÁ[1]

Institute of Preventive and Clinical Medicine and [1]Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovak Republic

Received August 7, 1995
Accepted August 28, 1996

Summary

Laboratory rats were exposed to the inhalation of dust from an agglomeration unit which is the greatest contributor to dust pollution in the vicinity of a mercury producing plant. The exposure lasted for 6 months (4 hours daily, 5 days per week), the concentration of aerosol in the chamber was 10 $mg.m^{-3}$. After finishing the exposure, the animals were examined and compared with the controls which were held under standard laboratory conditions. The number of alveolar macrophages was highly elevated ($P<0.001$) in the exposed animals, Mg^{2+} ATPase activity in the heart muscle was decreased. The alanine aminotransferase activity in the serum was not changed, the aspartate aminotransferase was slightly enhanced. No differences in the frequency of abnormal sperm and in the frequency of polychromatic erythrocytes in bone marrow were detected.

Key words

Inhalation exposure – Metal oxides – Lung – Cardiac Mg^{2+} ATPase – Genotoxic effect

Introduction

There is a mercury plant in Eastern Slovakia, where mercury is produced from the local ore and all mercury waste from the country is recycled. The whole plant consists of four production units, where not only mercury but also various secondary products are produced. The factory represents a serious ecological problem in that district because of the high mercury content in the environment and elevated dust pollution.

In order to differentiate the effect of the individual production units, an experiment was carried out in a laboratory inhalation chamber, where rats were exposed to the dust from the production unit called "agglomeration". In this unit, a fine-grained siderite concentrate is agglomerated in the presence of powdered coke, returns of agglomeration dust and powdered lime into an iron agglomerate. The product serves as raw material for the production of iron. This unit is the greatest contributor not of mercury, but of the dust pollution from the whole plant.

The aim of this research was to test, under laboratory conditions, the effect of inhalatory exposure of rats to real industrial dust, which is produced by an "agglomeration" unit in a mercury producing plant. The dust consists of various, predominantly heavy metal oxides.

The influence of inhalation exposure was studied on rats in a laboratory experimental chamber. We investigated several organs which might be affected. These included the lungs, specifically the number of alveolar macrophages; the heart, where Reichrtová *et al.* (1991) found high accumulations of metals; and the liver, where aminotransferase activities in the serum can reflect possible liver damage. Genotoxic effects were studied using micronucleus assay in the bone marrow and sperm abnormality assay.

Materials and Methods

Animals

Male albino Wistar rats (Velaz, Prague) weighing 200–220 g were randomly divided into two groups, each consisting of 10 animals. They were housed in plastic cages and were maintained on a 12 h light/dark cycle. Commercially prepared food and tap water were provided *ad libitum*.

Metal particles

The metal particles used for the chamber exposure of laboratory animals were obtained from electrostatic filters of the agglomeration unit. The chemical composition of the particles was as follows (in %): Fe_2O_3 34–35, FeO 18–22, SiO_2 9–13, BaO 7.5, MgO 4–6, S 1.3, Mn 2.27, Sb 0.145, As 0.044, Hg 0.0009. The aerosol was produced from the metal particles in an experimental chamber system. The geometric mean diameter (Dg) of the particles was 3.17 μm, the geometric standard deviation (Sg) 1.45, particle mass median aerodynamic diameter (Dgh) 4.80 μm (Reichrtová *et al.* 1992). The average concentration of mercury in the chamber was 0.58 ± 0.2 $\mu g.m^{-3}$.

Chamber exposure

Aerosol was produced in the experimental exposure system. Animals were exposed to 10 $mg.m^{-3}$ of metal aerosol for 4 hours daily, 5 days per week, for a period of 6 months. The dust aerosol concentration was estimated by the gravimetric method. Control animals were kept under standard laboratory conditions.

Isolation of alveolar macrophages (AM)

Animals were killed by severing the carotid artery. The lungs and trachea were removed *in situ* and washed with saline (Myrvik *et al.* 1961). AM were separated from the lavage fluid by centrifugation, resuspended in saline and counted.

Biochemical studies

Acid phosphatase was assayed by incubating a 2 ml mixture consisting of 200 μmol acetic buffer, pH 5.2, 3×10^5 AM, 1 μmol p-nitrophenylphosphate (pNPP) at 37 °C. After incubation for 30 min 1 ml of 0.5 $mol.l^{-1}$ NaOH was added to stop the reaction. Absorption was measured at 400 nm. The aminotransferase activities were determined in the serum using the Bio-La-Test (Lachema, Brno). The heart was homogenized in 0.25 M sucrose. The homogenate was centrifuged (10 min, 660 x *g*) and the ATPase activity was estimated in the supernatant by incubating a 1 ml mixture containing 30 μmol TRIS-HCl buffer, pH 7.5, 2 μmol $MgCl_2$, the heart homogenate and 2 μmol ATP at 37 °C. The reaction was stopped by trichloracetic acid and the liberated P_i was assayed in the supernatant according to Fiske and Subbarow (1925). The protein content was determined using the method of Lowry *et al.* (1951). Bovine serum albumin was used as standard.

Sperm abnormality assay

Slides for sperm abnormality assay were prepared and evaluated according to Wyrobek and Bruce (1978). They were examined under Amplival (Zeiss, FRG) microscope with oil immersion at 1000-fold magnification, 350 sperms per animal, i.e. 3500 sperms per group were evaluated.

Micronucleus assay

The micronuclei preparations were performed according to Schmid (1975). Slides were observed under an Amplival microscope. A total of 1000 polychromatic erythrocytes (PCE) were examined per animal for the presence of micronuclei (MN). The number of PCE was scored in a visual field of 2000 normochromatic erythrocytes (NCE) and the index PCE/(PCE+NCE) was calculated. Statistical analysis was made using tables of Kastenbaum and Bowman (1970).

Statistical analyses

Data are means ± S.D. Student's t-test was used for statistical evaluation for all data except for the incidence of micronuclei.

Table 1
Lung wet weights of exposed and control rats, relative lung weight/body weight, count of alveolar macrophages (AM) and their acid phosphatase activity

Group	Lung wet weight g	Lung relative weight x 10^3	AM count x 10^6	Acid phosphatase activity nmol pNP.mg $protein^{-1}.s^{-1}$
Control (n=10)	1.19±0.50	2.61±1.29	2.43±1.47	2.12±1.14
Exposed (n=10)	1.50±0.65	3.05±1.05	4.16±1.55*	2.38±1.00

** $P<0.001$, relative weight = weight of lung/ body weight.*

Results

The lungs from rats exposed to metal oxides were slightly grayish from the deposited dust, but otherwise they did not differ macroscopically from the controls. Both of the absolute lung wet weights and the relative lung weights are shown in Table 1. The AM from each animal were counted separately. The numbers of the recovered AM were significantly higher in the exposed group than in the control one ($P<0.001$). Acid phosphatase was not changed in the AM isolated from the exposed animals (Table 1).

The Mg^{2+}ATPase activity was estimated in the heart homogenate. The average value in exposed animals was $2.664 \pm 0.788 \times 10^{-2}$ nmol P_i.mg protein^{-1}.s^{-1}, which represents about 80 % of the activity measured in the group of control animals. The difference is close to the statistical significance ($P=0.06$). Both the wet weight of the heart (1.89 ± 0.51 g in the control and 1.97 ± 0.42 g in the exposed group) and the relative weights of hearts were the same in both groups.

Aminotransferase activities were estimated in the serum. Alanine aminotransferase was not changed in the exposed animals, while aspartate aminotransferase was enhanced to 116 % of the control value (Table 2).

Frequencies of abnormal sperm were counted in 3500 sperms from each group. Fifty abnormal sperms were found (1.42 %) in the exposed group. These were mostly sperms without a hook (46 %), or banana type (46 %) and only 8 % of the sperms were amorphous. In the control group 42 sperm were abnormal (1.20 %). Most of the abnormalities in this group were also sperms without hook (61.9 %), of the banana type (28.6 %) sperms and 9.5 % were amorphous sperms. The difference in frequency of abnormal sperms between the control and exposed group was not statistically significant.

Long-term exposure of Wistar rats to the metal aerosol originating from the agglomeration dust did not increase the frequency of micronuclei in polychromatic erythrocytes of their bone marrow (Table 3).

Table 2
Aminotransferase activities in the serum of Wistar rats

Group	ALT x 10^{-3}	AST x 10^{-3}
Control (n=10)	7.51 ± 1.33	6.17 ± 1.16
Exposed (n=10)	7.34 ± 1.16	7.18 ± 1.83

ALT alanine aminotransferase, AST aspartate aminotransferase, The aminotransferase activities are expressed in U.l^{-1}.

Table 3
Frequency of micronuclei in polychromatic erythrocytes of bone marrow of Wistar rats

Group	PCE with MN/1000 PCEs	PCE/(PCE + NCE) %
Control (n=10)	1.8 ± 0.632	49.63
Exposed (n=10)	2.0 ± 0.816	48.75

PCE – polychromatic erythrocytes, NCE – normochromatic erythrocytes, MN – micronuclei

Discussion

Piscator (1979) demonstrated that exposure to manganese dust produced pneumonia in workers and respiratory symptoms in persons living in the vicinity of a manganese emitting plant. Bencko and Symon (1977) reported about inhaled arsenic (in the form of $As^{3+}$and As^{5+} oxides) in the vicinity of non-ferrous smelter. There is substantial evidence that inhalation of inorganic arsenic may induce cancer of the respiratory tract. The cytotoxicity of heavy metals containing dust cannot be predicted by simple additive effects of single

elements. There exist positive as well as negative synergic effects which were observed by Fisher *et al.* (1986). Therefore, for evaluation of a real dust it is more logical to test the dust itself and not its individual components.

Dust particles smaller than 5 μm were used in this study. Particles of this size are easily inhaled into the lower airways, making them important from the point of view of toxicity. A stimulative effect of the dust after 6-month exposure was observed on the wet weight of rat lungs. The effect was most pronounced on the increased AM number in the exposed animals. The primary function of AM is to maintain the inner lung space sterile and free of particles. There is a positive correlation between the amount of dust cleared away and the number of phagocytic cells (La Belle and Brieger 1960). But the response of alveolar macrophages depends not only on the amount of the dust, but also on its chemical composition (Gulyas and Gercken 1988). Different elements are distributed in different locations between the surface and bulk phase of dust particles (Linton *et al.* 1977).

Another fact to be kept in mind is the different solubility of toxic compounds ingested by macrophages. In our study, the amount of AM had increased significantly ($P<0.001$), which points to a considerable load on the lung. The greater number of these phagocytic cells also means greater total activity of lysosomal enzymes (the acid phosphatase was tested as a representative of this), but the specific activity of acid phosphatase was the same in control and exposed animals. Normal AM are rich in lysosomal enzymes, which are involved in the processing of foreign materials. This system is crucial in the functional response of the macrophages. Any perturbation of the metabolic or enzymatic mechanisms of these cells may have important consequences on the ability of the lung to defend itself against disease (Gardner 1984). Lundborg *et al.* (1985) supposed that the dissolution of particles by alveolar macrophages should be one of the basic components in any model of alveolar clearance of inorganic particles. Even particles that are usually considered as insoluble are dissolved in the lung.

The components of dust are carried by the blood to other organs, where they accumulate and may exert a deleterious influence. The liver plays a central role in mediating the interaction between man and his chemical environment. The determination of serum aminotransferases provides one of the most sensitive tests for revealing damage to hepatocytes. Alanine aminotransferase is specific for the liver, whereas aspartate aminotransferase may also be affected in cardiac or skeletal diseases (Guzelian 1983). Serum analysis in our study showed no changes in alanine aminotransferase activity and only slight enhancement of aspartate aminotransferase activity, which points to possibly injury of the heart.

Combs and Acosta (1990) considered the decreased ability to produce ATP as the most important cardiotoxic mechanism. Our study showed a decrease in Mg^{2+} ATPase activity in the heart for which we have no explanation at present. It may be caused by lower substrate concentration, by enzyme inhibition by heavy metals, or some other factors. The above cited possibilities as well as our results point to a possible cardiotoxic effect, evoked by the inhalation of agglomeration dust.

As is clear from the results of the micronucleus test and sperm abnormality assay, no genotoxic effects of the tested dust were demonstrated. This agrees with the lack of reported genotoxic effects for metals which made up the dust used in our work (As, Hg, Mn and Sb).

The same analyses should be done in animals exposed in a bioindication station near the factory. The results could not only reveal the complex effect of the total environmental pollution near the mercury producing plant but, by comparing the results from the chamber and field exposure, we could deduce the toxic effect of a particular production unit.

Acknowledgments

This work was supported by SAV grant 382.

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Reprint requests
Dr. Z. Kováčiková, Institute of Preventive and Clinical Medicine, Limbová 14, 833 01 Bratislava, Slovak Republic.