INFLUENCE OF INHALED MANGANESE OXIDES NANOPARTICLES ON MASS OF INTERNAL ORGANS IN MICE

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Abstract

Results of the statistical analysis of a long-term inhalation experiment on laboratory mice are presented. During 17 weeks of the experiment, the experimental group was exposed to inhaled manganese oxides nanoparticles. Manganese oxides ($MnO.Mn_2O_3$) nanoparticles (MnONPs) were synthesized continuously via aerosol route in a hot wall tube flow reactor using a thermal decomposition of metal organic precursor manganese(II)acetylacetonate in the flow tube reactor (in vertical position) at temperature 750 °C in the presence of 30 vol% of oxygen. The concentration of produced MnONPs at the reactor output was in the range 1-3 × 10^7 particles/cm^3 and the size of generated nanoparticles $MnO.Mn_2O_3$ was in the range 7-50 nm. Before entering the inhalation chamber, MnONPs in the mixture of N_2, O_2 and air (at total flow rate of 3 L/min) were further diluted using filtrated humidified air (20 L/min) at temperature 21 °C resulting in the MnONPs concentration 2 × 10^6 particles/cm^3. At regular time intervals the mass of selected internal organs of mice from both experimental and control group was assessed. It has been proven that inhaled nanoparticles are able to influence the mass of internal organs of mice. Statistically significantly lower mass of kidneys, liver and spleen and higher mass of pancreas have been found in the experimental group compared to the control group.

Keywords:

inhaled nanoparticles; manganese oxides nanoparticles; changes in internal organ mass of mice; long-term inhalation experiment.

1. INTRODUCTION

Manganese is a trace element essential for the function of various enzymes [1]. It is a component of some metalloenzymes and also works as an enzyme activator [2]. In humans it is required in all tissues in concentrations of 0.3-2.9 μg/g [3]. The highest concentrations are found in the brain, bones, liver, kidneys and pancreas. However, when exposed to high doses of manganese its negative health effects may occur. Long-term exposure to high doses of manganese is proven to be neurotoxic. Accumulation of manganese in the brain tissue leads to irreversible progressive brain disease with similar symptoms like Idiopathic Parkinson's disease (IPD), which is also known as Manganism (Mn-induced Parkinson-like disease). Toxicity of manganese is probably associated with damage of basal ganglia, especially in the areas of globus pallidus and corpus striatum [4]. Neither the exact mechanism of manganese action nor the reason for damage of the basal ganglia is known. However, it is assumed that this could be a consequence of oxidative stress or inflammatory processes [1, 2, 5, 6]. It seems that high doses of manganese also induce changes in the levels of the neurotransmitters dopamine, glutamate (glutamic acid) and γ-aminobutyric acid, which may serve as another explanation for the neurotoxicity of manganese [7].

Manganese gets into the bloodstream primarily by ingestion and inhalation [8]. Given that the intake of manganese by absorption from the gastrointestinal tract is controlled by the current needs of the body and intoxications in this way are therefore rare, the ingestion is usually considered less dangerous way of exposure [9]. Inhaled particles of manganese compounds are partially trapped in the mucus of the lower respiratory tract, partly penetrate the alveoli and from there they get into the blood [10]. To get from the bloodstream into the brain manganese penetrates the blood-brain barrier or the choroid plexus [11]. The possibility of the passage of manganese compounds nanoparticles from the nasal cavity directly to the brain through the olfactory nerve is often discussed [9]. Research carried out on laboratory rodents confirmed the existence and importance of this exposure pathway [12, 13], although whether these findings also apply to humans is still unclear [10, 14].

The organism is able to remove some amount of manganese away from the body. The largest amount of manganese is excreted from the body via the bile [15, 16]. It has been proven that in laboratory rodents increased intake of manganese in diet causes decrease in gastrointestinal absorption, increase in metabolism of the liver and increase in excretion of manganese in the bile and pancreatic fluid [2, 3].

In the past neurotoxicity due to inhalation of Mn was observed in some specific occupations, particularly in miners in manganese ore mines, workers in the steel mills and foundries, especially those where ferromanganese and steel are melted, and dry cell battery factories. Also welders belong among highly exposed occupation groups [10]. Welding electrodes usually contain manganese which is also present in welding fume primarily in Mn^{2+} and Mn^{3+} oxidation states [17, 18, 19]. Furthermore, welding fume provably contains ultrafine particles of size <100 nm, which easily penetrate the respiratory system [20].

It had been proven that manganese from inhaled particles of manganese compounds can accumulate in the internal organs of laboratory animals, especially in the lungs, liver, pancreas and gall bladder [12, 13, 21, 22, 23]. Results of some studies [23, 24, 25] additionally suggest that inhaled manganese compounds particles may also influence the constitution of animals, but conclusive evidence has not been found yet. The aim of our research was to extend current knowledge on the effects of inhaled nanoparticles of $MnO.Mn_2O_3$ on the constitution of mice. We conducted a long-term inhalation experiment, during which laboratory mice were exposed to nanoparticles of $MnO.Mn_2O_3$ for 17 weeks, 24 hours a day, 7 days a week. On previously chosen days, mice were collected from the cages and autopsied. During the autopsy selected internal organs were weighed.

2. MATERIALS AND METHODS

2.1 Animals

Adult mice (males, ICR line) were obtained from Masaryk University (Brno, Czech Republic). All animals were allowed to acclimate to laboratory conditions for at least 1 week before the experiment began. The mice were provided with a commercial diet and water ad libitum. The experimental work was performed in accordance with the ethical approval of the Institute of Animal Physiology and Genetics (no. 081/2010). At the beginning of the experiment the males weighed about 24 g.

2.2 Experimental design

The experiment started with 80 laboratory mice which were randomly divided into 2 equally sized groups: a fresh-air control and the experimental group exposed to nanoparticles of $MnO.Mn_2O_3$. Both groups were placed in identical inhalation chambers and received identical feeding. During the experiment 2 mice from the control group had to be euthanized for health reasons and those were not further analyzed. At chosen time intervals (**Fig. 1**) mice were taken out of the cage, euthanized and autopsied one by one. During the autopsy the brain, lungs, heart, liver, kidney, spleen, pancreas, testes and thymus were weighed.

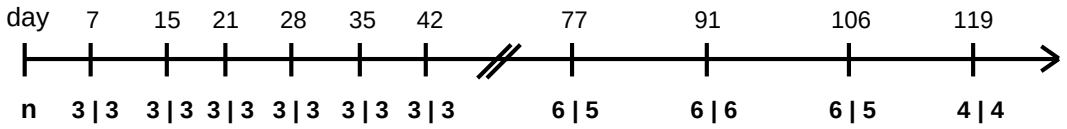

Fig. 1 Overview of the days when mice were collected from cages during the inhalation experiment, n marks the number of mice taken from experimental group | control group.

2.3 $MnO.Mn_2O_3$ nanoparticles generation and measurement

Manganese oxides ($MnO.Mn_2O_3$) nanoparticles (MnONPs) were synthesized continuously via aerosol route in a hot wall tube flow reactor using a thermal decomposition of metal organic precursor manganese(II)acetylacetonate in the flow tube reactor (in vertical position) at temperature 750 °C in the presence of 30 vol% of oxygen. The vapours of manganese(II)acetylacetonate were generated from solid form of acetylacetonate in saturator at temperature 160 °C and released vapours were transported by nitrogen (purity 5.5, flow rate 0.5 l/min) into the flow reactor. The total flow rate of nitrogen/oxygen mixture through the flow reactor was 2 l/min. At the output from the reactor, MnONPs transported in nitrogen flow were mixed with air (1 l/min). The concentration of produced MnONPs at the reactor output was in the range 1-3 × 10^7 particles/cm^3 and the size of generated nanoparticles $MnO.Mn_2O_3$ was in the range 7-50 nm. Before entering the inhalation chamber MnONPs in the mixture of N_2, O_2 and air (at total flow rate of 3 LPM) were further diluted using filtrated, humidified air (20 L/min) at temperature 21 °C resulting in the MnONPs concentration 2 × 10^6 particles/cm^3. Concentration of nanoparticles and particle size distribution in the inhalation chamber were continuously measured by SMPS (model 3936L72, TSI).

2.4 Statistical analysis

Differences between the group exposed to manganese nanoparticles and the control group in the mass of the organs were statistically evaluated. Statistical testing was carried out in two successive steps. First, the exact p-value was computed separately for each day when the mice were taken out and autopsied using one-tailed Wilcoxon Rank Sum test [26]. In case that the exposed and the control group do not differ in organ weight, these p-values computed for different collection days should be uniformly distributed over the interval [0, 1] with median being equal to 0.5 [27]. In the second step, this null hypothesis was tested at the 0.05 significance level using two-tailed Wilcoxon Sign Rank test [28]. Statistically significant results indicate that organ weight in both groups differs at least in one collection day. Software R [29] was used for all statistical computations.

3 RESULTS AND DISCUSSION

Statistically significant differences in organ mass of the kidney ($p < 0.05$), spleen ($p < 0.05$), liver ($p < 0.01$) and pancreas ($p < 0.05$) were found. The kidney, spleen and liver were considerably lighter in the mice from the experimental group compared to the mice from the control group (**Fig. 2**). The pancreas was also affected, but with the opposite effect.

Our results are significantly different from the results of the inhalation experiment performed by Dorman et al. [23]. They evaluated the weight of the brain, hypophysis, liver, lung, kidney, heart, pancreas and testes in monkeys (rhesus macaque) exposed to 0.18, 0.92 and 4.62 mg $MnSO_4/m^3$ for 6 hours a day, 5 days a week for 13 weeks. They did not find any statistically significant differences in the weights of these organs between the exposed groups and the control group in monkeys which were euthanized immediately after the end of the experiment. Only in monkeys which were exposed to the highest concentrations of $MnSO_4$ and after the exposure were left alive for other 90 days they found about 17% lower heart weight in comparison with the control group. However, the monkeys from the control group were killed immediately after the end of the inhalation experiment and were thus of different age. As noted by the authors themselves, monkeys were still growing during the experiment, so their result cannot be considered decisive.

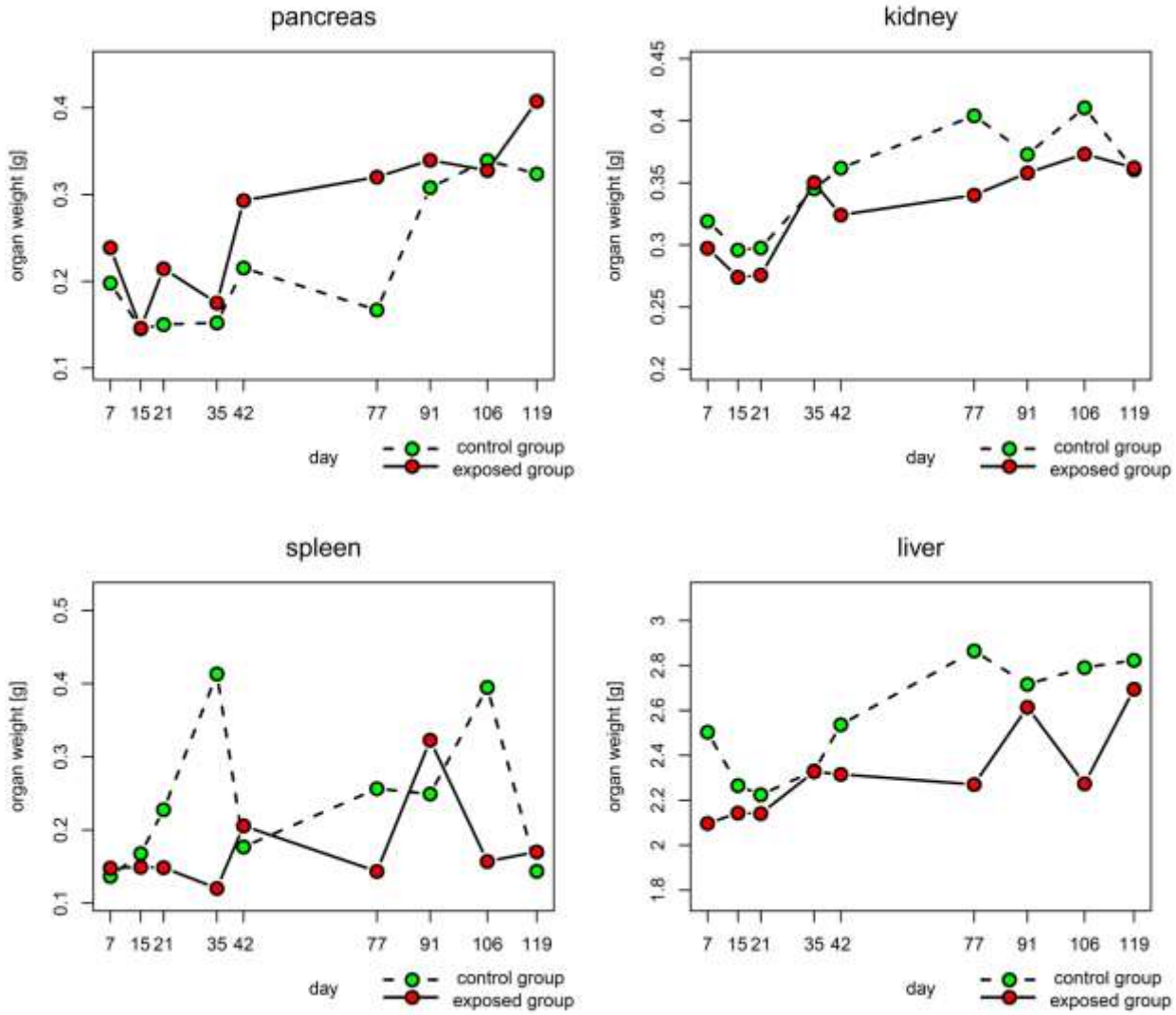

Fig. 2 Development of median weight of the pancreas, kidney, spleen and liver in mice during the 119 days of the inhalation experiment, green point marks the median of the control group; red point marks the median of the group exposed to $MnO.Mn_2O_3$ nanoparticles; solid lines connect adjacent medians of the control group; dashed lines connect adjacent medians of the group exposed to $MnO.Mn_2O_3$ nanoparticles.

Salehi et al. [24] conducted a long-term inhalation experiment in which rats were exposed to different concentrations of the mixture of manganese phosphate and manganese sulfate for 13 weeks, 6 hours a day, 5 days a week. They found that the exposed rats had significantly lower body weight than the rats from the control group. However, it is not possible to state whether there was also a corresponding change in the weight of internal organs, since the authors did not weigh the organs individually. The authors also stated that rats from the exposed groups showed a significantly higher level of locomotor activity, but they did not find any statistically significant difference in food intake between the groups.

Torrente et al. [25] gave rats 275 and 550 mg of $MnCl_2$/kg/day in drinking water for 19 weeks, during which the rats were weighed regularly. In the rats from the exposed groups a lower body weight and a lower weight gain together with less food and water intake were discovered in comparison with the control group. The rats exposed to higher doses of $MnCl_2$ also showed lower level of locomotor activity, which is in contradiction with the finding of Salehi et al. [24]. Regarding the cause of lower body weight of exposed rats, Torrente et al. [25] suggest that this could be a consequence of aphagia due to lesions in the mesolimbic dopaminergic system induced by excessive doses of manganese. However, given the disproportionality of organ weight

changes, which was observed in our sample, it seems unlikely that these changes could be fully explained only by the reduced food intake. Furthermore, there are also studies which did not prove any statistically significant decrease in body weight of laboratory animals following the exposure to manganese compounds [6, 30].

4 CONCLUSION

A long-term inhalation experiment, during which laboratory mice were exposed to $MnO.Mn_2O_3$ nanoparticles for 17 weeks, 24 hours a day, 7 days a week, was conducted. It was proven that inhaled nanoparticles of $MnO.Mn_2O_3$ affect weights of internal organs in mice. The resulting change in weight of different organs is disproportional. While the weight of the liver, kidney and spleen in the exposed group was lower than in the control group, in the pancreas it was the contrary. These results suggest that this phenomenon cannot be fully explained simply by reduced food intake in the exposed mice. Comparison of allocation level of Mn in the internal organs with the weight changes of these organs, which is being prepared, should provide further insight on the mechanism of manganese effect on the weight of internal organs.

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