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EFFECT OF MERCURY ON SELECTED HAEMATOLOGICAL PARAMETERS OF RABBITS *IN VITRO*

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ABSTRACT

The aim of this study was to examine possible toxic effect of mercury on selected haematological parameters of rabbit blood depending on time exposure. The blood of rabbits was treated with a mercuric chloride ($HgCl_2$) at the concentration of 5 mg .10 ml^{-1} of saline. The blood samples of the control (blood and saline) and experimental (blood and mercury addition) group were measured in dependence on the time of exposure to mercury: 0, 30, 60, 90, 150 and 210 minutes (T1, T2, T3, T4, T5 and T6, resp.). Selected haematological parameters (WBC – total white blood cell count, RBC – red blood cell count, HGB – haemoglobin, HCT – hematocrit and PLT – platelet count) were measured using haematology analyzer Abacus junior VET. Significant decrease in erythrocytes, non-significant increase in the content of haemoglobin and significant decrease in platelet counts in rabbit blood in the mercury exposed group compared to the control group (no mercury treatment) was observed. Our results suggest that mercury, as an environmental pollutant, causes the changes and imbalance in the blood and blood elements.

Key words: mercury; rabbits; blood; toxicity

INTRODUCTION

The exposure of animals and human to toxic elements is a factor that could be closely related to high cancer risk (Stawarz et al., 2009) and that increases mortality of animal and human organisms (Formicki et al., 2008; Kalafová et al., 2008; Koréneková et al., 2008; Skalická et al., 2008; Kacaniova et al., 2009). Mercury (Hg) is a non-essential element for plants and animals nutrition. Its presence in agricultural systems is of concern due to its high potential toxicity. Mercury is persistent in the environment and has been listed as a pollutant by several environmental organizations (Day et al., 2007; Tazisong and Senwo, 2009). It is also one of the major aquatic contaminants, even though emissions have been reduced over the years (Pereira et al., 2009), and is known to bioaccumulate in marine mammals (Das et al., 2008).

Mercury can be encountered in three main chemical forms (elemental, inorganic, and organic), which can affect the immune system in different ways (Vas and Monestier, 2008). Mercury and its compounds are very toxic. Occupational exposure and environmental pollution are the major sources of hazard to human health. In more than 50 professions, workers may be exposed to mercury, particularly in the mining and chemical industries and in agriculture (Affelska-Jercha, 1999). Mercuric chloride is an inorganic compound that has been used in agriculture as a fungicide, in medicine as a topical antiseptic and disinfectant, and in chemistry as an intermediate in the production of other mercury compounds. The widespread use of mercury has resulted in increased levels of mercury in rivers and lakes (National Toxicology Program, 1993).

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The symptoms of mercury exposure result from the damage to the central nervous system and the kidney, as well as from the impairment of erythrocyte metabolism, coagulation and immune response. Mercury may also induce allergic reactions (Affelska-Jercha, 1999), structural and functional damage in several organs (Massanyi et al., 2007; Penna et al., 2009), and its mutagenic effect has been reported (Moszczyński and Moszczyński, 1988). Mercury can cause autoimmune disease (Kazantzis, 2002; De Vos et al., 2007; Das et al., 2008; Hawley et al., 2009). Long-term exposure to mercury vapours may influence important haematological parameters of the peripheral blood in humans (Zabinski et al., 2000).

The aim of this study was to examine possible toxic effect of mercury on selected haematological parameters of rabbit blood in dependence on exposure time.

MATERIAL AND METHODS

Experimental design and animal management

Rabbits of meat line M91, maternal albinotic line, crossbreed (New Zeland White, Buskat rabbit, French Silver) and paternal acromalictic line, crossbreed (Nitra rabbit, Californian rabbit, Big Light Silver) were used in the experiment. Rabbits were healthy and their condition was judged as good at the commencement of the experiment. Animals were kept in individual sectors of rearing cages with feeding system *ad libitum*. Water for both groups was available from automatic drinking troughs at any time. Groups of animals were balanced for age and body weight (4±0.5 kg). Rabbits were fed a 12.35 $MJ.kg^{-1}$ of metabolizable diet composed of a pelleted concentrate.

Blood sampling and analyses

Blood samples from *vena auricularia* of the animals were taken by macromethods for three times at different days at the morning during one month. In order to restraint blood coagulation chelatone was added to the tubes. In blood samples selected haematological parameters (WBC – total white blood cell count, RBC – red blood cell count, HGB – haemoglobin, HCT – hematocrit and PLT – platelet count) were measured using haematology analyzer Abacus junior VET (Diatron MI LtD., Budapest, Hungary).

Mercury Treatment

Blood of rabbits was treated with a mercuric chloride ($HgCl_2$, Slavus Bratislava, Slovak Republic) at the concentration of 5 mg $HgCl_2$.10 ml^{-1} of saline to obtain 0.05 % solution of $HgCl_2$. Control group contained no mercury addition. Control group was represented by five tubes; 500 µl of rabbit blood and 500 µl of saline in each tube Experimental group was represented by five tubes; 500 µl of rabbit blood and 500 µl of 0.05 % solution of $HgCl_2$ in each tube. The blood of control and experimental groups was analyzed in dependence on the exposure time to mercury: after 0, 30, 60, 90, 150 and 210 minutes (T1, T2, T3, T4, T5 and T6, resp.).

Statistical Analyses

The data concerning the effects of each substance are presented as means of values obtained in three separate experiments performed on separate days. The analysis of variance and t-test were used to calculate basic statistic characteristics and to determine significant differences between experimental and control groups using the SAS statistical software. Differences were judged for statistical significance at the levels $P<0.05$ and $P<0.01$.

RESULTS AND DISCUSSION

In this study, selected haematological parameters were measured from blood samples in dependence on the time exposure to mercury. The results are summarized in Table 1. The organism acts as a one complex and therefore it is necessary to maintain the stability of its internal milieu. Mercury is a heavy metal that can cause a variety of toxic effects on the organism, such as haematological and immunological alterations (Brandao et al., 2008). Mercury in blood may represent a threat towards blood cells (Booth and Zeller, 2005).

Total white blood cell count in the control group (no mercury exposure) was balanced during whole time period. In the experimental group some fluctuations in observed parameters were found. Following 30 min of mercury exposure the increase in white blood cell count was determined in the experimental group versus control group. The values in the experimental group remained higher 90 minutes after mercury addition when compared with control group. After that time leukocyte counts decreased in the experimental group bellow the control group values and this tendency was retained. Mercury exposure caused a reduction in leukocyte count in mice (Brandao et al., 2008; Brandao et al., 2009). From leukocyte forms, monocytes are an important potential target of mercury because of its critical role in directing inflammatory and immune responses (Messer et al., 2005). Human monocytes have a capacity to resist trace concentrations of mercury (Wataha et al., 2008).

At the beginning of our experiment (T1) the red blood cells count was lower in the control group in comparison with the experimental group. After the mercury addition lower values of the observed parameter were measured in the experimental group for a whole time of the mercury exposure (T2, T3, T4, T5 and

Table 1: Haematological parameters in the control group and in the group with mercury addition in dependence on the exposure time

		T1	T2	T	T4	T5	T6
WBC $G.l^{-1}$	C	5.86±3.2	5.97±3.27	5.96±3.28	6.03±3.36	5.88±3.15	5.85±3.06
	E	6.17±2.13	7.88±2.23	6.74±2.37	5.8±2.61	5.57±2.77	5.63±2.79
RBC $T.l^{-1}$	C	3.12±0.05	3.18±0.10	3.17±0.04	3.18±0.07	3.26±0.05	3.18±0.06
	E	3.16±0.06	3.15±0.05	3.15±0.06	3.16±0.07	3.14±0.05*	3.17±0.08
HGB $g.l^{-1}$	C	54.33±2.89	54.00±4.36	53.00±3.46	53.33±3.79	54.33±3.79	53.33±3.06
	E	55.00±3.00	54.33±3.51	54.33±3.21	54.67±3.79	54.33±2.31	54.33±3.51
HCT %	C	20.62±0.92	22.92±1.6	22.38±0.85	21.98±1.48	22.35±0.28	21.55±1.25
	E	20.97±0.77	21.02±0.84	21.60±0.90	22.46±1.79	23.61±4.07	24.21±4.48
PLT $G.l^{-1}$	C	173.67±3.5	203.00±5.6	198.33±5.7	190.0±6.3	160.00±7.0	115.00±7.9
	E	69.67±8.9*	73.00±1.17***	85.00±1.18***	84.5±1.16***	57.33±9.2***	65.00±9.8***

C – Control group (no mercury addition), E – experimental group (mercury addition at 0.5 $mg.ml^{-1}$), T1, T2, T3, T4, T5, T6 – duration of mercury exposure in minutes (0, 30, 60, 90, 150, 210), *denote significant differences from control (P<0.05; ***P<0.001)

$G.l^{-1} = 10^{9}.l^{-1}$, $T.l^{-1} = 10^{12}.l^{-1}$

T6). Statistical evaluation showed significant decrease (P<0.05) in the experimental group after 150 minutes of the mercury addition (T5) (3.26±0.05 $T.l^{-1}$ in the control group vs. 3.14± 0.05 $T.l^{-1}$ in the experimental group). According to Brandao et al. (2008), mercury exposure caused a reduction in the erythrocyte count, what was in compliance with our experiment. Opposite results were observed in the worker occupationally exposed to mercury vapours (Zabinski et al., 2000; Zabinski et al., 2006). No induction of erythrocyte nuclear abnormalities was noted at highest blood total mercury concentration in fish, which may be explained by the alterations in haematological dynamics, as supported by a decreased immature erythrocyte frequency (Guilherme et al., 2008). Mercury can also result in changes in the metabolic processes occurring in the erythrocyte (Zabinski et al., 2006).

The content of haemoglobin was higher in the group with mercury addition during whole observation period (T1, T2, T3, T4, T5 and T6) in comparison with the control group, however, the difference was not statistically significant (P>0.05).

In case of hematocrit at the time points T1, T4, T5 and T6, the higher values were observed in the experimental group versus the control group. At remaining time points (T2 and T3) the situation was opposite so that higher values were measured in the control group. As it is shown in Table 1, this parameter in the experimental group showed a tendency to rise in dependence on the exposure time (the lowest values at the beginning and the highest ones at the end of the experiment). Statistical analysis revealed no significant differences (P>0.05). According to Brandao et al. (2008), mercury exposure caused a reduction in hematocrit and haemoglobin values in mice. In the present study different values were obtained, what could be explained by different character of the experiment (*in vivo* or *in vitro*), different animal species involved in the experiment, as well as the time of mercury exposure. Our results do not differ from those of Zabinski et al. (2006) in case of mercury-exposed workers, where values of hematocrit were higher when compared to the control group of workers.

A significant decrease (P<0.05 and P<0.01) in platelet count in rabbit blood from the mercury-exposed group was determined at whole time period (T1, T2, T3, T4, T5 and T6) in comparison with the control group (without mercury treatment). The platelet count levels in mice were modified by the mercury exposure (Brandao et al. 2008; Brandao et al., 2009). Similar results were obtained also in our study.

CONCLUSION

Our results suggest that mercury, as an environmental pollutant, can cause changes and imbalance in the blood and blood elements. Significant decrease in erythrocytes, non-significant increase in the content of haemoglobin and significant decrease in platelet counts in rabbit blood in the mercury exposed group compared to the control group (no mercury treatment) was observed.

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