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STUDY OF LEAD ACETATE GENOTOXICITY USING THE MICRONUCLEUS TEST IN MICE

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Genotoxicological characterization of lead acetate using the micronucleus test on BALB/c mice showed that the examined doses (50, 250 and 500 mg/kg body mass) induced a relatively large number of micronucleuses (8.8± 2.17, 9.5± 2.07, and 9.2± 2.28) in polychromatophilic erythrocytes of the bone marrow. These values are statistically highly significant (P<0.001) in comparison with the number of spontaneous micronucleuses (1.4± 0.89) in mice inoculated with physiological saline solution.

Considering the significant genotoxic potential of lead acetate, it is necessary to implement measures and procedures to prevent the exposure of animals and humans to lead compounds.

Key words: lead acetate genotoxicity, micronucleus test, bone marrow cells, polychromatic erythrocytes, BALB/c mice.

INTRODUCTION

Lead is distributed throughout the environment. It enters the biological cycle by incorporation into plants and animals which are consumed, making it possible for large quantities of lead to accumulate in the human organism. There are no data on an essential function for lead (Schroeder, 1968; Zielhius, 1971), and it has not been established which is the lowest dose of lead which induces signs of intoxication (Hammond, 1973).

Lead can be introduced into the organism perorally, percutaneously and by inhalation. Inhalation of gasolene fumes leads to serious signs of encephalopathy (Law, 1968; Boeckx, 1977; Hansen et al., 1978). Tetra ethyl lead (TEL) is considerably more toxic than non-organic lead. Thus, in rats the LD_{50} is 6.5 mg/kg for TEL (Schroeder et al., 1972) and 70 mg/kg for non-organic lead (Hammond, 1973).

Absorbed lead is transported into the bloodstream; about 95% of this leads is bound to erythrocytes and passes into the internal organs. The highest concentration is in the liver and kidneys, while a considerably lower concentration is present in the brain, lungs and spleen. It is deposited in the bones as well, in the form of relatively insoluble lead triphosphate. Most lead is excreted through

the urine, and less through sweat, feces and milk. In some cases, such as infections or disorders of the acidobasal balance, lead can be mobilized from the bones into soft tissue (Goldberg, 1975).

Experimental and clinical research have shown that lead, especially after long-term poisoning, leads to hypochromic microcytic anemia (Schooley and Mahlmann, 1974). In addition to the reduced number of erythrocytes and hemoglobin and hematocrit values, the number of leukocytes is also reduced. Snigs of hypochromia are visible on blood smears, as well as anisocytosis and poikilocytosis, together with normoblasts and basophilous punctured erythrocytes (Hass et al., 1964; Goyer, 1972; Mahaffey, 1973; 1974).

It has been established that lead, which can affect iron transport through the mitochondrial membrane (Kraimer-Birnbaum and Grinstein, 1965), can also cause erythroid hypoplasia and disorders in the mitochondria and ribosomes during proliferation and maturation of erythrocytes (Morse, 1972). Subclinical poisoning with lead and cadmium have a depressive effect on the formation of antibodies, i. e. sensitivity to infections is increased (Koller and Kovačić, 1974; Koller and Brauner, 1977).

Lead also acts as a cardiac depressant. It disrupts the conductive system in the heart and especially reduces the frequency and minute volume as well as leading to disorders in glucose metabolism (Schroeder, 1960; Morris, 1961; Vulović, 1982). In addition to its effect on the heart, lead also affects the nervous system, leading to the occurrence of peripheral myoneuropathy. Lead poisoning also causes changes in the kidneys (Cramer et al., 1974). Lead induced kidney insufficiency in turn causes decreased excretion of erythropoietin, which has an effect on the appearance of anemia (Abbrect et al., 1978).

With respect to the wide-spread presence of lead in nature, toxic and genotoxic effects of lead have been examined using in vivo and in vitro methods (Schlick and Friedberg, 1982; Maslat and Haas, 1989; Magos, 1991; Xu et al., 1992; Wise et al., 1992; Roy et al., 1992; Roy and Rossman, 1992; Windei and Bonin, 1993; Lin et al., 1994). Although, a high genotoxic potential of dissolvable lead compounds has been demonstrated, the mechanism of genotoxic and carcinogenic effects of lead has not been sufficiently clarified. Therefore, the genotoxic potential of various doses of lead acetate was examined in this work, using the micronucleus test on BALB/c mice.

MATERIALS AND METHODS

We determined LD_{50} over 7 days before the micronucleus assay was begun. The toxic effect of lead acetate in dose levels of 50, 250 and 500 mg/kg body weight was investigated in groups of 5 BALB/c female mice with a body weight of 20.2± 1.8 g.

The results showed that lead acetate in doses over 500 mg/kg caused mortality in mice.

For the micronucleus assay, the animals were divided into 5 groups with 5 BALB/c mice in each group. Their body weight was 21.4± 2.8 g. Physiological

saline solution was administered twice daily in doses of 0.1 ml per mouse during the 24-hour intervals between two treatments. The positive control group included animals treated with cyclophosphamide (Endoxan) in a single dose of 50 mg/kg b.w. Animals in three experimental groups were treated twice with lead acetate in doses of 25, 125, and 250 mg/kg b.w. in the 24-hour interval. All test compounds were applied intraperitoneally.

The mice were sacrificed 48 hours after the first treatment with test components. Their femurs were prepared for bone marrow sampling. Bone marrow was flushed from femurs into fetal calf serum. The method according to Schmid (1976) was used for the preparation of micronuclei. Two slides of sedimented bone marrow cells were made for each animal and stained with May-Grünwald Giemsa.

The frequency of micronuclei, their morphological and tinctorial caracteristics were established for each animal by counting a total of 1000 polychromatic erythrocytes (PCEs), as described in our previous paper (Rusov et al., 1988).

RESULTS AND DISCUSSION

The results obtained for genotoxicity of lead acetate and other test compounds in the micronucleus assay are shown in Table 1.

Table 1. Frequency of micronuclei in PCEs in BALB/c mouse marrow 48 hours after the first treatment of animals with test compounds.

Test compounds	Dose (mg/kg b.w.)	Number of micronuclei per 1000 cells	
Negative control (Physiological solution)	2 x 0,1 ml	1,4 ± 0,89	
Positive control (Cyclophosphamide)	50	7,6 ± 3,21	P < 0,005
Lead acetate	50	8,8 ± 2,17	P < 0,001
Lead acetate	250	9,5 ± 2,07	P < 0,001
Lead acetate	500	9,2 ± 2,28	P < 0,001

Data presented in Table 1 show that cyclophosphamide increased the number of micronuclei in polychromatic erythrocytes of bone marrow (7.6± 3.21). The value is statistically significant (P<0.005) when compared to the number of spontaneous micronuclei in mice inoculated with physiological solution only (1.4± 0,89).

All the administered test dioses of lead acetate (50, 250 and 500 mg/kg b.w.) also caused very significant changes in the number of micronuclei in PCEs of treated animals. All three examined test doses of lead acetate caused a very significant increase (P<0.001) in the number of micronucleusi (8.8± 2.17, 9.5± 2.07, and 9.2± 2.28) in comparison with the occurrence of spontaneous micronucleusi in the negative control group (1.4± 0.89).

Changes in erythropoiesis occur after the application of large doses of lead, and especially after long-term effects of lead. In these cases, this causes not only

decreased numbers of pluripotent stem cells, but also of other bone marrow cells which are in the phase of proliferation and maturation (Schlick and Friendberg, 1982), and disorders in hem synthesis (Goldberg et al., 1977) and hemolytic anemia (Secchi et al., 1973). The results of our investigations have shown that a lead acetate dose of 250 mg/kg b.w. causes slight effects on the erythrocytes of peripheral blood already 48 hours after the first intraperitoneal inoculation, and that a dose of 500 mg/kg b.w. causes strong effects. In addition to decreased numbers of erythrocytes, we also observed weaker or stronger hypochromia, polychromasia, anisocytosis and poikilocytosis in stained smears of peripheral blood.Polychromatophilic erythrocytes were often present but basophilically punctured erythrocytes were seldom seen. The slight increase in the number of induced micronuclei following inoculation with high doses of lead acetate could be explained by the fact that they damage and/or destroy large numbers of immature erythropoietic cells, thus reducing the possibility for the occurrence of larger numbers of micronuclei.

The results of our research on BALB/c mice showed the genotoxic effect of the lead acetate in high doses. Such results confirmed the findings of other authors in cell cultures from animals and human beings (Xu et al., 1992; Wise et al., 1992; Roy and Rossman, 1992; Windei and Bonin, 1993; Lin et al., 1994), in the micronucleus test on mice (Roy et al., 1992) as well as in the Ames test (Maslat and Haas, 1989). Those results demonstrated that dissolvable compounds of lead do have cytotoxic effects, causing neoplastic cell transformation in culture, DNA strand damage, induced micronuclei formation and chromosomal aberrations.

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IZUČAVANJE GENOTOKSIČNOSTI OLOVO ACETATA MIKRONUKLEUS TESTOM NA MIŠEVIMA

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SADRŽAJ

Genotoksikološka karakterizacija olovo acetata u mikronukleus testu na BALB/c miševima pokazuje da ispitivane doze (50, 250 i 500 mg/kg telesne mase) indukuju relativno veliki broj mikronukleusa (8,8± 2,17, 9,5± 2,07, 9,2± 2,28) u polihromatofilnim eritrocitima kostne srži. Ove vrednosti su statistički visoko

značajne ($P<0,001$) u odnosu na broj spontanih mikronukleusa ($1,4\pm0,89$) u kontrolnim miševima, inokulisanim fiziološkim rastvorom.

S obzirom na toksičnost i genotoksični potencijal visokih doza olova, neophodno je primenjivati odgovarajuće mere i postupke radi sprečavanja izlaganja životinja i ljudi olovu.