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A STUDY OF COPPER GENOTOXICITY IN THE MICRONUCLEUS TEST ON MICE

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Genotoxicological characterization of cupric acetate in the micronucleus test on BALB/c mice showed that small dose levels (0.5 and 5.0 mg/kg of body weight) and especially higher dose levels (10.0 and 25.0 mg/kg of b. w.) induce higher micronuclei counts (9.21± 1.30, 11. 0± 2.64, 15.6± 2.07 and 20.3± 2.58) in polychromatic erythrocytes of the bone marrow. The count of induced micronuclei was statistically significant ($P<0.001$) compared to that of spontaneous micronuclei (2.2± 1.30) found in control mice inoculated with physiological saline solution, but also somewhat lower than in mice treated with cyclophosphamide (23. 8± 1.79).

Thus, the results of the investigation showed that cupric acetate in the investigated dose levels, inoculated intraperitoneally in to mice had a mutagenic effect.

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

INTRODUCTION

Copper is an essential but also very toxic element when applied in large doses. Its role in enzymatic processes and hematopoiesis, as well as in the creation of humoral and cell-mediated immunity, is well known. Copper is also a component of cytochrome, cytochrome oxidase, arginase, tyrosinase, and can chelate to amino acids, ascorbic acid, etc. Copper also catalyses the implanting of iron during hemoglobin synthesis.

Disorders of copper metabolism are expressed through various pathologies.

Thus, cooper deficiency induces hematopoietic disorders, that is, the development of microcytic hypochromic or normocytic hypochromic anemia (Gubler et al., 1952; Weisenberg et al., 1980; Cohen et al., 1985). Deficiency also impairs neutrophil function (Boyne and Arthur, 1981), causes lymphocyte disorders with reduction of cell-mediated and humoral immunity (Lukasewycz and Prohaska, 1982) and increased susceptibility to bacterial infections (Jones and Suttle, 1983), as well as other disorders.

The primary biochemical lesions induced by copper toxicosis are manifested through Cu-initiated free radical-mediated membrane lipid peroxidation and protein and nucleic acid damage (Yourtee et al., 1992).

With respect to the physiological role of copper, and the pathological lesions induced by toxicosis, as well as certain results (Kostyniak et al., 1990; Sayato et al., 1990; Tkeshelashvili et al., 1991; Codina et al., 1995) showing that copper ions are mutagenic, the genotoxic effect of therapeutic doses of cupric acetate at lower and higher levels have been investigated in this study.

MATERIALS AND METHODS

Prior to the micronucleus test, groups of 5 BALB/c female mice, age 10-11 weeks, body weight (b.w.) 21. 25± 1.8 g, were tested for their sensitivity to copper toxicity.

Cupric acetate dissolved in 0.1 ml of physiological saline was inoculated into each of 5 mice intraperitoneally (i.p) in doses of 0.5, 5.0, 10.0 and 25.0 mg/kg b.w. The animals in the control group received 0.1 ml physiological saline i. p. per mouse.

The results of the toxicological assay showed that cupric acetate in the dose of 25.0 mg/kg b.w. induced mortality of 2 mice, one 6 and one 7 days after i. p. inoculation. The dose of 50.0 mg/kg b.w. induced the death of all 5 mice 18h subsequent to i. p. inoculation.

For the micronucleus test 6 experimental groups of 5 BALB/c female mice, age 11-12 weeks and b. w. 22.5± 2.7 g were formed. The animals in the negative control group were treated twice with physiological saline at a dose of 0.1 ml per mouse, at 24-hour intervals. Animals in the positive control group were treated with cyclophosphamide (Endoxan), administered as a single dose of 50.0 mg/kg b. w. In the four experimental groups, animals were treated twice with cupric acetate at the total dose level of 0.5, 5.0, 10.0 and 25.0 mg/kg b. w. at 24-hour intervals respectively.

The mice were sacrificed 48 hours subsequent to the first application of test material. Bone marrow was taken from their femurs and flushed into fetal calf serum. Preparation of the slides of the bone marrow and staining by the May-Grünwald Giemsa method for the determination of micronuclei in polychromatic erythrocytes, was performed using the method of Schmid (1976) somewhat modified.

The occurrence and frequency of micronuclei, their morphological and tinctorial characteristics were determined for each animal by counting a total of 1000 polychromatic erythrocytes, as described in our previous papers (Rusov et al., 1988, 1990, 1996).

RESULTS AND DISCUSSION

The results concerning the genotoxicity of cupric acetate and other test compounds in the micronucleus assay are presented in table 1.

Table 1. Frequency of micronuclei in polychromatic erythrocytes in BALB/c mouse bone 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	P
Negative control (Physiological saline)	2 x 0.1 ml per mouse	2.2 ± 1.30*	
Positive control (Cyclophosphamide)	50	23.8 ± 1.79	< 0.001
Cupric acetate	0.5	9.2 ± 1.30	< 0.001
Cupric acetate	5.0	11.0 ± 2.64	< 0.001
Cupric acetate	10.0	15.6 ± 2.07	< 0.001
Cupric acetate	25.0	20.3 ± 2.58	< 0.001

* Data are expressed as mean ± SD for 5 mice

The data presented in table 1 show that cyclophosphamide induced an increase of the micronuclei count in polychromatic erythrocytes of the bone marrow (23.8 ± 1. 79). This increase was statistically highly significant ($P<0.001$) compared to the count of spontaneous micronuclei in mice treated with physiological saline (2.2 ± 1.30).

Cupric acetate at all investigated dose levels (0.5, 5.0, 10.0 and 25.0 mg/kg b.w.) induced a corresponding increase of the micronuclei count in polychromatic erythrocytes of the treated mice (9.2 ± 1.30, 11.0 ± 2.64, 15.6 ± 2.07 and 20.3 ± 2.58). The increases in micronuclei counts were also statistically highly significant ($P<0.001$) compared to the frequency of spontaneous micronuclei found in the negative control group of mice (2.2 ± 1.30).

Copper levels directly affect the interaction of copper ions with specific sequences of nucleotides in DNA. Copper ions are the most mutagenic. Tkeshelashvili et al. (1991) established that the frequency of micronuclei in mice treated with copper is equal to or greater, namely 1-2 times greater than that induced by Fe2+ (Rusov et al., 1995).

Mutagenesis by Cu+ was diminished by catalase, mannitol, and superoxide dismutase suggesting the involvement of H_2O_2, hydroxyl ions, and superoxide, respectively. However, the finding that Cu+ and Cu2+ are nearly equally mutagenic and that the mutagenic activities are not completely inhibited by oxygen free radical scavengers make it unlikely that the mechanism for mutagenesis is simply the production of hydroxyl free radicals (Tkeshelashvili et al., 1991).

Codina et al., (1995). investigated heavy metal genotoxicity using four genotoxicity assays, and all the metals tested, including copper, were found to be genotoxic by the Mutatox super (TM) and the SOS tests.

With respect to the significant mutagenic potential of copper ions, special attention to dose determination, application mode and duration of use of copper preparations in animals is suggested.

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IZUČAVANJE GENOTOKSIČNOSTI BAKRA U MIKRONUKLEUS TESTU NA MIŠEVIMA

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SADRŽAJ

Genotoksikološka karakterizacija bakar acetata u mirkonukleus testu na BALB/c miševima pokazuje da i niske doze (0.5 i 5.0 mg/kg telesne mase), a naročito visoke doze (10.0 i 25.0 mg/kg) indukuju veliki broj mikronukleusa (9.2± 1.30, 11.0 ± 2.64, 15.6 ± 2.07 i 20.3 ± 2.58) u polihromatofilnim eritrocitima kostne srži. Broj mikronukleusa prouzrokovan bakar acetatom je vrlo visoko signifikantan ($P<0.001$) u odnosu na broj spontanih mikronukleusa (2.2 ± 1.30) u kontrolnih miševa, tretiranih fiziološkim rastvorom, i samo nešto niži nego u miševa tretiranih ciklofosfamidom (23.8 ± 1.79).

Rezultati istraživanja u mikronukleus testu na miševima pokazuju da bakar acetat u ispitivanim dozama, aplikovan miševima intraperitonealno, deluje mutageno.