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Evaluation of the Genotoxicity of Cadmium Chloride in Mice Using the Micronucleus Test

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ABSTRACT : **In order to determine the safety of chemicals and pharmaceutical products, various methods can be used to evaluate the toxicity. In this study the genotoxic effect of the widely used industrial chemical, cadmium chloride, was assessed using the micronucleus test in peripheral blood of mice. The presence of micronucleated reticulocytes by microscopic observation following acridine orange staining indicated a potential genotoxic effect. The genotoxicity of intraperitoneally (i.p.) administered cadmium chloride (0.5, 1, 2 mg/kg) appeared to be dose dependent, with the maximum tolerated dose (MTD) found to be 2 mg/kg. Compared to the negative control (saline), cadmium chloride (2 mg/kg) exhibited statistically significant genotoxic potential ($P<0.05$) but was found to be less than the positive control of mitomycin C (0.5 mg/kg) and was not statistically significant compared to historical negative controls ($P>0.05$).**

Keywords : **genotoxicity, cadmium chloride, micronucleus test**

Introduction

In recent years the prevalence of metals and other inorganic compounds has increased significantly in the human environment. Some of these compounds may be carcinogenic and following exposure could produce cancer but until relatively recently the mechanism of action has not be elucidated.

Cadmium is an inert inorganic compound which is widely used within various industrial processes (e.g. battery manufacturing, anti-rust coating, welding alloys, pigment in paint and plastic). It has been reported that cadmium can result in lung, kidney, liver, intestine and bone marrow toxicity [1-3]. It was found to inhibit protein synthesis and also interfere in calcium metabolism potentially causing calcium deficiency leading to osteoporosis.

The micronucleus test is one of the most reliable, sensitive and simple techniques for evaluating genotoxicity and has been recognized by the 4th International Conference on the Harmonization of Genotoxicity Guidelines. Micronuclei are produced in the anaphase of cell division as a result of chromosomal alterations (e.g. changes in numbers or breakage, by aneugens or clastogens, respectively). The presence of micronuclei thus indicates a mutagenic/genotoxic effect of the compound tested. Usually mice are the preferred animals used for this procedure, as it is possible to use both bone marrow and peripheral blood [4]. The assay can also be performed concurrently with other mutation tests [5]. In 1993, the International Workshop on Standardization of Genotoxicity Tests suggested that in mice 2000 reticulocytes should be counted in order to determine any genotoxic effect [6].

This paper describes the evaluation of the potential genotoxic effect of cadmium chloride in mice as evidenced using the micronucleus test.

Materials and Methods

Cadmium chloride and acridine orange were obtained from Merck, Germany. Mitomycin C was purchased from Lenery Co., Germany. Wistar albino male mice were purchased from Razi Institute, Karaj, Iran.

For this experiment, it was necessary to determine the Maximum Tolerated Dose (MTD). This was achieved by administering cadmium chloride intraperitoneally (i.p.) at 0.5-4 mg/kg. 2 mg/kg was found to be the most suitable MTD for the mice tested [7-9].

In this study five groups of mice (each group consisting of five mice) were used. Mice were fasted overnight prior to receiving i.p. normal saline (negative control) or

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0.5 mg/kg mitomycin C (positive control) or cadmium chloride at 0.5 mg/kg, 1 mg/kg and 2 mg/kg (based on the MTD). The mice were weighed and kept in their cages. A second dose was given 24 hours after the first administration. 24 hours later, peripheral blood was obtained from the tail of each mouse by microsyringe.

Blood was pipetted on to the centre of pre-prepared acridine orange coated microscopic slides, a coverslip was placed on the slide and left overnight for improved staining and fixation of reticulocytes. Following acridine orange staining, non-nucleated reticulocytes produce red/orange fluorescence, whilst micronucleated reticulocytes produce yellow/green fluorescence. This allows easy identification of micronuclei contained in the reticulocytes [10-11]. Subsequent observation used a fluorescence microscope with blue and yellow to orange filters. Following initial observation at 10x magnification, counting of the reticulocytes was performed using 40x power magnification. The number of micronucleated reticulocytes were counted per 2000 reticulocytes and statistically analysed. The methodology and subsequent interpretation of data were performed in accordance with the guidelines of the 5th Collaborative Study Genotoxicity Micronucleus Test (CSGMT).

Results

The results of the microscopic observations of mice blood in the presence of; normal saline, 0.5 mg/kg mitomycin C and 2 mg/kg cadmium chloride, respectively are shown in Photographs 1-3. Microscopic inspection following acridine orange staining showed yellow/green fluorescence of micronucleated reticulocytes for the group receiving 0.5 mg/kg mitomycin C (positive control) and cadmium chloride (0.5, 1 and 2 mg/kg), however more micronucleated reticulocytes were observed at increasing cadmium chloride dosage. No micronuclei were observed in the normal saline, negative control group.

Table 1 shows the number of micronucleated reticulocytes per 2000 reticulocytes in the five mice of the five different test groups. In general, the negative control and 0.5 mg/kg dose of cadmium chloride showed very few (1-3) micronucleated reticulocytes. In the presence of a known genotoxic agent (mitomycin C) 28-44 micronucleated reticulocytes were observed. Compared to the negative control, higher doses of cadmium chloride (1 and 2 mg/kg) produced more micronucleated reticulocytes (2-5 and 3-7, respectively). Figure 1 shows the apparent dose dependent genotoxic potential of cadmium chloride, compared to the negative control and is consistent with the previously determined MTD. Figure 2 shows the mean number of micronucleated reticulocytes for each group studied.

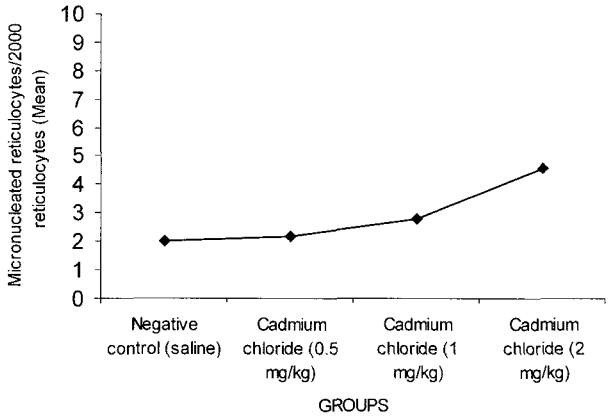


Fig. 1. Dose dependent increase in the mean number of micronucleated reticulocytes per 2000 reticulocytes observed in mice receiving i.p. cadmium chloride.

Discussion

As previously mentioned, the micronucleus test has been demonstrated to be a simple, rapid and accurate technique for the determination of genotoxicity and is a useful tool in the assessment of pharmaceutical, herbal and chemical products. Following analysis of the results, the International Workshop in Biostatistics recommends interpretation in the form of positive, negative or equivocal (undetermined in this context) [12].

Table 1. Number of micronucleus reticulocytes per 2000 counted reticulocytes in different mice groups (n=5)

Groups (Dose)	No. of micronucleated reticulocytes per 2000 reticulocytes				
Negative control (saline)	1	2	2	3	2
Positive contril (mitomycin C 0.5 mg/kg)	37	44	28	38	33
Cadmium chloride (0.5 mg/kg)	2	3	3	2	1
Cadmium chloride (1 mg/kg)	2	2	3	5	2
Cadmium chloride (2 mg/kg)	3	6	4	3	7

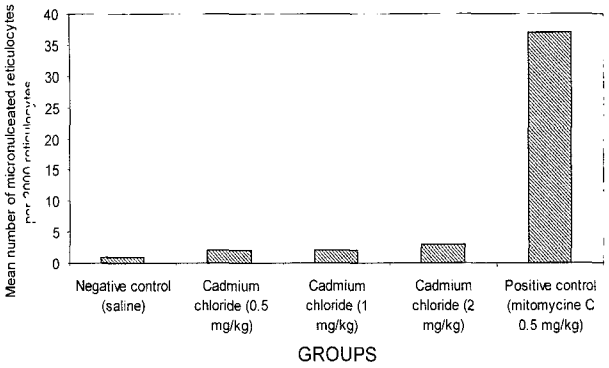
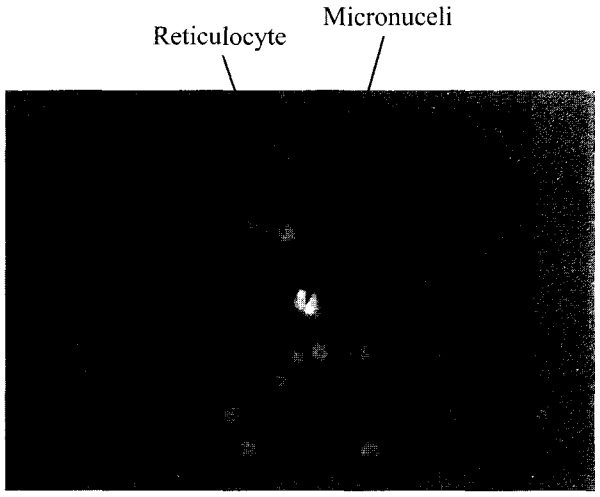
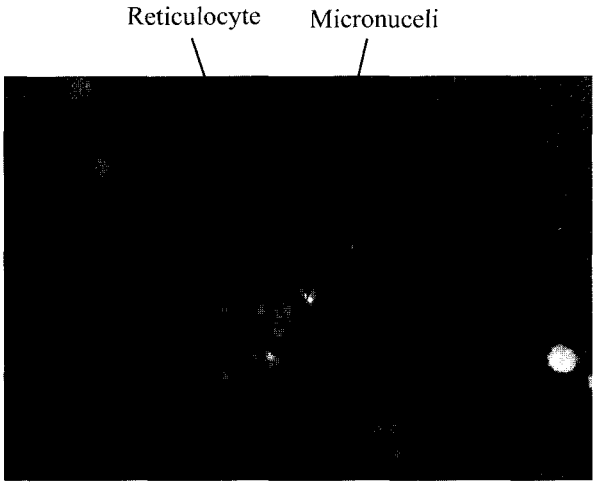


Fig. 2. Mean number of micronucleated reticulocytes per 2000 reticulocytes in mice receiving i.p. normal saline, cadmium chloride (0.5, 1 and 2 mg/kg) and mitomycin C (0.5 mg/kg).

Photograph 1. Microscopic observations following acridine orange staining in peripheral blood of mice receiving normal saline solution (negative control group).



Photograph 2. Microscopic observations following acridine orange staining in peripheral blood of mice receiving 0.5 mg/kg mitomycin C (positive control group).



Photograph 3. Microscopic observations following acridine orange staining in peripheral blood of mice receiving 2 mg/kg cadmium chloride (test group).

Based on the mean number of micronucleated reticulocytes per 2000 reticulocytes as observed by the micronucleus test, the results indicate that cadmium chloride has a genotoxic potential that appears to be dose dependent. Although the number of micronuclei produced is less than that of a known genotoxic agent (mitomycin C), the number is greater than that observed in the negative control group receiving no genotoxic agents. In the saline control group, a mean of 2 micronucleated reticulocytes per 2000 reticulocytes was observed, whilst a mean of 4.6 micronucleated reticulocytes were observed following 2 mg/kg cadmium chloride i.p. administration (Fig. 2).

Statistical analysis of the data showed that the difference between the 2 mg/kg cadmium chloride dose and the negative control was statistically significant (P=0.0088, therefore P<0.05). However, compared to historical negative controls, the results were statistically insignificant (P=0.13, therefore P>0.05). Therefore, although the findings in this study indicate cadmium chloride has undetermined genotoxic potential, further work is necessary in order to prove a significant/positive genotoxic effect.

Conclusion

The results of the micronucleus test indicated a potential genotoxic effect of cadmium chloride when administered intraperitoneally to mice. Genotoxicity appeared to be dose dependent, with a maximum tolerated dose (MTD) of 2 mg/kg. Compared to the negative control (saline),

cadmium chloride (2 mg/kg) exhibited statistically significant genotoxic potential ($P<0.05$) but was found to be less than the positive control of mitomycin C (0.5 mg/kg) and was not statistically significant compared to historical negative controls ($P>0.05$).

References

[1] Timbrell, J.A. (Ed.) (1995): *Introduction to Toxicology*, 2nd Edition. New York, 135-137.

[2] Hinkle, P.M., Kinsella, P.A., Osterhoudt, K.C. (1987): Cadmium uptake and toxicity via voltage-sensitive calcium channels. J. Biol. Chem., **262**(34), 16333-16337.

[3] Shaikh, Z.A., Blazka, M.E., Endo, T. (1995): Metal transport in cells: cadmium uptake by rat hepatocytes and renal cortical epithelial cells. Environ. Health Perspect., **103** (Suppl 1), 73-75.

[4] Micronucleus test with mouse peripheral blood erythrocytes by acridine orange supravital staining: the summary report of the 5th collaborative study by CSGMT/JEMS.MMS (1992): The Collaborative Study Group for the Micronucleus Test. Mutat. Res., **278**(2-3), 83-98.

[5] Suzuki, T., Hayashi, M., Sofuni, T., Myhr, B.C. (1993): The concomitant detection of gene mutation and micronucleus induction by mitomycin C *in vivo* using lacZ transgenic mice. Mutat. Res., **285**(2), 219-224.

[6] Protocol recommended by the CSGMT/JEMS.MMS for the short-term mouse peripheral blood micronucleus test (1995): The Collaborative Study Group for the Micronucleus Test (CSGMT) (CSGMT/JEM.MSS, The Mammalian Mutagenesis Study Group of the Environmental Mutagen Society of Japan). Mutagenesis, **10**(3), 153-159.

[7] Volkova, N.A, Karpliuk, I.A., Emelianova, E.V. [Study of the mutagenic acitivity of cadmium by the method of dominant lethal mutation]. Vopr. Pitan. 1995, **2**, 24-25.

[8] Satarug, S., Haswell-Elkins, M.R., Moore, M.R. (2000): Safe levels of cadmium intake to prevent renal toxicity in human subjects. Br. J. Nutr., **84**(6), 791-802.

[9] Habeebu, S.S., Liu, J., Lui, Y., Klaassen, C.D. (2000): Metallothionein-null mice are more sensitive than wild-type mice to liver injury induced by repeated exposure to cadmium. Toxicol. Sci., **55**(1), 223-232.

[10] Hayashi, M., Morita, T., Kodama, Y., Sofuni, T., Ishidate, M. (1990): The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange coated slides. Mutation Res., **245**(4), 245-249.

[11] Hayashi, M., Sofuni, T., Ishidate, M. (1983): An application of acridine orange fluorescent staining to the micronucleus test. Mutation Res., **120**, 241-247.

[12] Hayashi, M., Hashimoto, S., Sakamoto, Y., Hamada, C., Sofuni, T., Yoshimura, I. (1994): Statistical analysis of data in mutagenicity assays: rodent micronucleus assay. Environ. Health Perspect., **102** (Suppl 1), 49-52.