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METAL IONS

EFFECT OF CADMIUM (II) AND LEAD (II) ON THE RADIATION ADAPTIVE RESPONSE VALUE IN RAT BONE MARROW CELLS

S.I. Zaichkina, O.M. Rozanova, G.F.Aptikaeva, A.Kh. Akhmadieva, M.Ed. Zaichkin

Institute of Theoretical and Experimental Biophysics, Pushchino, Moscow region 142290 Russia, e-mail: Zaichkina@mail.ru

SUMMARY. It was shown that treatment with Cd (II) and Pb (II) led to increase in frequency of chromosome aberrations in bone marrow cells of non-irradiated rats and slightly enhanced the radiation effect. Exposure to chronic g-irradiation induced significant adaptive response (AR) to subsequent acute irradiation. In the presence of Cd (II) and Pb (II) the magnitude of the AR was decreased 1.5- and 2-fold, respectively.

Introduction

Heavy metals are the most widespread and harmful contaminants of environment. They induce DNA lesions that either are not repaired or are repaired very poorly. Cadmium and plumbum compounds have a particularly high genotoxic action (Carla Campa et al., 1993; Mukherjee et al., 1998). Normal cells are also capable of forming AR, an enhancement of cellular resistance to high challenge doses of ionising radiation (IR) by preliminary exposure to low priming doses. The literature data about the AR in animals exposed to IR in vivo are contradictory (Cai, Liu, 1990; Muller et al., 1992; Wojcik et al., 1992). Only in some works the authors used chronic pre-irradiation (Tuschl et al., 1980, 1983; Liu et al., 1992). Along with the wide distribution of metals in environment, prolonged chronic exposure to IR at low dose rates often takes place. Therefore, the study of the ability of animals to form AR under similar conditions is of great interest.

The goal of the present work was to study the effect of salts of heavy metals, lead and cadmium, on the cytogenetic damage in bone marrow cells of rats exposed to acute and chronic γ-irradiation, as well as on the magnitude of the AR induced by chronic irradiation.

Materials and Methods

Adult male rats (120–200 g) were exposed to chronic γ-irradiation (^{137}Cs, dose rate of 0.0013 Gy/h, cumulative doses were 0.03, 0.09, 0.2 and 0.4 Gy). Solutions of heavy metals Pb $(CH_3COO)2x3H_2O$ and $CdCl_2x2.5H_2O$ at a rate of 50 mg of pure substance per 1l of boiled water were given to animals as a drinking throughout the period of chronic irradiation. Rats subjected to chronic irradiation with an appropriate dose were immediately subjected to single acute dose of 4 or 6 Gy (dose rate of 0.47 Gy/min). Four hours later, the animals were injected with colchicine intraperitoneally (4 mkg/g body weight). After twq hours, the rats were anaesthetized with nembutal administered intraperitoneally (60 mkg/g body weight). Chromosome preparations of bone marrow cells were carried out by standard procedure (Macgregor, Varley, 1983). Percent of cells with aberrations and all types of chromosome aberrations were recorded. Five animals were used for each experimental point. From each animal, 30–50 cells were analysed.

Results

The results of analysis of chromosomal aberrations induced by acute irradiation are presented in Table 1.

Chromatid aberrations were observed (mainly acentric fragments and deletions). The data of Table 2 show that preliminary chronic irradiation of animals at a total dose of 0.03–0.4 Gy can induce a pronounced AR. The AR was estimated as the ratio of chromosomal damage expected with the additive effect to the damage observed. Response was shown to increase with the dose of chronic irradiation. The most effective dose for induction of the AR was 0.4 Gy. It is seen from Table 2 that the AR was due to the reduction of fragment number.

The data of Table 3 show that treatment with Cd (II) and Pb (II) led to increase in frequency of chromosome aberrations in bone marrow cells of non-irradiated rats and slightly enhanced the radiation effect.

In the presence of Cd (II) and Pb (II) the magnitude of the AR was decreased 1.5- and 2-fold, respectively (Table 4).

TABLE 1. FREQUENCY OF CHROMOSOMAL ABERRATIONS INDUCED BY DIFFERENT DOSES OF ACUTE γ-IRRADIATION.

Dose, Gy	Metaphases scored	Cells with aberrations, %	Aberrations, %	Fragments, %	Exchanges, %
0	524	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	–
4	204	25 ± 0.15	28 ± 0.18	21 ± 0.16	7 ± 0.07
6	400	42 ± 0.18	48 ± 0.20	36 ± 0.15	12 ± 0.10

Table 2. Frequency of chromosomal aberrations induced by various doses of chronic (D1) and acute (D2) γ-irradiation.

D1+D2, Gy	Metaphases scored	Metaphases * with aberrations, %		Aberrations, %		Fragments, %		Exchanges, %	
		1	2	1	2	1	2	1	2
0+6	154	42.0±3.9	–	48.0±4.0	–	36.0±3.9	–	12.1±2.6	–
0.03+6	113	21.0±3.8	42.0	26.0±4.1	48.1	19.0±3.7	36.0	6.8±2.4	12.0
0.09+6	221	28.0±3.0	42.1	31.9±3.1	48.1	24.0±2.9	36.1	8.0±1.8	12.0
0.2+6	155	27.7±3.6	42.2	37.2±3.9	48.2	25.9±3.5	36.2	11.1±2.5	12.0
0.4+6	154	13.0±2.7	42.2	14.0±2.8	48.2	10.0±2.4	36.2	4.0±1.6	12.0
0.4+4	140	8.0±2.3	26.2	9.1±2.4	28.2	6.0±2.0	21.2	3.0±1.4	7.0

* data are shown without background level of chromosome aberrations;
1 — experimental data;
2 — data expected with the additive effect.

Table 3. Frequency of chromosomal aberrations induced by combined action of cadmium(II) and lead (II) with chronic or acute γ-irradiation.

Treatment	Metaphases scored	Total aberrations, no	Average yield of aberrations per cell	Yield of deletions per cell	Yield of exchanges per cell
0	524	7	0.014±0.002	0.014	–
CdCl2	130	5	0.038±0.003	0.038	–
Pb (II)	196	8	0.04±0.005	0.04	–
0.4 Gy	176	6	0.03±0.015	0.03	–
6 Gy	400	192	0.48±0.04	0.36	0.12
Cd (II) + 0.4 Gy	139	8	0.06±0.02	0.06	–
Pb (II) + 0.4 Gy	199	18	0.09±0.01	0.09	–
Cd (II) + 6 Gy	171	104	0.6±0.07	0.39	0.21
Pb (II) + 6 Gy	227	113	0.5±0.05	0.43	0.07

Table 4. Frequency of chromosomal aberrations induced by pretreatments with chronic radiation and cadmium (II) and lead (II) and subsequent acute γ-irradiation with a dose of 6 Gy.

Treatment	Metaphases scored	Total aberrations, no	Average yield of aberrations per cell	Yield of deletions per cell	Yield of exchanges per cell
0.4 Gy	176	6	0.03±0.015	0.03	–
6 Gy	400	192	0.48±0.02	0.36	0.12
0.4 Gy + 6 Gy	154	21	0.14±0.03	0.10	0.04
Cd (II) + 0.4 Gy + 6 Gy	146	26	0.18±0.02	0.15	0.03
Pb (II) + 0.4 Gy + 6 Gy	135	28	0.2±0.02	0.16	0.04

Discussion

In our previous study we have shown that acute irradiation of mice with adapting doses of 0.1, 0.2 and 0.4 Gy induced the AR in bone marrow cells as assessed by the micronucleus test. The yields of micronuclei in polychromatic erythrocytes (MNPCE) in adapted mice were less than those in non-adapted mice. The values of the AR were much the same for all the adapting doses (Zaichkina et al., 1999). The results of the experiments on the induction of the AR in mice by chronic g-irradiation at doses of 0.1 and 0.5 Gy have shown that the AR induced by 0.5 Gy dose is more pronounced than that induced by 0.1 Gy dose (Zaichkina et al., 2001). This result is consistent with the literature data showing reverse relationship between an adapting dose and a dose-rate for the maximum AR to be induced.

We obtain in the experiments with rats that preliminary chronic irradiation of animals at a total dose of 0.03–0.4 Gy induced the AR too. As in our experiments

with mice, the response was shown to increase with the dose of adapting irradiation.

In both papers by Shadley (Shadley, Weincke, 1989), Liu and Cai (Liu et al., 1992), and by using different objects this distinguishing feature of an AR was demonstrated — the lower dose-rate of an adapting dose, the higher this dose should be to induce the maximum AR. The most effective for the induction of the AR in our experiments under in vivo conditions was the dose of 0.4 Gy given at a dose rate of 0.0013 Gy/h. When rats were irradiated by a challenging dose of 4 Gy the value of the AR was 3.1, and, at a challenging dose of 6 Gy, the AR was 3.4.

The study of the modifying action of cadmium and lead administered chronically with the drinking water on radiation-induced cytogenetic effects have shown that this treatment increased the frequency of chromosome aberrations in cells of non-irradiated animals. Also, these metals increased the cytogenetic damage induced by chronic irradiation with a dose of 0.4 Gy, though statistically significant result was observed only in the lead-treated group.

Similarly, slightly enhanced levels of cytogenetic damage were recorded in cadmium- and lead-pretreated animals upon irradiation with an acute dose of 6 Gy.

We believe that the effect of the increase in the radiosensitivity by heavy metals is associated with the alterations in cell defence systems, such as antioxidant and DNA repair systems. In support of this point, there are data by Russian authors (Medvedev et al., 2000) who used cadmium and lead treatment conditions very close to ours and demonstrated that chronic exposure of mice to these metals inhibited the repair of DNA breaks induced by subsequent γ-irradiation. Besides, the yield of MNPCE after γ-irradiation in bone marrow of those mice was increased as compared to mice that did not receive cadmium and lead.

We studied the action of cadmium and lead pretreatment on the cytogenetic AR in rats and revealed that the chronic dose of 0.4 Gy induced the pronounced AR. Administration of metals with the drinking water throughout the period of chronic adapting irradiation, namely 14 days, did not abolish the AR completely. However, we can see the slight increase in the frequency of chromosome aberrations as compared to this group. Thus cadmium and lead, given to rats with the drinking water, decreased the relative value of the cytogenetic AR in bone marrow cells.

The data available in the literature on combined action of cadmium, lead and IR are conflicting. On the one hand, cross-resistance to IR was reported to be induced after acute injection of cadmium in mice (Meliksetova et al., 1997), on the other hand, abrogation of a cytogenetic AR due to chronic administration of dietary cadmium for 50 days was shown (Chernikov et al., 1998). In this work, authors used the concentration of cadmium similar to that used in our study. We believe that some controversy between their and our results exists due to different period of metal uptake. It is supported by a correlation between the period of chronic occupational and environmental exposure to heavy metals, including lead, and severity of cytogenetic effects in human reported by several researchers.

As for the mechanisms of cadmium and lead action, it is known that they deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of reactive oxygen species. As a consequence enhanced lipid peroxidation, DNA damage and altered calcium and sulfhydryl homeostasis occur. Also, cadmium can affect the p53-dependent responses to DNA damage, because it can readily substitute for zinc in p53 protein which is a multi-function nuclear factor. This protein is activated in response to various forms of stress and controls the proliferation, survival, DNA repair and differentiation of cells (Beyersmann, 1994; Muller et al., 1994; Skorczynska, 1997).

Conclusions

Our results presented here demonstrate that chronic low-dose γ-irradiation of rats induces a cytogenetic adaptive response in bone marrow cells. The administration of cadmium (II) and lead (II) salts to the diet of rats: enhances the cytogenetic damage in non-irradiated animals; slightly enhances the effect of chronic and acute γ-irradiation; decreases the cytogenetic AR induced by chronic γ-irradiation.

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