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Effects of Cadmium on Haemopoiesis in Irradiated and Non-Irradiated Mice: 2. Relationship to the Number of Circulating Blood Cells and Haemopoiesis

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Summary

The effect of administration of cadmium alone in non-irradiated mice as well as the effect of pre-irradiation administration of cadmium on the reparation processes of haemopoiesis were investigated in mice irradiated by a dose of 7.5 Gy. The pre-irradiation administration of cadmium accelerated the reparation processes in the bone marrow and spleen as well as the number of leukocytes and thrombocytes in the peripheral blood. The administration of cadmium alone caused a temporary weight decrease of the thymus and reduced number of erythrocytes, reticulocytes and haemoglobin values in the peripheral blood. The temporary rapid increase in the number of leukocytes on the 21st day after cadmium administration was investigated.

Key words
Cadmium – Haemopoiesis – Gamma radiation

Introduction

The risk factors caused by industrialization are increasing in our environment. Ionizing radiation belongs to the most dangerous types of pollution known worldwide. Among others very serious pollutants which have attracted increased attention in recent times, concerns the contamination of ecological systems by chemical substances, especially heavy metals. Pollution of the environment by heavy metals originates mainly from industrial waste and phosphorous fertilizers, etc. (Foulkers and McMullen 1986, Bache *et al.* 1986), and includes lead and cadmium which cause serious damage to very important organs which are essential for life and the process of haemopoiesis. It has been reported that cadmium causes pulmonary oedema and fibrosis, bone decalcification (Fassett 1980), liver (Dudley *et al.* 1982) as well as renal damage (Nordberg *et al.* 1975, Cherian *et al.* 1976) when it is accumulated rapidly (Sakata *et al.* 1988). The immune system (Dean *et al.* 1982, Koller 1980), especially thymocytes (Xu *et al.* 1989), react very sensitively to heavy metals. After administration of cadmium, the suppression of lymphocyte proliferation was observed in the spleen (Ohsawa *et al.* 1986). The effects of risk factors of chemical and physical pollution can be cumulative, causing more severe damage to the organism. On the other hand, it has been reported that a single dose of cadmium before irradiation increase the survival rates of irradiated animals (Matsubara *et al.* 1987, Fedoročko *et al.* 1996).

Based upon above mentioned research, the present report deals with the effect of cadmium itself or irradiation (single exposures to 7.5 Gy) as well as their combined application on the haemopoietic system of laboratory mice.

Materials and Methods

Mice

Conventionally bred ICR female mice, aged 8–10 weeks, with an average body mass of 30 g were used. They were housed in rodent cages (five to seven animals per cage) at about 22 °C, and were given Velaz/Altromin 1320 ST (Velaz, Prague, Czech Republic) laboratory chow and tap water acidified to pH 2.4 *ad libitum*. Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals", prepared by the State Veterinary Office of the Slovak Republic, Bratislava.

Cadmium chloride administration

Cadmium chloride, as $CdCl_2x2.5\ H_2O$, (Lachema, Brno, Czech Republic) was administered subcutaneously (s.c.) in the dorsal midthoracic area in 0.9 % sterile saline. Mice received 5 mg Cd/kg body weight. Control animals received an injection of saline.

Irradiation

Mice were placed in plexiglass containers and whole-body (unilaterally) exposed to 7.5 Gy of gamma rays (at a dose rate of 0.4 Gy/min), 24 h after cadmium injection. A Chisostat ^{60}Co source (Chirana, Czech Republic) was used for all irradiations.

Haematological assessment

Animals (5–10 mice per group) were examined at various time intervals after injection of cadmium and after irradiation. Leukocyte and erythrocyte counts, haematocrit and haemoglobin were measured using an automatic Coulter counter Model ZF; thrombocytes were counted using a Bürker chamber. Reticulocytes were evaluated after staining blood smears with Brilliant cresyl blue. White cell differentials were performed by counting 100 white blood cells on May-Grünwald-Giemsa stained smears. The spleen and thymus were excised, weighed and processed by routine histological methods. Histological sections of 5–6 μm were stained with haematoxylin-eosin. Bone marrow smears were stained with May-Grünwald-Giemsa. Statistical significance of differences in organ weight and peripheral blood parameters was evaluated by Student's t-test.

Results

Bone marrow

Three days after irradiation, a range from expressive hypoplasia to aplasia appeared in both irradiated animal groups (irradiated by 7.5 Gy with and without cadmium). In the Cd+7.5 Gy group, mitoses and myeloblasts were often observed in the marrow as evidence of proliferation processes. These were accompanied 7 days after irradiation by an increase of the total cell number mainly with proliferation and differentiation of myeloid cells. In the control irradiated group, haemopoiesis damping continued in most of the examined animals. However, reparation processes were also observed in this group 10 days after irradiation. In both groups (irradiated alone or Cd+7.5 Gy) these effects were accompanied by temporary cummulation of mononuclear cells of the lymphoid type (MCLT) which continued in the control irradiated animals until the 15th or 21st day after irradiation. In the Cd+7.5 Gy group, reparation processes continued more rapidly and they led to granulocyte hyperplasia in the marrow as well as restoration of erythropoiesis and megakaryocytopoiesis on the 21st day after irradiation. Similar morphological pictures in the control irradiated group occurred on the 31st day after irradiation. The administration of cadmium alone slightly stimulated granulopoietic activity in the bone marrow on the 10th day.

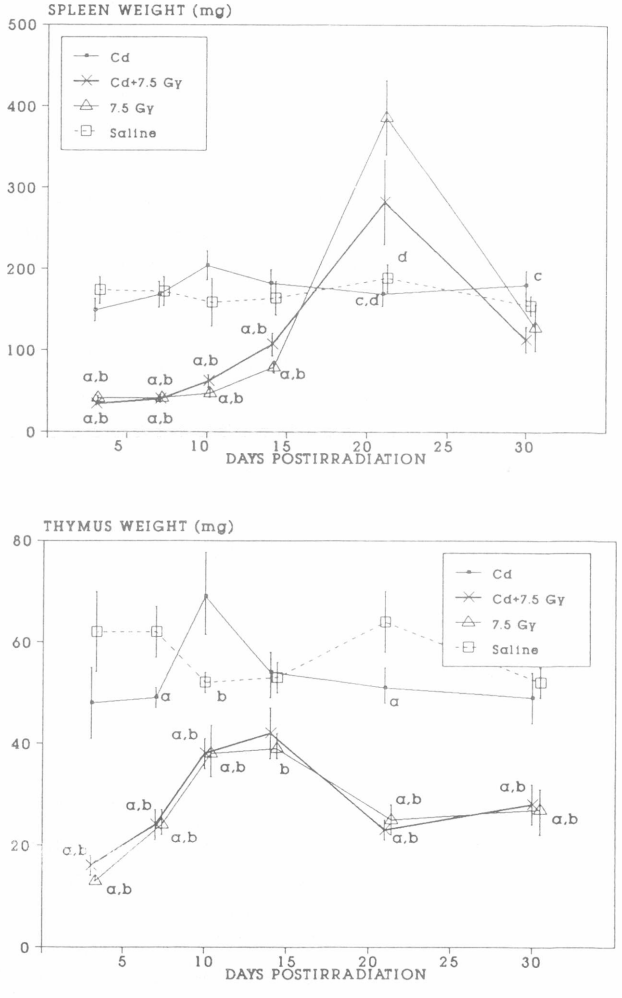

Fig. 1

Effects of cadmium on spleen and thymus weight in non-irradiated and irradiated mice. Symbols denote statistical significance when compared to the controls (a), group treated with cadmium alone (b), group treated with Cd+7.5 Gy (c) and group irradiated by 7.5 Gy (d). For the sake of simplicity significance at the 0.05 level was used for all comparisons.

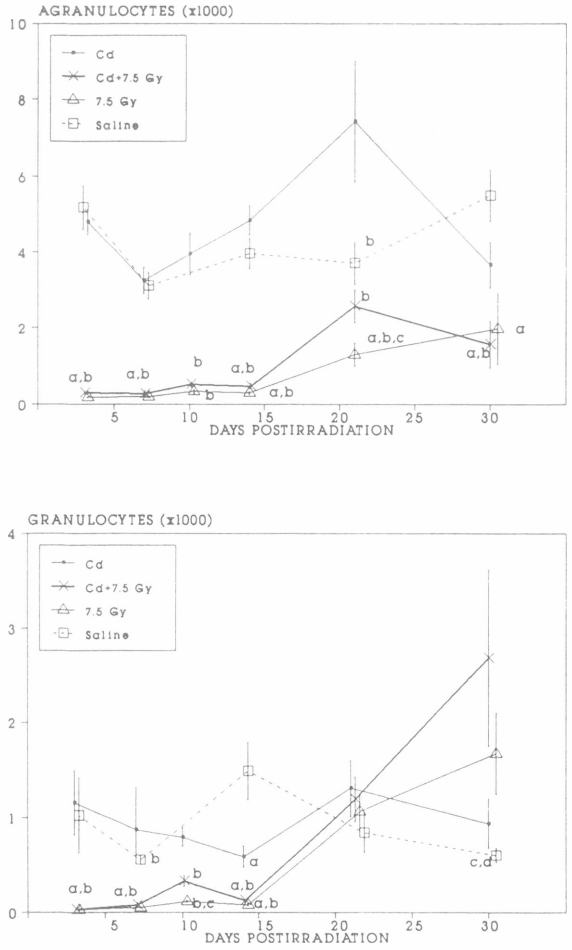

Fig. 2
Effects of cadmium on leukocyte and agranulocyte and granulocyte numbers in peripheral blood in non-irradiated and irradiated mice. For other legend see Fig. 1.

Spleen

The spleen also responded very sensitively to the above-mentioned irradiation conditions. Weight of the spleen in both irradiated groups decreased by 80 % compared to control non-irradiated animals (Fig. 1, $P<0.001$) by the 3rd day after irradiation. This was caused by a rapid reduction in lymphocyte numbers and a profound damping of erythropoietic activity. During the 10th to 14th day after irradiation, cadmium administration 24 h before irradiation accelerated the increase in spleen weight which was greater by 32 to 35 % than the spleen weight of animals which were only irradiated (Fig. 1). From the morphological examination of the spleen it was evident that in the Cd+7.5 Gy group the endocolonies already appeared in the red spleen pulps on the 7th day after irradiation, their number evidently being higher in the Cd+7.5 Gy group than in the control irradiated group on the 10th day after irradiation. After the 14th day, the restoration of lymphopoiesis was observed in animals which had received pre-irradiation administration of cadmium, and megakaryocyte occurrence increased rapidly at this time. In spite of this, the increase in spleen weight on the 21st day after irradiation was greater in the group which was only irradiated compared to the group which received cadmium before irradiation (Fig. 1), with rapid increases mainly in erythropoietic activity. The administration of cadmium alone increased the activity of germinal centres of lymphatic follicles in the spleen on the 10th day, and this was also accompanied by slight increase of spleen weight.

Thymus

The pre-irradiation administration of cadmium did not influence the reparation processes of the thymus, the course of which was the same in both irradiated groups of animals during the investigated period (Fig. 1). After the administration of cadmium alone thymus weight was significantly lower on the 7th day compared to the thymus weight of non-irradiated controls (Fig. 1, $P<0.05$). In this period, the thymocyte number also decreased in the thymus cortex. The thymus cortex was again densely occupied with thymocytes on the 10th day when thymus weight also increased temporarily (Fig. 1, $P<0.05$).

Peripheral blood

In the both irradiated groups of animals, expressive agranulocytopenia and granulocytopenia were observed and the total number of leukocytes also decreased below the control non-irradiated levels (Fig.

2, $P<0.001$). Cadmium application accelerated their reparation following the 7th day after irradiation, manifested by granulocyte number increases. On the 21st day after irradiation, the acceleration of reparation was also observed in agranulocyte number (Fig. 2). On the 31st day after irradiation, the granulocyte number in the control irradiated group was higher by 174 % ($P<0.05$) and in the Cd+7.5 Gy group by 341 % ($P<0.001$) than the granulocyte number of non-irradiated controls (Fig. 2). After administration of cadmium alone the number of leukocytes temporarily increased as compared to control values (Fig. 2) because the number of agranulocytes increased ($P<0.05$) on the 21st day after the injection.

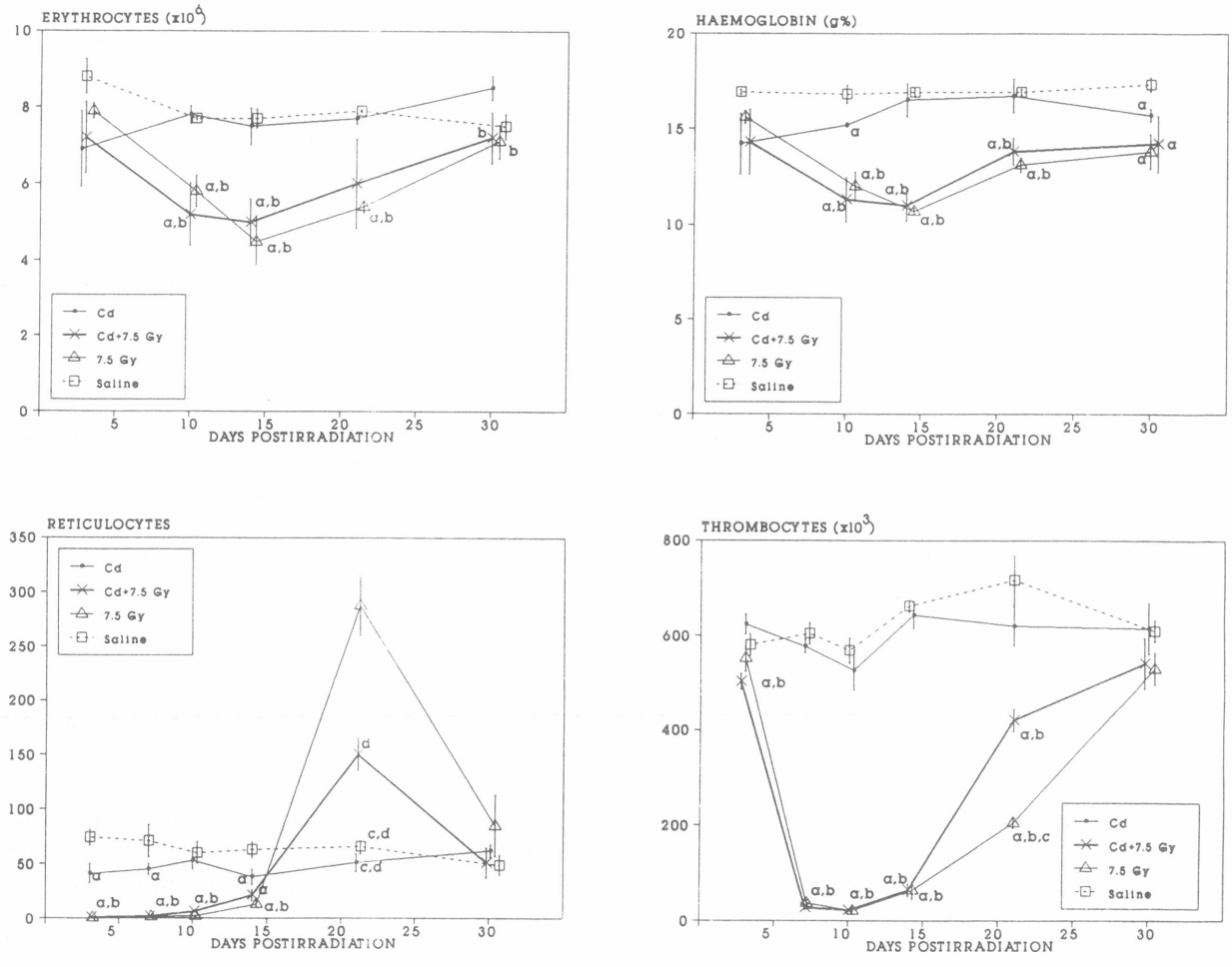

Fig. 3

Effects of cadmium on erythrocyte, reticulocyte and thrombocyte numbers and haemoglobin values in peripheral blood in non-irradiated and irradiated mice. For other legend see Fig. 1.

Other blood elements included reticulocytes which reacted very sensitively to irradiation and which were almost absent in the blood within 3 days after irradiation. The reticulocytopenia persisted until the 10th day after irradiation when values in both irradiated groups increased. On the 21st day after irradiation, reticulocytosis appeared which was observed in irradiated group only (Fig. 3). After the administration of cadmium alone, the reticulocyte number remained at a significantly lower level than that in the non-irradiated controls until the 15th day, with the exception of day 10 (Fig. 3).

The time course and reparation of the number of erythrocytes as well as the haemoglobin values occurred similarly in both irradiated groups (irradiated alone and Cd+7.5 Gy) (Fig. 3). After the administration of cadmium alone, the erythrocyte numbers were temporarily lower compared to control values on the 3rd day and compared to haemoglobin values up to the 10th day after cadmium administration (Fig. 3).

Thrombocytes reacted in a way directly proportional to the level of irradiation. Their counts decreased rapidly until the 3rd day after irradiation (Fig. 3). The observed thrombocytopenia persisted

from the 7th day up to the 15th day when pre-irradiation administration of cadmium accelerated thrombocyte reparation (Fig. 3). The administration of cadmium alone had no further effect on the thrombocyte counts (Fig. 3).

Discussion

From the morphological results, it can be concluded that pre-irradiation administration of cadmium did not prevent the general haemopoietic damage within the first 3 days after irradiation. On the other hand, it mainly accelerated myelopoiesis and megakaryocytopoiesis reparation, and partly also lymphopoiesis in the spleen which was manifested by blood element recovery in peripheral blood. It is to be assumed that the above mentioned reparation processes are connected with the higher number of the haemopoietic stem cells which survived irradiation without any serious damage. It was observed that pre-irradiation administration of cadmium caused that the number of the progenitor GM-CFC in the bone marrow was 50 % higher than that in the bone marrow of animals which had only been irradiated (Fedoročko *et al.* 1996). Because the frequency of the endocolony occurrences in the spleens of mice pretreated with cadmium increased from the 7th day after irradiation, it can be assumed that part of the stem cells were very rapidly mobilized from the bone marrow into the spleen by recirculation. As is generally known, the spleen has a suitable microenvironment for stem cell settlement, proliferation and further differentiation (Tavassoli 1975). The endothelial cells are apparently very important and mainly influence erythroid colony formation in the spleen (Yanai *et al.* 1989, 1991).

On the basis of present knowledge about the effect of heavy metal on the organism, the protective action of cadmium administration before irradiation is connected with the induction of metallothionein synthesis (MT) which is rich in cysteine and which provides protection by scavenging free radicals (Shiraishi *et al.* 1983). MT synthesis increases after heavy metal application, just as MT synthesis increases after administration of immunomodulatory substances such as interleukin-1, interferon or the application of various stress factors such as cold, contusion or surgical procedures (Oh *et al.* 1978, Matsubara *et al.* 1983, 1987, Karin 1985). Matsubara *et al.* (1987) put forward the hypothesis that MT, which was also detected in pulmonary macrophages after intoxication with heavy metals (Hart and Garvey 1986), is the main substance in the chemical protection mechanisms of organisms exposed to physical, chemical or biological stress.

Under our irradiation conditions no important changes in the reparation process of erythroidal system were found. The temporary decrease in the erythrocyte counts on the 3rd day, as well as in haemoglobin values up to the 10th day and reticulocyte counts up to the 14th day after the administration of cadmium alone may be caused either by the direct toxic effect on CFU-E stem cells (Sakata *et al.* 1988) or by the inhibition of growth at later stages of progenitor cells, which was observed under *in vitro* conditions (Lutton *et al.* 1984). Cadmium accumulates not only in the liver (Dudley *et al.* 1982, Matsubara *et al.* 1987) but also in the kidney (Nordberg *et al.* 1975, Matsubara *et al.* 1987) both of which are the main producers of the erythropoietin growth factor (Jacobson *et al.* 1957, Jelkmann *et al.* 1983, Jelkmann 1986). The possibility of its temporarily decreased production can not to be excluded. The transiently decreased production of this factor could influence erythropoietic activity and lead to temporary changes in the erythrocytes of peripheral blood after a single administration of cadmium. The haemopoietic damage, namely impairment of its erythropoietic system, could be connected with the inhibiting effect of cadmium on the intestinal resorption of iron (Hamilton and Valberg 1974), which is necessary for the synthesis of haemoglobin (Bainton and Finch 1964).

As can be seen from the present results, the administration of cadmium prior to irradiation, similarly as the administration of immunomodulatory substances before irradiation (Macková and Fedoročko 1993), did not influence the post-irradiation reparation processes of the thymus. The administration of cadmium alone, as seen from our results, transiently reduced thymus weight. However, the relatively rapid regeneration which followed is, in principle, in accordance with the results published by Xu *et al.* (1989). It is being assumed that the above mentioned changes are mainly an expression of the reaction to an alien substance which can affect not only the direct toxic effects, but can also act as a stress factor. It is generally known that the thymus reacts very sensitively to this factor as well as to other stress factors.

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Reprint Requests

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