Physiol. Res. 45: 93 – 100, 1996

Effects of Cadmium on Haemopoiesis in Irradiated and Non-Irradiated Mice: 1. Relationship to the Number of Myeloid Progenitor Cells

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Received September 22, 1995
Accepted December 12, 1995

Summary

The effects of single subcutaneous injection of cadmium chloride on haemopoiesis in normal (non-irradiated) or irradiated mice were investigated. Cadmium doses used ranged from 1–8 mg/kg body weight. Twenty-four hours after treatment with cadmium (doses from 3 to 8 mg/kg) there were no significant changes in bone marrow cellularity and the granulocyte-macrophage progenitor cell (GM-CFC) number per femur in non-irradiated female ICR mice. Similarly, during the 30-day postinjection period bone marrow cellularity and marrow GM-CFC number in mice treated with a cadmium dose of 5 mg/kg were not significantly different from the control values. Cadmium significantly reduced the lethal effects of gamma rays. In addition, increasing the doses of cadmium administered 24 h prior to sublethal irradiation increased the number of endogenous haemopoietic stem cells (endoCFU-S) in a concentration-dependent manner. Pretreatment with cadmium also decreased the radiation damage to endoCFU-S and haemopoietic progenitor cells committed to granulocyte/macrophage development (GM-CFC). The survival of stem cells was higher and the regeneration of cellularity and GM-CFC of irradiated bone marrow was accelerated in mice pretreated with 5 mg Cd/kg body weight in comparison with saline-injected mice.

Key words
Cadmium – Haemopoiesis – Gamma-radiation – Stem cells

Introduction

Cadmium is a toxic element of continuing concern because environmental levels have risen steadily with progressing worldwide industrialization. Sublethal cadmium exposures produce a wide variety of species-related and dose-related toxic effects including carcinogenicity, teratogenicity, mutagenicity and endocrine and reproductive damage (Waalkes and Goering 1990). It has been estimated that the average cadmium concentration in the renal cortex of populations living in industrialized countries has increased about 50-fold (Drasch 1983).

The effects of another common environmental factor, ionizing radiation, have been extensively examined during the last three decades (Hendry and Lord 1983, Walker 1988).

The effect of cadmium on haemopoietic stem cells in non-irradiated or irradiated mice has not been fully studied yet. Lutton *et al.* (1984) and Sakata *et al.* (1988) reported that cadmium suppressed *in vitro* growth of rat marrow late erythroid progenitor cells (CFU-E) in a dose-dependent fashion. Sakata *et al.* (1988) demonstrated that the oral administration of cadmium produced bone marrow hyperplasia at the CFU-E level due to iron deficiency. Hays and Margaretten (1985) reported that long-term oral

cadmium administration reduced the number of pluripotent or various progenitor haemopoietic cells in the murine bone marrow.

The protein most frequently associated with cadmium exposure is metallothionein – a cytoplasmic, heat-stable, low-molecular weight, metal-binding protein with a high content of cysteine residues (Dunn *et al.* 1987). It is well known that one of the biological roles of metallothionein is the detoxification or tolerance for heavy metals (Min *et al.* 1987, 1991). On the other hand, Matsubara *et al.* (1986a,b, 1987a,b, 1988) reported a marked tolerance to lethal damage from high radiation dose of mice pretreated with metallothionein-inducing metals, e.g. cadmium, manganese or zinc salts, 24 h prior to irradiation. These results indicated that the induction of metallothionein may be a significant factor modulating the response of the organism to irradiation.

The present study was performed to investigate the relationship between cadmium and haemopoiesis and the relationship between cadmium and radiation-induced haemopoietic damage.

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 $CdCl_2x2.5\ H_2O$, was obtained from Lachema (Brno, Czech Republic) and administered subcutaneously (s.c.) in the dorsal thoracic midline area as a solution in 0.9 % sterile saline. For the dose-response studies, mice received 1, 3, 5 or 8 mg Cd/kg body weight. Control animals received an injection of saline.

Irradiation

Twenty-four hours after cadmium injection mice were placed in plexiglass containers and whole-body (unilaterally) exposed to 0.5–3 Gy (survival of haemopoietic progenitor cells), 7.5 Gy (haemopoietic recovery) or to 7–10 Gy (animal survival assay) of gamma rays in a dose of 0.4 Gy/min. A Chisostat ^{60}Co source (Chirana, Czech Republic) was used for all irradiations.

Survival

Survival was monitored daily and was assessed as the percentage of animals surviving 30 days after irradiation. Ten mice per group were used in each experiment. Moribund animals in this experiment were killed. On day 31, surviving mice were euthanized by cervical dislocation. The experiments were repeated twice. The dose reduction factor (DRF) was calculated by dividing the treatment $LD_{50/30}$ by the control $LD_{50/30}$. Survival rates were compared among groups using the chi-square test with Yates' correction.

Haemopoietic stem cell assays

Two primary assays were used to assess the effects of environmental pollutants on haemopoietic stem cells. They included the *in vivo* endogenous spleen colony-forming unit (endoCFU-S) assay and the *in vitro* granulocyte-macrophage (GM-CFC) progenitor cell assay. The endoCFU-S formation was assayed by the method of Till and McCulloch (1963). Briefly, mice received 6.5–9 Gy of radiation. They were killed by cervical dislocation at 10 days postirradiation and their spleens were removed and fixed in Bouin's solution. The number of macroscopic colonies per spleen was determined. The D_o values were determined by the method previously described by Vacek *et al.* (1985).

Haemopoietic progenitor cells committed to granulocyte/macrophage development (GM-CFC) were assayed as described by Vacek *et al.* (1991). Bone marrow cells ($9x10^4$–$6x10^5$, depending on the radiation dose) were plated in triplicate in a semisolid environment created by a plasma clot, containing Iscove's modification of Dulbecco's medium (IMDM, TechGen Int. Ltd., UK), supplemented with antibiotics (penicillin, 100 U/ml and streptomycin, 1000 μg/ml) and L-glutamine (Calbiochem, Behring, La Jolla, USA) in a concentration of 1.2 mg/ml plus 15–20 % newborn bovine calf serum (TechGen Int. Ltd., UK), 10 % murine lung-conditioned medium (LCM), 10 % citrate bovine plasma and 3 % $CaCl_2$ (Biotika, Slovenská Ľupča, Slovak Republic). The cultures were incubated at 37 °C in a fully humidified atmosphere of 5 % CO_2 in air for 7 days. Colonies of at least 50 cells were counted at 30x magnification. The cell suspension used for these assays represented each time a pool of tissues from 5 mice. The D_o values were determined for each separate radiation survival experiment from the linear portion of the curve fitted by least-squares linear regression.

Statistics

The values given in the Figures and Table 1 represent the means ± S.E.M. The statistical significance of the differences was evaluated using Peritz's F-test, Student's t-test and the chi-square test with Yates' correction. $P<0.05$ was considered to be statistically significant.

Table 1
Effect of various cadmium doses on bone marrow cellularity and number of GM-CFC 24 h after administration

Dose of Cd (mg/kg body weight)	Number of cells (x10^6 per femur[a])	n	Number of GM-CFC (x10^3 per femur[a])	n
0	27.81 ± 1.30	3	26.72 ± 1.45	2
3	28.85 ± 2.03	3	27.70 ± 1.72	2
5	26.67 ± 1.61	3	28.48 ± 2.38	2
8	24.90 ± 1.52	3	25.75 ± 1.56	2

Mice were injected with saline or cadmium (3, 5, or 8 mg/kg body wt) 24 h before assay. Cells were pooled from both femurs of five mice per group in each study, values represent the means ± S.E.M. from individual studies. n = number of experiments.

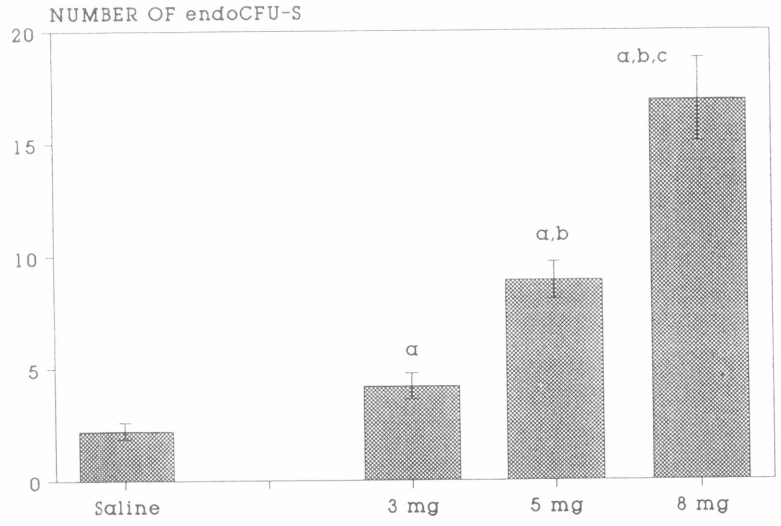

Fig. 1
The effect of a single subcutaneous dose of cadmium chloride on the number of 10-day endogenous spleen colonies (endoCFU-S). Mice received an injection 24 h before 8 Gy irradiation. The data are expressed as the means ± S.E.M. of three separate experiments and each column represents 20–37 mice. Symbols denote statistical significance when compared to the controls (a), 3 mg Cd/kg group (b), and 5 mg Cd/kg group (c). For the sake of simplicity the significance at the 0. 05 level was used for all the comparisons.

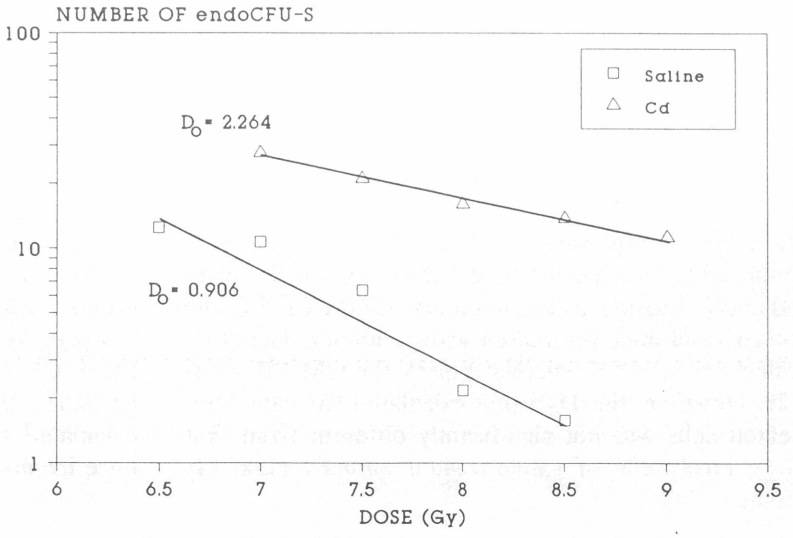

Fig. 2
Number of endogenous spleen colonies as a function of the radiation dose, and the mean values of spleen colonies in control mice and in mice treated with cadmium (5 mg/kg body weight) 24 h before irradiation at the indicated radiation doses. Each point represents 10 mice.

Results

Effects of cadmium on haemopoiesis in normal (non-irradiated) mice

The bone marrow cellularity and content of GM-CFC per femur was measured in the bone marrow from non-irradiated mice 24 h after cadmium or saline injection (i.e. at the time of presumed irradiation) (Table 1). At 24 h after injection of 3, 5 or 8 mg Cd/kg body weight these values did not change significantly as compared to control mice. During the 30-day postinjection period after administration of 5 mg Cd/kg the number of nucleated cells and GM-CFC number in the femur was the same as that in control animals (Figs 6, 7).

Effects of cadmium on haemopoiesis in irradiated mice

Pretreatment of mice with 3, 5 or 8 mg Cd/kg body weight 24 h before irradiation increased the number of endogenous spleen colony-forming units (endoCFU-S). The number of surviving endoCFU-S was dependent on the dose of cadmium. As can be seen from Fig. 1, the 8 mg Cd/kg dose was more effective than 5 or 3 mg Cd/kg doses ($p<0.001$) and a 5 mg Cd/kg dose was also more effective than a 3 mg Cd/kg dose ($p<0.001$). Treatment with 3 mg Cd/kg resulted in endoCFU-S numbers greater than those observed in control (saline-treated) mice ($p<0.001$). Fig. 2 shows the dependence of the number of endoCFU-S on the radiation dose in control and cadmium-treated mice. In both groups the number of colonies decreased as the dose of radiation increased. However, the number of spleen colonies was higher in the cadmium-treated mice (for doses of 7.0–8.5 Gy, cadmium versus saline $p<0.001$). The radiation sensitivity of endoCFU-S (Do) indicated enhanced radiation tolerance of endoCFU-S in mice irradiated after the administration of cadmium, because the Do calculated for cadmium-treated mice was greater than that for control mice (2.24 versus 0.93 Gy). The equieffective exposure to gamma rays (De), after which 5 endoCFU-S (calculated from the regression curves) grew in the spleen, increased by 1.85 Gy after the administration of cadmium (saline De=7.78 versus cadmium De=9.63). Thus, the protective factor (PF) for this equal-effect dose ratio was 1.23.

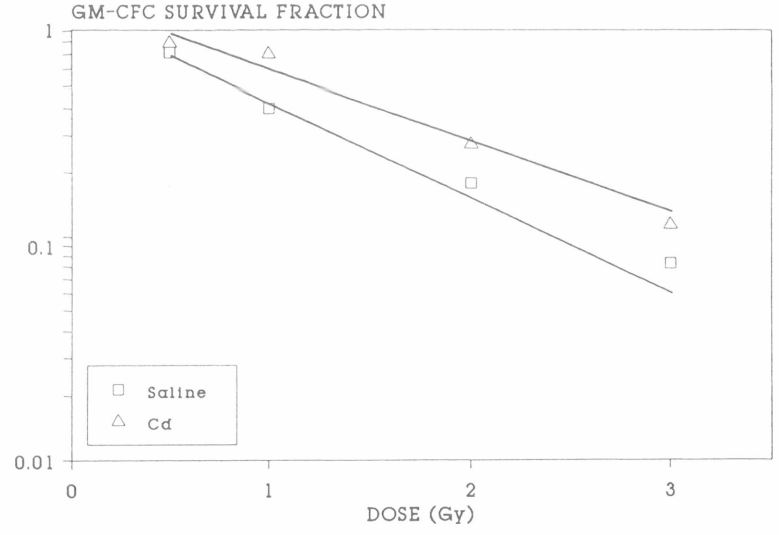

Fig. 3
Radiation survival curve for bone marrow GM-CFC. Animals received an injection of saline or 5 mg Cd/kg body weight 24 h before irradiation at the indicated radiation doses. Twenty minutes postirradiation, animals were killed and their bone marrow was assayed for GM-CFC. The data given as the mean ± S.E.M. GM-CFC values of three separate experiments.

To further investigate the cadmium-induced protection of haemopoietic cells against radiation injury, the ability of cadmium treatment to alter GM-CFC survival following *in vivo* irradiation was evaluated by employing radiation survival curves. Fig. 3 summarizes the results of experiments which relate radiation exposure to the numbers of GM-CFC in the controls and mice pretreated with cadmium. The DRF (calculated at 37 % survival point) for GM-CFC was 1.29. However, the D_o value calculated for cadmium-treated cells was not significantly different from that for control cells of saline-treated animals (1.40 Gy versus 1.15 Gy).

The percentage of mice surviving more than 30 days after exposure to 8.5 Gy was compared between cadmium- and saline-pretreated mice. The cumulative 30-day survival data from these experiments are presented in Fig. 4. In comparison with only 20 % survival of the control group, the 80 % survival of the group treated with 5 mg Cd/kg should be noted. The $LD_{50/30}$ for cadmium-pretreated mice was 8.79 Gy (95% CL 8.41; 9.19). This was significantly greater ($p<0.05$) than the value of 7.97 Gy (7.56; 8.40) calculated for the saline controls (Fig. 5). For ICR mice irradiated 24 h after s.c. injection of 5 mg Cd/kg, the DRF was 1.10 (1.03; 1.18).

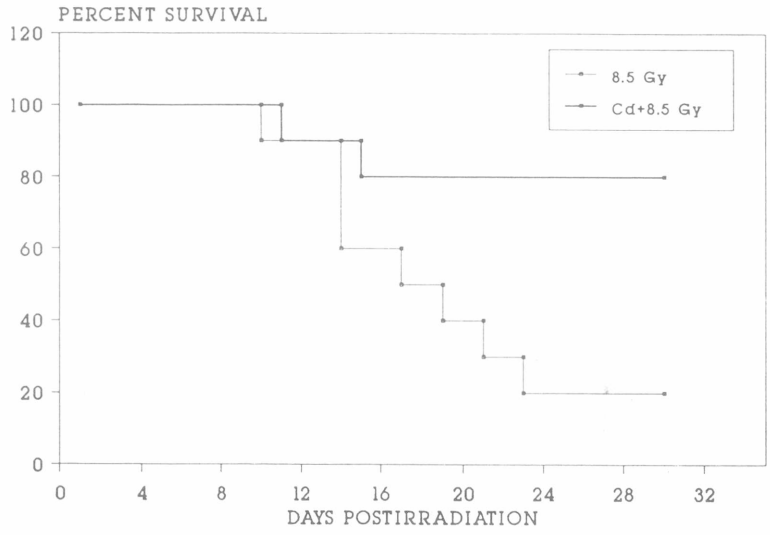

Fig. 4
Survival of lethally irradiated mice (n = 18–20) injected with saline or 5 mg Cd/kg body weight 24 h before irradiation (8.5 Gy). The values are pooled results of two separate experiments. The survival of mice treated with 5 mg Cd/kg body weight was significantly increased when compared to the controls (p < 0.01).

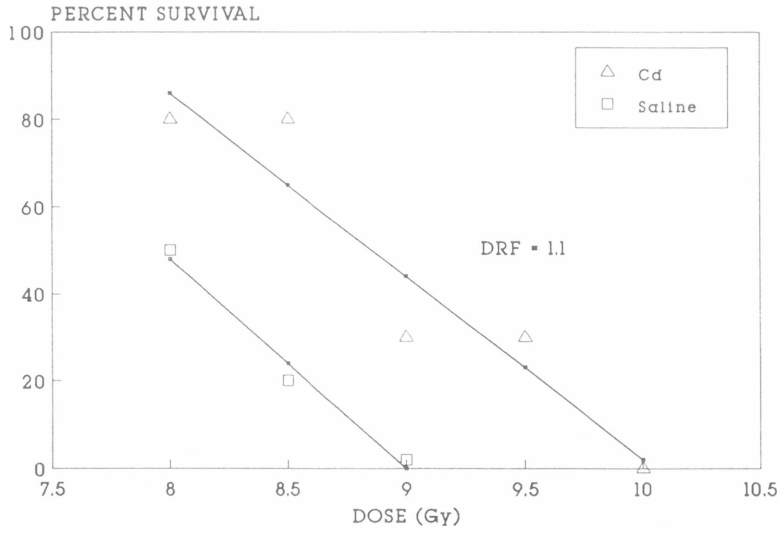

Fig. 5
Animal survival as a function of radiation dose for mice injected saline or 5 mg Cd/kg body weight 24 h before irradiation. DRF = $LD_{50/30}$ (cadmium + irradiation)/$LD_{50/30}$ (saline + irradiation), using probit analysis. Each data point represents 18–20 mice. Based on $LD_{50/30}$ values: cadmium versus saline, $p < 0.05$.

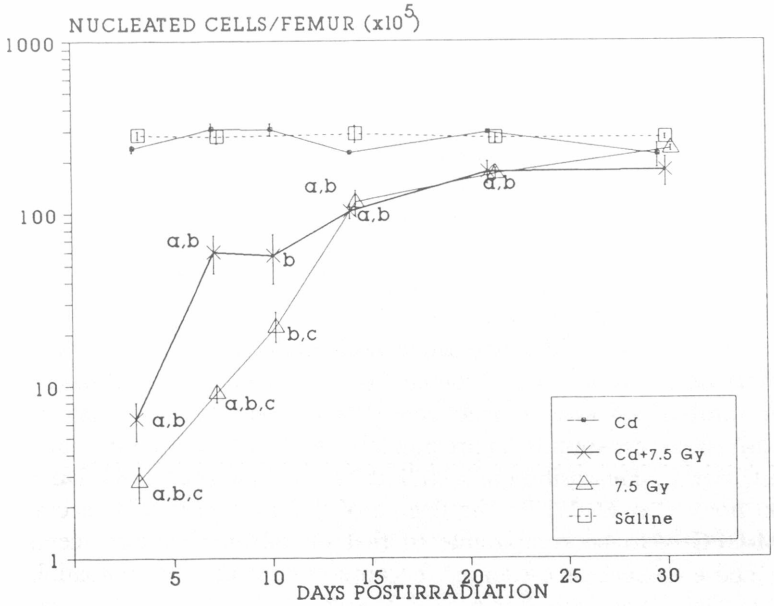

Fig. 6
Effects of a single subcutaneous dose of cadmium (5 mg/kg body weight) on the number of nucleated cells per femur in normal (non-irradiated) and in irradiated mice (7.5 Gy). Ten mice per point were used. Symbols denote statistical significance when compared to the controls (a), group treated with cadmium alone (b) and group treated with Cd plus 7.5 Gy (c). For the sake of simplicity the significance at the 0.05 level was used for all comparisons.

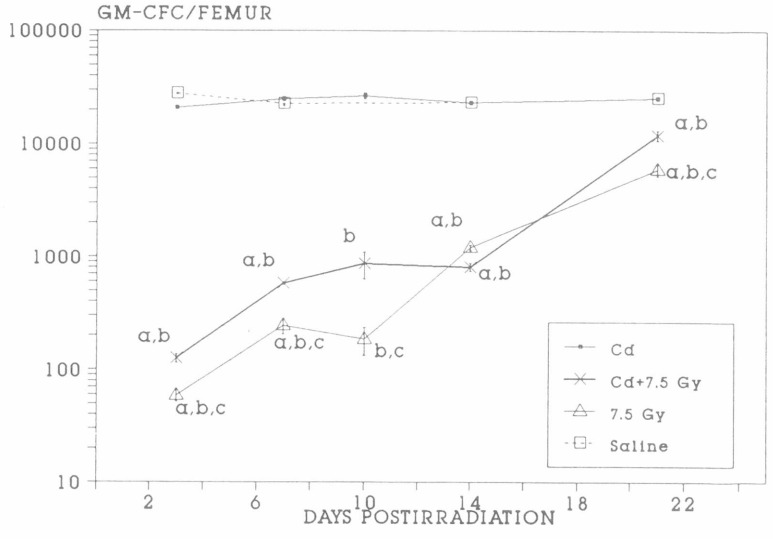

Fig. 7
Effects of a single subcutaneous dose of cadmium (5 mg/kg body weight) on the number of GM-CFC in femoral marrow on different days in normal (non-irradiated) and in irradiated mice (7.5 Gy). For legend see Fig. 6.

Parameters of GM-CFC are good indications of myeloid haemopoietic activity in animals recovering from exposure to radiation. After total body sublethal irradiation (7.5 Gy), bone marrow cellularity and the number of GM-CFC decreased markedly. In contrast, Figures 6 and 7 show that 3, 7, 10 and 21 days after exposure to 7.5 Gy there was evidence of an earlier recovery of bone marrow cellularity and bone marrow GM-CFC number, respectively, in cadmium-treated mice. Three, 7 and 10 days after irradiation in saline-injected mice, the number of bone marrow cells was less than 1 %, 3 % and 8 %, respectively, and number of GM-CFC was less than 0.1–0.5 % of the number in normal non-irradiated mice. At these time intervals the values increased 2 to 6-fold in bone marrow from mice which had received an injection of cadmium before irradiation.

Discussion

In our experiments, we have not found any differences (in cellularity or GM-CFC number) between the controls and animals pretreated with various doses of cadmium or between the controls and cadmium-pretreated mice during the 30-day postinjection period. Hays and Margaretten (1985) showed that the drinking of water containing 300 mg Cd/l for 1 year induced in mice bone marrow hypoplasia, which was characterized by a significant reduction of pluripotent haemopoietic stem cells (CFU-S) and progenitor cells (GM-CFC, CFU-E). On the other hand, Sakata *et al.* (1988) observed marrow hyperplasia with increases of CFU-E after oral administration of cadmium to rats (100 mg Cd/l in drinking water for 12 to 100 days). In contrast, our results that single-dose cadmium administration does not influence bone marrow cellularity and GM-CFC number, are different from these authors. These inconsistencies seem to have been caused by the differences in the dose, route of cadmium administration and the postinjection examination period. Long-term administration of cadmium would produce marrow hypoplasia through direct cytotoxicity of cadmium (Sakata *et al.* 1988), which has not been revealed by our experiments.

The increased survival of endoCFU-S and GM-CFC and accelerated recovery of the cellularity and GM-CFC number per femur after irradiation to the level of the control group was greater and more rapid in the group of cadmium-pretreated mice. Matsubara *et al.* (1987b) presumed that the induction of metallothionein in the mouse liver was an important factor in the mechanism of radioresistance induced by cadmium. The pretreatment of mice with cadmium produced an 8-fold increase in liver metallothionein prior to irradiation and this level in the cadmium-treated animals increased further after irradiation (Shiraishi *et al.* 1983a, Matsubara *et al.* 1987b). The formation of free radicals following irradiation causes extensive tissue damage. Shiraishi *et al.* (1983b) found that metallothionein can scavenge superoxide radicals. Thornally and Vašák (1985) reported that metallothionein was an efficient scavenger of free hydroxyl radicals. On the other hand, Matsubara (1987) suggested that metallothionein may act as a substitute for glutathione when the cells are glutathione-deficient whereby glutathione levels in the cell are a significant factor for tissue resistance to radiation (Mitchel *et al.* 1988, Vos and Roos-Verhey 1988, Bump and Brown 1990). The increase in radiation resistance may be due to increased content of SH substances in tissues sensitive to radiation (Bacq 1965), because cysteine constitutes approximately 30 % of the amino acid composition of metallothionein (Kagi *et al.* 1984, Dunn *et al.* 1987). The degree of radiation protection seems to be comparable to that of known chemical agents such as cystamine, because our results are compatible with the findings of Vacek *et al.* (1971, 1985), who

showed a similar increase in Do, PF and DRF values for mice exposed to cystamine. When evaluating the radiosensitivity of marrow GM-CFC, no significant modification of Do was observed. The reason for the discrepancy in the response of splenic and marrow cells to the protective action may be due to either a different degree or a different ability of cadmium to modify the radiosensitivity of splenic endoCFU-S and marrow GM-CFC. Our results indirectly support Matsubara's hypothesis that metallothionein is a crucial substance in the protective mechanism. Increased radiation resistance of the self-renewing haemopoietic cell populations is manifested by an increased survival rate of stem cells and an acceleration of regeneration from radiation damage. A change in the slope of the regression lines of the number of endoCFU-S in dependence on the radiation dose showed that the radiation resistances of this haemopoietic stem cell population increased after pretreatment with cadmium. The survival of a greater number of haemopoietic stem cells in animals given cadmium injection forms the basis for a more rapid regeneration of haemopoiesis destroyed by the action of ionizing radiation, which was manifested by a significant decrease in the lethality of gamma irradiation. These results demonstrated that cadmium is capable of interacting with gamma irradiation and suppresses its unfavourable effects.

Acknowledgments
The authors gratefully acknowledge the excellent technical assistance of Mrs. Zuzana Kubičková.

References

BACQ Z.M.: *Chemical Protection Against Ionizing Radiation*. C.C. Thomas, Springfield, Illinois, 1965, 178 p.

BUMP E.A., BROWN J.M.: Role of glutathione in the radiation response of mammalian cells in vitro and in vivo. *Pharmacol. Ther.* **47:** 117–136, 1990.

DRASCH G.A.: An increase of cadmium body burden for this century. An investigation of human tissues. *Sci. Total Environ.* **26:** 111–119, 1983.

DUNN M.A., BLALOCK T.L., COUSINS R.J.: Metallothionein. *Proc. Soc. Exp. Biol. Med.* **185:** 107–119, 1987.

HAYS E.F., MARGARETTEN N.: Long-term oral cadmium produces bone marrow hypoplasia in mice. *Exp. Hematol.* **13:** 229–234, 1985.

HENDRY J.H., LORD B.I.: The analysis of the early and late response to cytotoxic insults in the hematopoietic cell hierarchy. In: *Cytotoxic Insult to Tissue: Effects on Cell Lineages*. C.S. POTTEN, J.H. HENDRY (eds), Churchill Livingstone, Edinburgh, 1983.

KAGI J.H.R., VASAK M., LERCH K., GILG D.E.O., HUNZIKER P., BERNHARD W.R., GOOD M.: Structure of mammalian metallothionein. *Environ. Health Perspect.* **54:** 93–105, 1984.

LUTTON J.D., IBRAHAM N.G., FRIEDLAND M., LEVERE R.D.: The toxic effects of heavy metals on rat bone marrow in vitro erythropoiesis: protective role of hemin and zinc. *Environ. Res.* **35:** 97–105, 1984

MATSUBARA J.: Alteration of radiosensitivity in metallothionein induced mice and a possible role of Zn-Cu-thionein in GSH-peroxidase system. In: *Metallothionein II.* J.H.R. KÄGI, Y. KOJIMA (eds), *Experientia* **52** (Suppl.): 603–612, 1987.

MATSUBARA J., ISHIOKA K., SHIBATA Y., KATOH K.: Risk analysis of multiple environmental factors: radiation, zinc, cadmium, and calcium. *Environ. Res.* **40:** 525–530, 1986a.

MATSUBARA J., SHIDA T., ISHIOKA K., EGAWA S., INADA T., MACHIDA K.: Protective effect of zinc against lethality in irradiated mice. *Environ. Res.* **41:** 558–567, 1986b.

MATSUBARA J., TAJIMA Y., KARASAWA M.: Promotion of radioresistance by metallothionein induction prior to irradiation. *Environ. Res.* **43:** 66–74, 1987a.

MATSUBARA J., TAJIMA Y., KARASAWA M.: Metallothionein induction as a potent means of radiation protection in mice. *Radiat. Res.* **111:** 267–275, 1987b.

MATSUBARA J., TAJIMA Y., IKEDA A., KINOSHITA T., SHIMOYAMA T.: A new perspective of radiation protection by metallothionein induction. *Pharmacol. Ther.* **39:** 331–333, 1988.

MIN K.-S., HATTA A., ONOSAKA S., OHTA N., OKADA Y., TANAKA K.: Protective role of renal metallothionein against Cd nephropathy in rats. *Toxicol. Appl. Pharmacol.* **88:** 294–301, 1987.

MIN K.-S., FUJITA Y., ONOSAKA S., TANAKA K.: Role of intestinal metallothionein in absorption and distribution of orally administered cadmium. *Toxicol. Appl. Pharmacol.* **109:** 7–16, 1991.

MITCHEL J. B., BIAGLOW J. E., RUSSO A.: Role of glutathione and other endogenous thiols in radiation protection. *Pharmacol. Ther.* **39:** 269–274, 1988.

SAKATA S., IWAMI K., ENOKI Y., KOHZUKI H., SHIMIZU S., MATSUDA M., MORIYAMA T.: Effects of cadmium on in vitro and in vivo erythropoiesis: erythroid progenitor cells (CFU-E), iron and erythropoietin in cadmium-induced iron deficiency anemia. *Exp. Hematol.* **16:** 581–587, 1988.

SHIRAISHI N., AONO K., UTSUMI K.: Increased metallothionein content in rat liver induced by X-irradiation and exposure to high oxygen tension. *Radiat. Res.* **95:** 298–302, 1983a.

SHIRAISHI N., UTSUMI K., MORIMOTO S., JOJA I., IIDA S., TAKEDA Y., AONO K.: Inhibition of nitroblue tetrazolium reduction by metallothionein. *Physiol. Chem. Phys.* **14:** 533–537, 1983b.

THORNALLY P. J., VAŠÁK M.: Possible role for metallothionein in protection against radiation-induced oxidative stress. *Biochim. Biophys. Acta* **827:** 36–44, 1985.

TILL J. E., McCULLOCH E. A.: Early repair process in marrow cells irradiated and proliferating in vivo. *Radiat. Res.* **18:** 96–101, 1963.

VACEK A., SEMENOV L. F., ROTKOVSKÁ D., RAKOVÁ A., ZEYTUNYAN K. A., FYODOROV B. A., DIKOVENKO E. A.: Protective effect of cysteamine-S-phosphate (Cystafos) on haemopoietic stem cells in irradiated mice. *Folia Biol. (Prague)* **17:** 340–347, 1971.

VACEK A., BARTONÍČKOVÁ A., PALYGA G. F., ZHUKOVA N. A.: Increased protective effect of cystamine and mexamine against radiation after activation of the haemopoiesis by dextran sulphate in mice. *Acta Radiol. Oncol.* **24:** 193–198, 1985.

VACEK A., MICHURINA T. V., SEROVA L. V., ROTKOVSKÁ D., BARTONÍČKOVÁ A.: Decrease in the number of progenitors of erythrocytes (BFUe, CFUe), granulocytes and macrophages (GM-CFC) in bone marrow of rats after a 14-day flight on board the Cosmos-2044 biosatellite. *Folia Biol. (Prague)* **37:** 35–41, 1991.

VOS O., ROOS-VERHEY W. S. D.: Endogenous versus exogenous thiols in radioprotection. *Pharmacol. Ther.* **39:** 169–177, 1988.

WAALKES M. P., GOERING P. L.: Metallothionein and other cadmium-binding proteins: recent developments. *Chem. Res. Toxicol.* **3:** 281–288, 1990.

WALKER R. I.: Acute radiation injuries. *Pharmacol. Ther.* **39:** 9–12, 1988.

Reprint Requests

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