Use of Zinc Metallothioneine to Protect Mice from Ionizing Radiation

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Injecting mice with exogenous zinc-metallothioneine from rat liver at 2 mg/kg 5-10 min before their total-body irradiation resulted in a significantly increased 30-day survival rate. The radioprotective effect of zinc-metallothioneine was not associated with the influence of its constituents, since a model mixture composed of these (albumin, cysteine, and zinc) failed to protect.

Key Words: *zinc metallothioneine; mice; protection; ionizing radiation*

Metallothioneines (MT) are low-molecular-weight proteins containing up to 30% cysteine and capable of binding heavy metal ions. They are synthesized in response to toxic or genotoxic agents, and an increase in the content of these proteins enhances the body's resistance to adverse factors. MT have been shown to reduce the toxicity of heavy metals, to take part in the regulation of zinc and copper levels, and to act as scavengers of free radicals [14]. Mice administered compounds that elevate MT levels in tissues exhibited enhanced resistance to ionizing radiation [11]. In rodents, injection of a bismuth salt followed by induction of MT synthesis in their bone marrow returned leukocyte counts to normal levels after irradiation [13]. A cadmium salt raised the number of endogenous cell colonies in spleens of radiation-exposed mice [1]. We found that the survival of mice irradiated at different times after receiving cadmium chloride was closely associated with the level of MT induced by cadmium ions in the bone marrow (but not in the liver) [2]. *In vitro* experiments produced conflicting results; for example, cells with a high basal or induced MT level displayed increased radioresistance in some experiments [5,12] but not in others [9,12,14]. There are virtually no data on radioprotective effects of exogenous MT, except for one study [8] which demonstrated a moderate radioprotective effect of cadmium-zinc-MT in Chinese hamster cells incubated with this protein prior to irradiation. However, the unique structure of MT [4] suggests that they can also act as effective radioprotectors *in vivo*. We showed previously that zinc-MT reduce the acute toxicity of ethanol [3]. In the present study, a moderate but distinct radioprotective effect of zinc-MT is demonstrated in mice.

MATERIALS AND METHODS

Male (CBA×C57Bl)F_1 mice weighing 24-28 g were used. The purification of an electrophoretically homogeneous composite zinc-MT preparation (MT1 + MT2) used in this study and its characteristics were described by us previously [3]. Zinc-MT was injected in 0.3 ml of Tris-HCl, pH 7.4, 5-10 min before irradiation. Control mice were injected, prior to radiation exposure, with either 0.3 ml of a buffer solution or a mixture modeling the composition of zinc-MT and consisting of human serum albumin (Reanal, Hungary), cysteine hydrochloride (also Reanal), and zinc chloride (the pH of the mixture was adjusted to 7.4) [3]. All three solutions were administered intraperitoneally.

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The mice were irradiated with ^{137}Cs gamma-rays from an IGUR installation at a dose rate of 1.74 Gy/min, and 30-day survival rates and mean survival times were recorded in the test and control groups. The χ^2 test was used for statistical treatment of the results.

RESULTS

Survival rates in both control groups and in the test groups preinjected with zinc-MT at 2 mg/kg are shown in Fig. 1. It can be seen that zinc-MT produced a clearly defined, albeit moderate, radioprotective effect in the groups irradiated with 850 and 875 rads. The group exposed to 900 rads showed some tendency toward an increased survival compared with the control groups. In a lower dose (0.5 mg/kg), zinc-MT virtually failed to protect mice irradiated with 875 rads (the survival rate in this group was only 11% higher than in the respective controls).

The protective effect of zinc-MT was not associated with the influence of its constituents, including zinc and cysteine, although these substances are themselves radioprotectors. Thus, as shown in Fig. 1, no increases in survival rates were recorded in the groups injected with the model mixture (albumin + cysteine + zinc) [3] in a dose equivalent to 2 mg/kg zinc-MT (1.4, 0.6, and 0.106 mg/kg albumin, cysteine, and zinc, respectively).

The mean survival of mice was distinctly improved by zinc-MT at both dose levels (0.5 and 2 mg/kg) in the groups irradiated with 850 or 875 rads and at 2 mg/kg in the group exposed to 900 rads (Table 1).

Thus, this is the first study in which exogenous zinc-MT is shown to afford well-defined radioprotection *in vivo*.

The mechanism of the radioprotective action exerted by MT when present at elevated levels in cells and tissues remains unknown, and, as mentioned above, *in vitro* tests have yielded conflicting results [5,9,12,14]. In terms of our findings, the existing hypotheses may be classified into four groups.

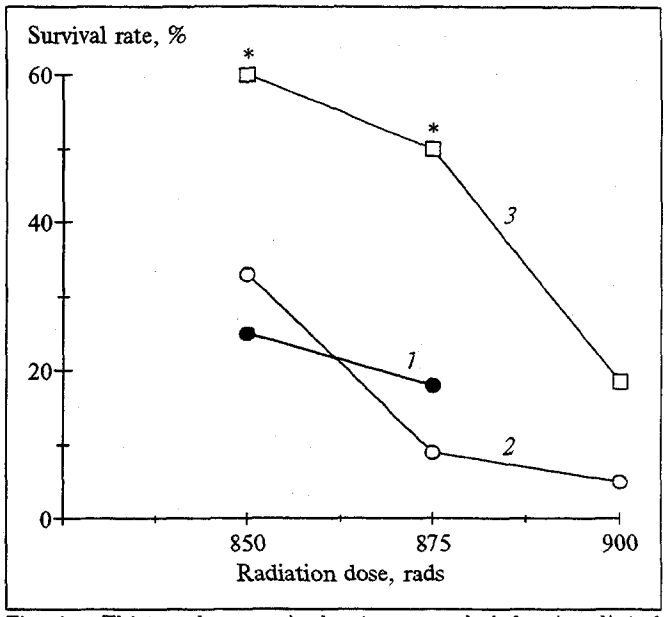

Fig. 1. Thirty-day survival rates recorded for irradiated mice preinjected with the buffer solution (*1*), model mixture at 2 mg/kg (*2*), or zinc-MT at 2 mg/kg (*3*). Asterisks denote significant differences from the control groups ($p<0.05$).

1. MT are scavengers of free radicals. Indeed, scavenging effects have been demonstrated for purified MT in model systems (inactivation of the hydroxyl and superoxide radicals) [15]. Cadmium-MT protected DNA from damage by the hydroxyl radical and did so 5 times more efficiently than free cysteine [4] (this may explain why in our study the cysteine contained in the model mixture failed to protect). The reactivity of thiol groups in MT proved to be 39-fold higher than in reduced glutathione [4]. Also, CHO cells with an elevated level of MT gene expression were found to generate far fewer injuries in the DNA when exposed

TABLE 1. Mean Survival Times of Mice after Irradiation

Group	Radiation dose, rads	Number of mice	Mean survival time, days
Control (buffer)	850	30	13.9
Model mixture		22	12.9
Zinc-MT (2 mg/kg)		30	17.83
Control (buffer)	875	45	11.22
Model mixture		20	12.76
Zinc-MT (0.5 mg/kg)		20	12.5
Zinc-MT (2 mg/kg)		30	12.53
Control (buffer)	900	16	10.6
Zinc-MT (2 mg/kg)		14	11.38

Note. The model mixture was given in the dose corresponding to 2 mg/kg zinc-MT.

to hydrogen peroxide than did control cells [7]. Elevated MT concentrations were recorded in oxidative stresses of various origin [6], these proteins evidently reducing the damaging effects of oxidants by scavenging free radicals [4,6,14,15]. This latter effect of MT resulted in lowered lipid peroxidation levels in the bone marrow of irradiated rodents and thus led to stabilization of cell membranes [13]. The extremely high efficacy with which MT scavenge free radicals is most likely due to the unique structure of these proteins. The sulfur-containing groups in conjunction with metal ions form unique metal-thiol clusters that display greater reactivity than free cysteine or glutathione [4].

2. Zinc-MT is possibly a donor of thiol groups, whose numbers are decreased during the postirradiation period [11,14].

3. Zinc-MT may act as a donor of zinc for various reactions leading to enhanced radioresistance, and this zinc is more active biologically than that entering the body when zinc salts are injected. Indeed, zinc-MT has been shown to release free metal during oxidation [11] (which may occur in the postirradiation period). It has been suggested that this protein is a zinc donor for the glutathione peroxidase inactivating peroxide radicals following radiation exposure [11]. Moreover, the liberated zinc appears to be capable of stabilizing biological membranes [6] and to act as a cofactor for various proteins and enzymes (including DNA polymerases) effecting postradiation repair.

4. Zinc-MT is probably able to replenish the leukocyte pool after irradiation. For example, exogenous apo-MT, zinc-MT, and zinc-cadmium-MT considerably stimulated the proliferation of murine splenic lymphocytes *in vitro* [10]. The ability to stimulate lymphocyte proliferation correlated with levels of available thiols and was most strongly marked in zinc-MT. Interestingly enough, zinc within the zinc-MT was effective in concentrations 7 times lower than within a zinc salt. The authors of this study [10] believe that the observed rise in proliferative activity was associated with MT modification of thiol groups on the cell surface so that membrane permeability for calcium ions was increased and DNA synthesis was stimulated.

The results of this and our previous [3] studies indicate that exogenous zinc-MT is able to enhance the body's resistance both to chemical compounds (ethanol [3]) and to genotoxic agents (radiation). In view of the low toxicity of MT preparations [3], further studies may be of practical interest.

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