BIOPHYSICS AND BIOCHEMISTRY

Stimulation of the DNA Replication and Murine Bone Marrow Cell Proliferation with Exogenous Zinc-Metallothionein

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It is shown that zinc-metallothionein from rat liver increases 1.5-fold the *in vitro* incorporation of ^{3}H-thymidine in murine bone marrow cells. The same concentrations of zinc chloride and a mixture imitating zinc-metallothionein (zinc, cysteine, and albumin) inhibit DNA synthesis. In mice receiving an intraperitoneal injection of zinc-metallothionein 10-15 min before γ-irradiation, the incorporation of ^{3}H-thymidine and the content of nucleated cells in the bone marrow is 1.5- to 2-fold higher than those in unprotected animals, the number of endogenous splenic colonies in pretreated mice being 2.7-fold higher.

Key Words: *zinc; metallothionein; DNA synthesis; proliferation; bone marrow; mice*

Metallothioneins (MT) are low-molecular-weight proteins containing up to 30% cysteine and binding up to 7 atoms of heavy metals per one molecule. The role of MT in the detoxication of heavy metals and in the regulation of zinc and copper levels in the organism has been demonstrated. A protective effect of MT against free radicals has been discussed [4]. For instance, we found that exogenous zinc-MT injected into mice inhibits lipid peroxidation in the plasma and liver [5] and protects the animals against the adverse effects of ethanol [2], bromobenzene [5], and ionizing radiation [3]. In the latter case, the protective effect of zinc-MT may be attributed to inactivation of free radicals and the ability to restore cellular pool in hemopoietic organs. It was hypothesized that MT acting as a zinc depot participates in the synthesis of DNA and in cell proliferation [4]. This hypothesis was corroborated by the following findings:

1. The MT content is high in actively proliferating tissues (fetal and neonatal tissues and some tumors).

2. In these tissues MT is located predominantly in the nuclei.

3. In regenerating liver, the content of MT increases in parallel with an increase in the synthesis of DNA [4]; the concentration of MT increases primarily in the nuclei [4,12].

4. Exogenous MT stimulates proliferation of splenocytes *in vitro* [10]. We failed to find any other report on the effect of zinc-MT on DNA replication and cell proliferation. It was demonstrated that zinc salt enhances the expression of MT genes in the bone marrow, which is important for hemopoiesis [8]. However, the information regarding the effect of MT on the proliferation of bone marrow cells is scarce. The aim of the present study was to examine the effect of zinc-MT on DNA replication and proliferation of bone marrow cells *in vitro* and *in vivo*.

MATERIALS AND METHODS

Experiments were carried out on (CBA×C57Bl/6) F_1 mice weighing 24-28 g. Preparation of electrophore-

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tically pure zinc-MT from mouse liver and characterization were described previously [2]. Bone marrow was isolated from the femur. The animals were killed by cervical dislocation under ether narcosis, femurs were excised, and the bone marrow was washed out with 1-2 ml 0.15 M NaCl containing 20 mM HEPES, pH 7.4 (Serva). Nucleated cells were calculated after the addition of 5% acetic acid to an aliquot of cell suspension. DNA replication in the bone marrow cells was assayed by ^{3}H-thymidine (Izotop, specific activity 740 TBq/mol) incorporation as described elsewhere [9]. The samples in the above-mentioned buffer (1 ml) contained the suspension of nucleated bone marrow cells (2.5-12×10^6), 13.3 μCi ^{3}H-thymidine and varied concentration of zinc-MT, zinc chloride, or mixture imitating zinc-MT [2,3,5] (zinc chloride, cysteine hydrochloride (Reanal), and bovine serum albumin (Serva) in the corresponding proportions, pH 7.4). After a 45-min incubation at 37°C, 20 μl aliquots were withdrawn. Processing of samples and radioactivity measurements were performed as previously [9]. There was a linear relationship between the incorporation of ^{3}H-thymidine and the number of cells in the sample in the above-mentioned range and incubation time (15-45 min).

Endogenous colonies in the spleen (CFU-S) were counted as described elsewhere [1]. The mice were irradiated in an IGUR installation (^{137}Cs, 1.74 Gy/min, total dose 6 Gy). Zinc-MT was injected intraperitoneally 10-15 min before irradiation. On day 9, the spleen and bone marrow were removed, CFU-S [1] and nucleated cells were counted per femur, and ^{3}H-thymidine incorporation into bone marrow cells was determined.

The results were analyzed using the Student—Fisher *t* test.

RESULTS

The effect of zinc-MT, zinc chloride, and the model mixture on DNA replication in bone marrow cells *in vitro* is illustrated in Fig. 1. The concentrations of the substances (abscissa) were standardized to physiological levels of zinc-MT, assuming that these substances are the constituents of zinc-MT. Therefore, the real concentration of zinc chloride ranged from 3.74 to 95.6 μM (or 0.51-12.9 μg/ml for the salt). The contents of zinc and cysteine were 6% and 30%, respectively, for both native zinc-MT [4] and the model mixture [2,3,5] (the metal content in zinc-MT was assumed to be maximal — 7 atoms per molecule [4]).

As Fig. 1 shows, starting from the concentration of 4 μg/ml zinc-MT stimulates ^{3}H-thymidine incorporation. Within the concentration range of 12-100

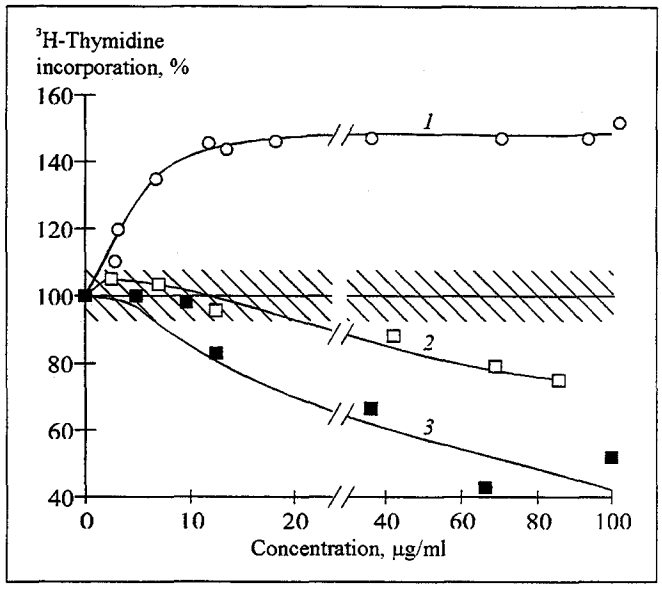

Fig. 1. Effect of zinc-metallothionein (*1*), zinc chloride (2), and zinc-MT-modeling mixture (*3*) on DNA replication in the bone marrow cells *in vitro*. Shaded area: control (772±62 cpm/10^6 nucleated cells, *n*=12).

μg/ml this effect is close to a plateau (Fig. 1, *1*). The model mixture, which contains free active zinc and cysteine, did not activate but inhibited DNA synthesis (Fig. 1, *3*). It is likely that this is associated with the action of cysteine. Aminothiols suppress DNA replication due to inhibition of deoxyribosyl transferase [6]. Since free zinc stimulates DNA replication and cell proliferation [13,14], we studied the effect of zinc chloride on ^{3}H-thymidine incorporation. No activation of DNA synthesis was observed in the presence of low concentrations of $ZnCl_2$, while higher concentrations (>50 μM) inhibited this process (Fig. 1, *2*). It was found that zinc stimulates *in vitro* DNA synthesis in mammalian lymphocytes (optimal concentration 80 μM) [13]. At 37-150 μM zinc inhibits production of interleukin-2 and proliferation of splenic T cells and stimulates ^{3}H-thymidine incorporation in a nonfractionated suspension of splenocytes [14]. Thus, the effect of zinc salts on DNA synthesis and cell proliferation depends on both the type of hemopoietic cells and zinc concentration in the medium. None of the studied zinc concentrations stimulated DNA synthesis (Fig. 1, *2*). The observed incorporation of ^{3}H-thymidine represents an integral characteristic for a mixed cell population. It can be suggested that zinc activates DNA synthesis in some cells and inhibits in others (as it does in T cells [14]). The latter effect is predominant in the bone marrow. It can be hypothesized that suppression of interleukin production in some cells [14] leads to inhibition of DNA synthesis in others.

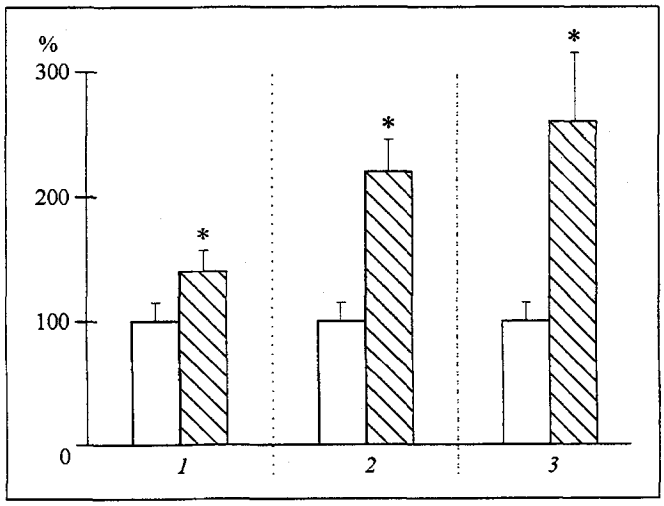

Fig. 2. ^{3}H-thymidine incorporation in bone marrow cells (*1*), number of nucleated cells per femur (*2*), and number of endogenous colonies in the spleen (*3*) on day 9 postirradiation (n=5-12). Light bars: irradiated controls; hatched bars: injection of zinc-MT (2 mg/kg) 10-15 min prior to irradiation. Control values for *1-3* were 179±22 cpm/10^6 nucleated cells, 3.0±0.5×10^6 nucleated cells per femur, and 5.67±0.71 colonies per spleen. *p<0.05 in comparison with the control.

Zinc-MT containing both zinc and cysteine markedly stimulated DNA replication in the bone marrow cells *in vitro* (Fig. 1). Previous studies showed that zinc-MT, zinc-cadmium-MT, and cadmium-MT activate proliferation of splenocytes *in vitro* [10]; however, the role of their constituents is unclear. Our findings show that stimulation of DNA replication is specific for zinc-MT and cannot be attributed to the effects of zinc and cysteine.

Figure 2 illustrates the effect of exogenous zinc-MT on DNA synthesis and cell proliferation in hemopoietic organs after irradiation. On day 9, we observed a 1.5- to 2-fold increase in ^{3}H-thymidine incorporation (Fig. 2, *1*) and in the number of nucleated cells in the bone marrow (Fig. 2, *2*) of zinc-MT-protected mice in comparison with the control. Moreover, zinc-MT increased 2.7-fold the number of CFU-S (Fig. 2, *3*). An increase in the number of bone marrow cells and CFU-S is probably associated with the protection of stem hemopoietic cells by zinc-MT against free radicals after irradiation, which preserves a greater number of stem cells. However, from this viewpoint it is difficult to explain the stimulation of DNA replication by zinc-MT *in vitro* (Fig. 1) and *in vivo* (Fig. 2). In addition to the antioxidant properties [4,5], zinc-MT can restore cellular pool in hemopoietic organs through activation of the DNA synthesis.

The mechanism of the effect of exogenous zinc-MT remains unclear. Certain amounts of zinc-MT may appear in the cytoplasm as a result of pinocytosis (similarly to transferrin [7]) and donate zinc to DNA-synthesizing enzymes. Zinc is a constituent of numerous DNA-binding proteins and replicating enzymes (for example, DNA polymerase α) [4]. It is more likely that exogenous zinc-MT acts at the level of the external cell membranes. This protein may transport zinc ions to specific membrane sites, which modulates the membrane permeability for calcium that serves as a mitogenic signal [7]. It was hypothesized that free thiol groups of zinc-MT formed due to the splitting of zinc atoms in oxidative reaction [11] cross-link the surface proteins of cell plasma membrane, which also modulates the membrane permeability for calcium [10]. It is most likely that both stimulating effect of exogenous zinc-MT on the DNA synthesis and its radioprotective activity are mediated by a complex mechanism.

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