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Experimental basis for increasing the therapeutic index of *cis*-diamminedicarboxylatocyclobutaneplatinum(II) in brain tumor therapy by a high-zinc diet*

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Summary. Metallothionein (MT), a ubiquitous intracellular protein, confers resistance to the toxic effects of platinum compounds. Since a high-zinc diet has been shown to induce MT synthesis in extracerebral tissues but not in brain, we investigated whether it could provide an experimental basis for decreasing the hematotoxicity of carboplatin without impairing its activity against brain tumors. After 2 weeks on either a high-zinc diet or a control diet (zinc content, 180 vs 10 ppm), mice and rats received various doses of carboplatin or Hanks' balanced salt solution by i.p. injection. The hematotoxicity of carboplatin was evaluated with an assay of colony-forming units of granulocytes and mononuclear cells in mice. The high-zinc diet enabled a 50% increase in the carboplatin dose without increasing hematotoxicity. The antitumor activity was evaluated with an assay of the colony-forming efficiency of gliosarcoma cells from 9L brain tumors in rats. The high-zinc diet did not alter the efficacy of carboplatin against this brain tumor. Northern blot analysis confirmed that the high-zinc diet induced MT mRNA in the kidney but not in the brain of mice and rats; it also showed MT mRNA induction in bone marrow cells of mice but not in rat 9L brain tumors. These results suggest that increasing the dietary intake of zinc might increase the therapeutic index of carboplatin in the treatment of brain tumors.

Introduction

In the treatment of malignant brain tumors, the efficacy of cytotoxic agents is limited by their ability to cross the blood-brain barrier, by tumor cell resistance, and by systemic toxicity [51]. Each of those factors contributes to decrease the therapeutic index of *cis*-diamminedichloroplatinum (cisplatin), an agent that is active against brain tumors in children [58] and, at least after intracarotid administration, in adults [36]. However, cisplatin has limited access to the brain [33], which reduces its activity against brain tumors, and its cumulative nephrologic, neurologic, and, to a lesser extent, hematologic toxicities limit dose escalation [57]. Furthermore, the ototoxicity of cisplatin seems to be enhanced by central nervous system irradiation [47].

cis-Diamminedicarboxylatocyclobutaneplatinum(II) (carboplatin), a platinum analog, is also active against childhood brain tumors [2] but produces much less severe renal and neurotoxicity [39]. Furthermore, animal studies have shown that brain levels of platinum are higher after treatment with carboplatin than after cisplatin therapy [8, 54]. In addition, we have shown that at clinically achievable doses, cisplatin and carboplatin have comparable cytotoxic effects against human glioma cell lines in vitro [12]. In view of these characteristics, carboplatin may be preferable to cisplatin for the treatment of brain tumors. Since carboplatin's dose-limiting toxicity is myelosuppression [39], we designed an experimental strategy to decrease its hematologic toxicity without compromising its activity against brain tumors.

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Abbreviations used: MT, Metallothionein; CFU-GM, colony-forming unit of granulocytes and mononuclear cells; CFE, colony-forming efficiency; HBSS, Hanks' balanced salt solution; SDS, sodium dodecyl sulfate

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Our aim was to induce resistance to carboplatin in normal tissues but not in the brain tumors. Elevated intracellular levels of metallothionein (MT) are thought to be a mechanism of resistance to cisplatin [3]. MT, an intracellular protein with a molecular weight of 6,000–7,000 Da, has a high content of thiol groups (30% cysteine) [25] that bind physiologic heavy metals such as copper [19] and zinc [31] as well as toxic metals such as cadmium and mercury [60]. MT is expressed in most tissues, reaching its highest level in the kidneys and liver [25]. Cisplatin [27, 34, 53, 61] is also bound by MT, probably after competition with zinc [53], and cells with a high MT content are resistant to its cytotoxic effects [26]. MT induction has protected mice against lethal toxicity from cisplatin, mainly by reducing nephrotoxicity, and has also decreased its hematotoxicity [41]. In human subjects, MT concentrations in erythrocyte lysates have been increased by daily dietary supplementation with zinc [23], which suggests that zinc supplementation induces MT synthesis in erythocyte progenitor cells. Furthermore, in peripheral-blood leukocytes, MT synthesis has been induced in vitro [24]. Finally, in animals fed a high-zinc diet or i.p. or s.c. injections of zinc salts, MT given was induced in numerous tissues but not in the brain [7, 14, 30].

We therefore hypothesized that a high-zinc diet could induce MT in bone marrow cells, which would protect them against the hematotoxic effects of carboplatin and enable an increase in the carboplatin dose. A high-zinc diet does not induce MT in the brain and therefore should not decrease the activity of carboplatin against brain tumors. To test this hypothesis, we used two in vitro clonogenic assays after in vivo treatment: hematotoxicity was evaluated by assaying colony-forming units of granulocytes and mononuclear cells (CFU-GM) in mice, and antitumor activity was evaluated by assaying the colony-forming efficiency (CFE) of gliosarcoma cells from 9L brain tumors in rats. These assays have been extensively characterized in these two species [1, 6, 44]. The extent of MT induction was verified by Northern blot analysis of MT mRNA in various tissues.

Materials and methods

Animals. After approval by the Animal Experimentation Committee, University of California, San Francisco, the experiments were performed in 6-week-old DBA/2 mice weighing approximately 20 g (Simonson Labs, Gilroy, Calif.) and in male Fisher 344 rats weighing approximately 200 g (Bainten-Kingman, Fremont, Calif.). The rodents were housed in cages in a room maintained at 22–25° C on a 12-h light-dark cycle.

Diets. The mice and rats were fed a purified diet (Research Diets, New Brunswick, N. J.) containing either the minimal zinc requirement (control diet, 10 ppm) [42] or a high-zinc content (180 ppm) for 12–14 days. Deionized, distilled water was provided ad libitum. The rodents were weighed once or twice a week.

Drug. Carboplatin (kindly provided by Bristol Myers Squibb Inc., Syracuse, N. Y.) was reconstituted from lyophilized powder with distilled water on the day of treatment. The in vivo doses were 150–300 mg/kg. All treatments were given by i.p. injection in the morning.

Bone marrow toxicity as determined by CFU-GM assay. After 2 weeks on the high-zinc or control diet, four to six mice per experimental group received i.p. injections of Hanks' balanced salt solution (HBSS) or various doses of carboplatin and were killed by cervical dislocation 24 h later. The bone marrow cells from both femurs were collected by purging with Eagle's minimum essential medium, pooled, and counted by the procedure for leukocyte enumeration using 3% acetic acid as a diluent (Becton-Dickinson, Rutherford, N. J.). As previously described [1], the cells (5×10^4–20×10^4 cells/well, 4 replicates for each mouse) were suspended in minimum essential medium supplemented with 20% fetal calf serum (HyClone, Logan, Utah), 20% mouse-lung conditioned medium, and 0.3% agarose type VII (Sigma Chemical Co., St. Louis, Mo.) and were placed on pregelled agarose layers in 4-well plates (Nunc, Roskilde, Denmark) in 5% CO_2:95% humidified air at 37° C. After 5–7 days, colonies containing at least 40 cells were counted with an inverted microscope; at least 30 colonies/control plate were required for evaluation. The mean CFU-GM was calculated for each mouse, and the means within each experimental group were averaged. Fractional survival was calculated as the ratio of the mean CFU-GM for each experimental group divided by the mean CFU-GM for untreated mice fed the control diet. In a preliminary experiment in which mice were fed the control diet and then treated with carboplatin, the fractional survival of CFU-GM showed a first-order dose-response curve (data not shown).

Activity against 9L intracerebral brain tumor as determined by CFE assay. To evaluate antitumor activity after in vivo treatment, we used an assay of in vitro clonogenic growth similar to that used to evaluate bone marrow toxicity in mice. At the beginning of the diet period, 4×10^4 9L gliosarcoma cells were implanted intracerebrally in each rat [6]. After 12–14 days, the rats received i.p. injections of HBSS or various doses of carboplatin (six rats per experimental group) and were decapitated 24 h later. The tumors were immediately removed and dissociated to single cells, which were plated on irradiated 9L feeder cells (6–12 replicates for each rat) [44]. After 12–14 days' incubation in 5% CO_2:95% humidified air at 37° C, the cultures were fixed with ethanol and stained with crystal violet. Colonies containing at least 50 cells were counted under a stereo dissection microscope. The CFE for each rat was calculated, and the means within each experimental group were averaged. Fractional survival was calculated as the ratio of the mean CFE for each experimental group divided by the mean CFE for untreated rats fed the control diet.

RNA extractions. At the end of the diet period, the rodents were killed without having undergone treatment with HBSS or carboplatin. Femoral bone marrow cells were harvested and pooled from two mice of the same group, and RNA was extracted by cytoplasmic lysis in buffer containing Nonidet-P40 and vanadyl-ribonuclease complexes and by repeated phenol-chloroform extractions [46]. Tissue specimens (kidneys, brains, and rat brain tumors) were resected, immediately frozen in liquid nitrogen, and kept at –70° C. Frozen fragments of kidney or brain were placed in 5 M guanidium monothiocyanate supplemented with 10% β-mercaptoethanol and 1% *N*-lauroylsarcosine and were immediately disrupted with a tissue homogenizer (Brinkmann, Westbury, N.Y.). RNA was extracted by ultracentrifugation on a cushion of 5.7 M cesium chloride. For rat brain tumors, we used a single-step method to isolate RNA by acid guanidium thiocyanate and phenol-chloroform extraction [9] (RNAzol, Cinna/Biotech Laboratories International, Inc., Friendswood, Tex.). In some cases, two to three small tumors from the same group were pooled for RNA extraction.

Northern blot analysis. After precipitation in ethanol, RNA was resuspended in 10 mM TRIS-HCl (pH 7.4) and 1 mM ethylenediaminetetraacetic acid (EDTA) and quantitated by absorption at 260 nm. Depending on the tissue analyzed, 10–25 μg of RNA from each sample was loaded onto a 1.5% agarose gel and size-fractionated using formaldehyde as a denaturating agent [32]. The evenness of loading was verified by monitoring the amount of RNA in each lane by ethidium bromide visualization. RNA was then blot-transferred to a nylon membrane (Genescreen-Plus, NEN/DuPont, Boston, Mass.), baked for 2 h at 80° C, and hybridized at 50° C to the mouse MT-I c-DNA probe [13]. The extensive homology between mouse and rat MT [50] allowed the use of the same probe for both species. The probe was labeled by random-primed DNA labeling with [^{32}P]-deoxycytidine triphosphate to a specific activity of about 10^9 cpm/μg DNA. After hybridization to the labeled

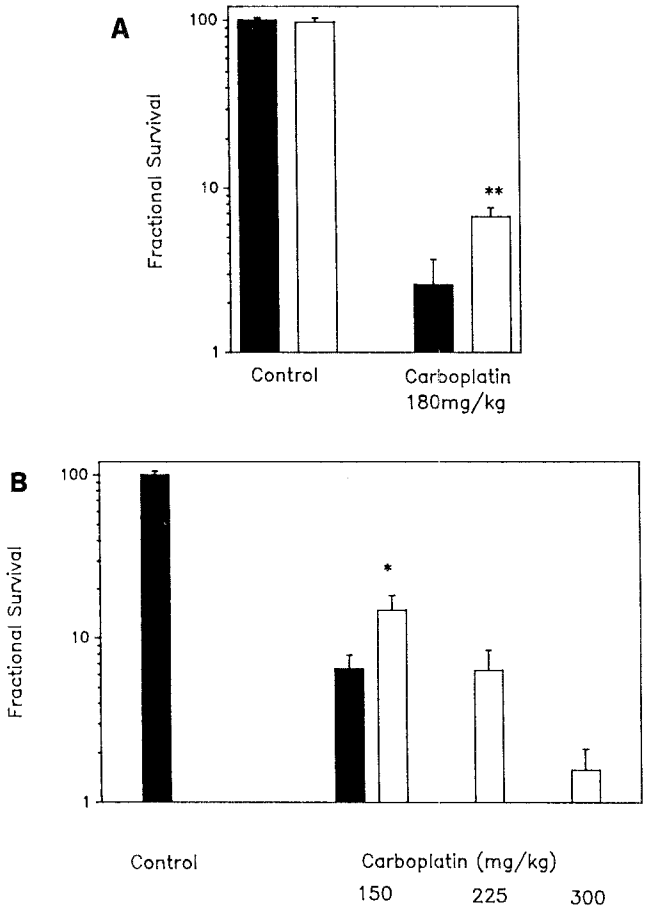

Fig. 1 A. Myelotoxicity and **B** dose advantage of carboplatin in mice fed the control diet (*filled bars*) or the high-zinc diet (*open bars*). **A** Zinc intake did not affect the in vitro clonogenic growth of granulocytes and mononuclear progenitor cells in untreated mice (data from one of two replicate experiments). Fractional survival was calculated as the ratio of the mean number of CFU-GM in each experimental group divided by that in untreated mice fed the control diet. After treatment with 180 mg/kg carboplatin, the fractional survival was 2.5 times higher in mice fed the high-zinc diet than in those fed the control diet (** $P < 0.02$) **B** The high-zinc diet decreased the hematotoxicity of 150 mg/kg carboplatin (* $P < 0.05$), enabling a 50% increase in the dose of carboplatin (to 225 mg/kg) with equivalent hematotoxicity (data from one of two similar replicate experiments). *Error bars* indicate 1 SE

probe, blots were sequentially washed in 2 × SSC (1 × SSC = 0.15 M NaCl + 0.015 M sodium citrate) plus 0.1% sodium dodecyl sulfate (SDS) at room temperature for 10 min, in 0.1 × SSC plus 0.1% SDS at room temperature for 10 min, and in 0.1 × SSC plus 0.1% SDS at 42° C for 10–20 min. The blots were air-dried and exposed to X-ray film in a lightproof cassette.

The optical density of each band on the X-ray films was quantified with a laser densitometer (LKB Instruments, Inc., Gaithesburg, Md.). After the first exposure in certain cases, the hybridization membranes were stripped in just-boiled water and rehybridized to a probe complementary to 18s rRNA. The ratio of the optical density measurement of the MT mRNA signal to that of the 18s rRNA signal was determined for each lane.

Statistical analysis. The differences in mean values obtained for clonogenic cell growth (CFU-GM and CFE), for the optical density of MT mRNA in kidney, and for the MT mRNA/18s rRNA ratio in brain were analyzed by unpaired *t*-test. The weight gain was analyzed by paired *t*-test.

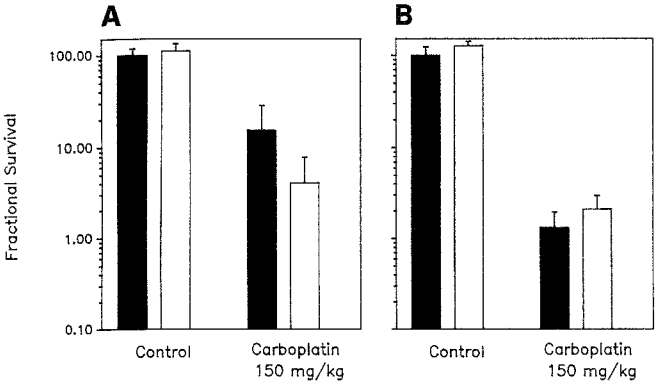

Fig. 2 A, B. Comparison of the sensitivity of intracerebral 9L gliosarcoma cells to carboplatin in rats fed the control diet (*filled bars*) and those fed the high-zinc diet (*open bars*). Zinc intake did not affect spontaneous tumor cell growth. Fractional survival was calculated as the ratio of the mean CFE in each experimental group divided by that in untreated rats fed the control diet. After treatment with 150 mg/kg carboplatin, the fractional survival in rats fed the control diet was not different from that in rats fed the high-zinc diet in two replicate experiments. **A** $P = 0.46$. **B** $P = 0.38$

Results

Nutritional status

Among mice and rats, respectively, there was no difference in weight gain after 12–14 days between those fed the control diet and those fed the high-zinc diet. There were no signs of zinc toxicity in any group.

Bone marrow toxicity

The CFU-GM assay showed that zinc intake did not influence the in vitro clonogenic growth of granulocyte-monocyte progenitor cells (Fig. 1A). After treatment with 180 mg/kg carboplatin, the fractional survival of CFU-GM was 150% higher in mice fed the high-zinc diet than in those fed the control diet ($P < 0.02$, Fig. 1A). After treatment with carboplatin at 150 mg/kg, the fractional survival of CFU-GM in one experiment was 73% higher in mice fed the high-zinc diet ($P = 0.13$, data not shown) and was 130% ($P < 0.05$, Fig. 1B) and 140% ($P < 0.05$, data not shown) higher in two other experiments. In the latter two experiments, in which the dose advantage of carboplatin was investigated, the high-zinc diet allowed a 50% increase in the carboplatin dose (from 150 to 225 mg/kg) without increasing hematotoxicity (Fig. 1B).

Activity against 9L intracerebral rat gliosarcoma

The CFE assay showed that zinc intake did not influence the in vitro clonogenic growth of 9L rat gliosarcoma cells (Fig. 2). After treatment with carboplatin (150 mg/kg), there was no significant difference in the CFEs of rats that were fed the control diet and those of animals that were fed the high-zinc diet (Fig. 2).

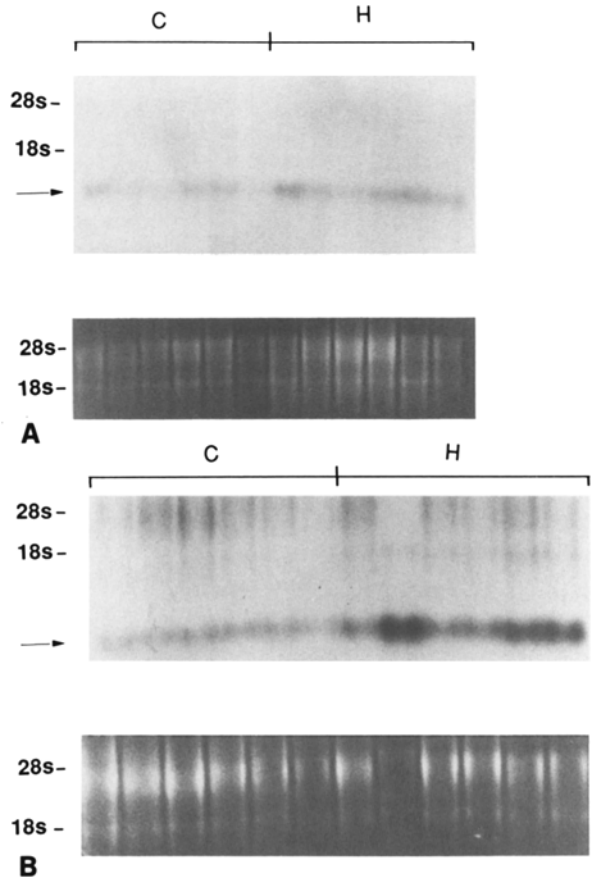

Fig. 3 A, B. MT mRNA levels in kidneys from **A** mice and **B** rats fed the control (*C*) or high-zinc (*H*) diet (6 animals/group, 1 animal/lane). *Upper panel:* the migration positions of the two ribosomal RNA bands are 28s and 18s, respectively. The *arrow* indicates the migration position of the MT mRNA as detected after hybridization to the [^{32}P]-labeled MT-I cDNA probe. *Lower panel:* ethidium bromide visualization of the ribosomal RNA bands. Equivalent amounts of RNA were present in each sample. In both mice and rats, higher amounts of MT mRNA were detected in the kidneys of the animals receiving the high-zinc diet

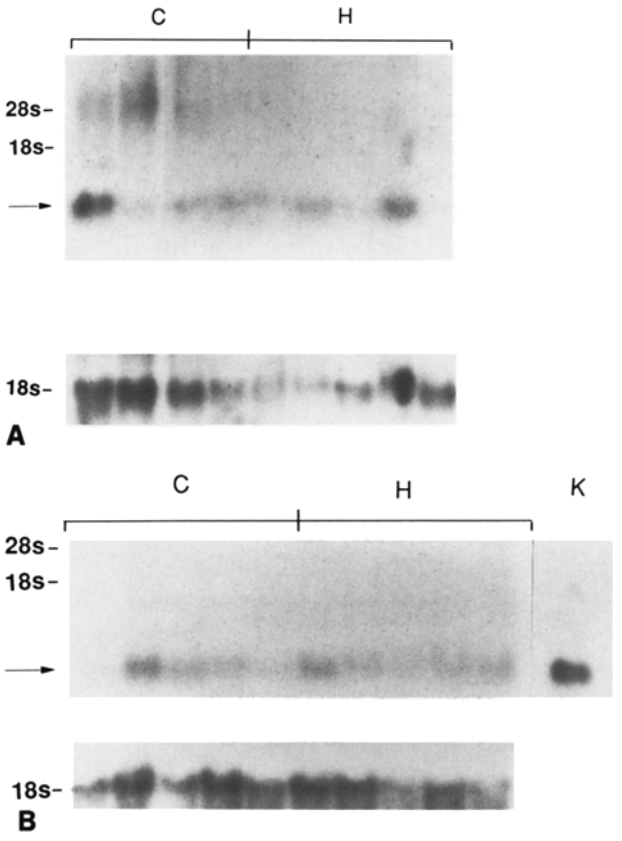

Fig. 4 A, B. MT mRNA levels in the brains of **A** mice and **B** rats fed the control (*C*) or high-zinc (*H*) diet (5 animals/group, 1 animal/lane, except for the group of mice fed the control diet, which comprised 4 animals). *Upper panel:* the migration positions of the two ribosomal RNA bands are 28s and 18s, respectively. The *arrow* indicates the migration position of the MT mRNA as detected after hybridization to the [^{32}P]-labeled MT-I cDNA probe. As shown in **B**, RNA from kidney (*K*) was incorporated in the blot as an internal control to verify the migration position of MT mRNA. *Lower panel:* autoradiogram of the same blot, rehybridized to the cDNA probe complementary to 18s rRNA

MT mRNA induction

As expected, the high-zinc diet induced higher MT mRNA concentrations in the kidneys of mice (Fig. 3A) and rats (Fig. 3B). MT mRNA levels in the kidney were 1.4 times higher in mice that were fed the high-zinc diet than in those that received the control diet ($P < 0.05$) and were 2.2 times higher in rats that were fed the high-zinc diet than in controls ($P < 0.05$). MT mRNA was also detected in bone marrow cells in mice, in 9L tumors in rats, and in brain tissue from both species, but at much lower levels than in the kidneys. No increase in MT mRNA was observed in the brains of mice (Figs. 4A and 5A) or rats (Figs. 4B and 5B) fed the high-zinc diet. Similarly, no significant difference in MT mRNA levels was observed in the brain tumors of rats fed control and high-zinc diets, but the MT mRNA signals in both groups were too weak to photograph clearly. Finally, MT mRNA was markedly increased in the bone marrow cells of mice that received the high-zinc diet, but very little MT mRNA was detected in these cells in control mice (Fig. 6); because of the very low expression of MT mRNA in the control group, laser densitometry was not performed.

Discussion

Chemomodification is a concept that attempts to improve the therapeutic index of cytotoxic drugs by decreasing their toxicity to normal tissues and increasing their antitumor activity [10]. The intent is to enable an escalation of the dose of the cytotoxic agents to levels that are normally not tolerated by the host [21]. There are three potential limitations to the clinical application of this concept. First, if it is not effective, chemomodification would not protect normal tissues against the toxicity of increased doses of cytotoxic drugs. Second, if it is not specific, it might decrease the antitumor activity as well as the systemic toxicity. Third, the chemomodifier itself might be toxic. The experimental strategy tested in the present study appears to avoid these risks.

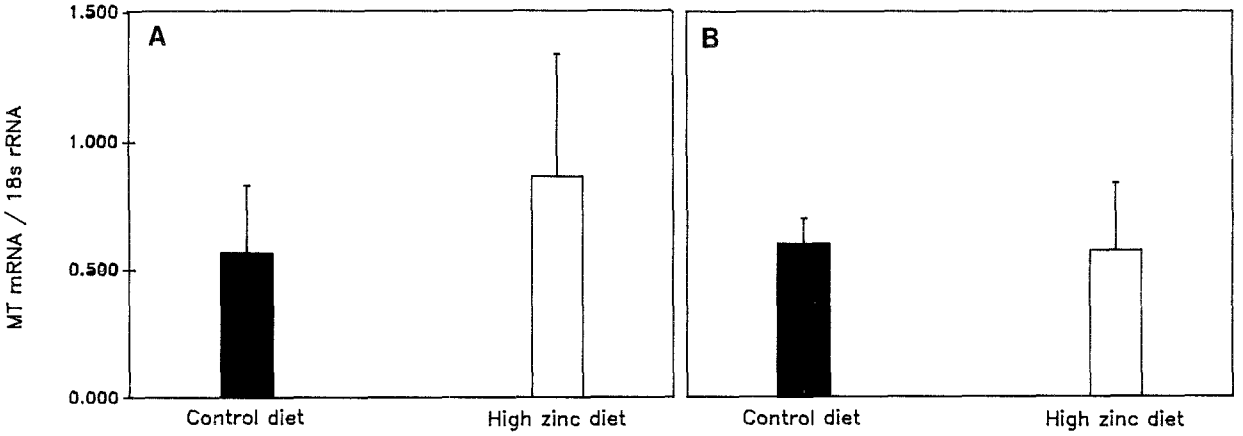

Fig. 5 A, B. Quantitative measurements of MT mRNA in brain samples from **A** mice and **B** rats that were fed each diet. The results are expressed as the ratio of the optical density measurements of the MT mRNA signal vs the 18s rRNA signal. No significant difference in this ratio was observed between the two diet groups in mice ($P = 0.62$) or rats ($P = 0.93$)

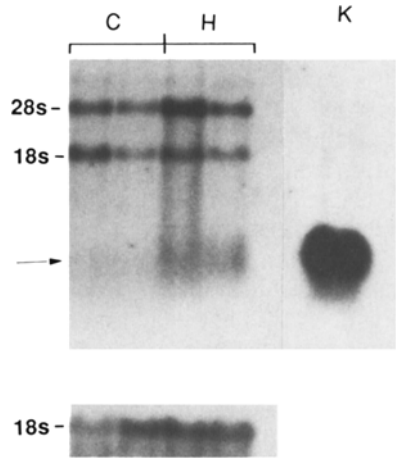

Fig. 6. MT mRNA levels in bone marrow cells of mice that were fed the control (*C*) or high-zinc (*H*) diet (4 animals/group, 2 animals/lane). *Upper panel:* the migration positions of the two ribosomal RNA bands are 28s and 18s, respectively. The *arrow* indicates the migration position of the MT mRNA as detected after hybridization to the [^{32}P]-labeled MT-I cDNA probe. RNA from kidney (*K*) was incorporated in the blot as an internal control to verify the migration position of MT mRNA. Note that the MT cDNA probe also hybridized nonspecifically to the ribosomal RNA bands in the case of bone marrow RNA. *Lower panel:* autoradiogram of the same blot, rehybridized to the cDNA probe complementary to 18s rRNA. The high-zinc diet induced increases in MT mRNA concentrations in the mouse bone marrow cells

Protection of normal tissue

Naganuma et al. [41] showed that cisplatin's hematotoxicity as measured by leukocyte counts was decreased after MT induction by bismuth in mice. In the present study, a high-zinc diet protected bone marrow cells from the cytotoxic effects of carboplatin and enabled a 50% increase in the dose without increasing hematotoxicity. The assay we used measures only the bone marrow progenitors of the monocytes and polymorphonuclear cells and does not directly assess the toxicity to megakaryocytes, which is very often reflected by a low platelet count after carboplatin treatment [39]. However, the number of CFU-GM is related to the number of other progenitor cells and is even used to determine the overall quality of the bone marrow in patients receiving high-dose chemotherapy [45].

Although the increased cellular levels of MT induced by preadministration of heavy metals appear to protect normal tissues from the toxic effects of cisplatin [41], it has been suggested that these metals themselves may produce independent protective effects [40, 52, 56]. To our knowledge, binding of carboplatin to MT has not been studied. The diamminoplatin radical of cisplatin that binds MT is the same as the hydrolytic product of carboplatin [22]. The binding of carboplatin by MT is therefore most likely to occur as it does for cisplatin [27, 34, 53, 61]. However, the kinetics of carboplatin's binding to MT might be different from that of cisplatin, because the hydrolysis of carboplatin is much slower [22]. In our experiments, a high-zinc diet protected rodents against carboplatin's hematotoxicity; the 50% dose advantage observed might be further increased by optimizing MT induction. The markedly increased MT mRNA levels in bone marrow cells, which has not to our knowledge been reported previously, is consistent with the increase in MT in erythrocyte lysates in humans after dietary zinc supplementation [23]. Other studies have shown a good correlation between MT mRNA induction and protein production [7]. In fact, the increased production of MT seems to be proportionally higher than the increased production of MT mRNA [49]. However, to ensure that the observed bone marrow protection is due to the increased level of MT, it would be necessary to compare the amount of platinum bound to MT in the rodents fed the control diet and those fed the high-zinc diet.

The induction of MT mRNA we found in the kidney not only serves as a positive control of MT mRNA induction by a high-zinc diet but also has clinical implications. Higher doses of carboplatin may increase the risk of nephrotoxicity [37]; the increased levels of MT in the kidneys might decrease renal susceptibility to carboplatin, as reported for cisplatin [41]. It is also possible that MT mRNA induction in the kidney might modify the pharmacokinetics of carboplatin and thereby affect its hematotoxicity.

Specificity of the protective effect

Some authors have shown that the in vivo response of several implantable tumors was not changed by pretreatment with bismuth to induce MT in normal tissues [41]; other authors have observed that MT induction in cancer cells is possible and may increase resistance to platinum compounds [4, 26]. However, in the treatment of brain tumors, this risk appears to be minimal because induction of MT-like protein in the brain has been reported only after intraventricular injection [14] and not after dietary intake [7] or i.p. [14] or s.c. [30] injection of zinc. This shows that the failure of a high-zinc diet to induce MT in the brain and in 9L cells reflects more a function of their location than an intrinsic property of the tissue. In fact, we showed that the high-zinc diet did not protect the intracerebral 9L gliosarcoma from the cytotoxic activity of carboplatin.

The physiologic importance of MT in resistance to platinum compounds is controversial [3]. Increased MT levels have been associated with cisplatin resistance in cancer cells [4, 5, 17, 26, 29] as well as with resistance to alkylating agents [15, 16, 26, 35]. However, a lack of correlation between MT content and resistance to platinum has also been reported [18, 48]. The role of MT in resistance to platinum compounds or alkylating agents might vary according to tumor or tissue type. Furthermore, there are several types of MT with highly analogous amino acid sequences, and the respective roles of these different types of MT in resistance to chemotherapeutic agents might also differ [4, 28]. In the present study, we used the mouse MT-I c-DNA probe to detect global induction of MT-s in the different tissues but did not determine whether there was a tissue-specific regulation of specific MT genes or whether one type of MT was primarily involved [28].

At present, there is no clear explanation for the lower induction of MT in cancer cells than in normal cells after pretreatment with heavy metals in some studies [28, 41]. To our knowledge, the induction of MT in brain tumor cells has not been studied. However, the intracerebral location of these cells should decrease the risk of MT induction by pretreatment with a high-zinc diet [7]. In fact, we did not detect a significant difference in MT mRNA levels in the brain tumors of rats fed a control or high-zinc diet.

Risk of zinc toxicity

The zinc intake provided by the modified diet in our experiments in rodents was relatively well below the levels that have been reported to cause the digestive and neurologic symptoms associated with acute zinc toxicity in humans [20]. However, it was relatively higher than the recommended dietary allowance. If taken over several months, the amount of zinc in our high-zinc diet could induce copper deficiency (with attendant symptoms of anemia and neutropenia), impair immune functions, and increase the ratio of low-density to high-density lipoprotein [20].

These adverse effects may not result when an equivalent dose is given sporadically over shorter periods. MT synthesis is rapidly induced after an increase in the dietary intake of zinc [11]. Therefore, short periods of high zinc intake before chemotherapy might effectively induce MT and protect normal tissue from the toxic effects of carboplatin without causing zinc toxicity. Furthermore, zinc supplementation may correct a possible zinc deficiency that has been reported after cisplatin administration [55].

Limitations of the therapeutic strategy

Increasing the therapeutic index of carboplatin in brain tumor chemotherapy by extracerebral MT induction has some limitations. The first is the lack of protection in normal tissues such as the brain [7, 14, 30] and testis [43], where MT synthesis cannot be induced by a high-zinc diet and antitumor therapy would be limited by the toxic effects of carboplatin. In the brain, carboplatin's toxicity to normal tissue would not be reduced by this strategy. Although platinum compounds are thought to exert their ototoxic effects through damage to the auditory nerve, it is possible that some component of ototoxicity results from damage within the CNS. If so, it is possible that higher doses of carboplatin would increase the risk of ototoxicity; however, the ototoxicity of carboplatin is lower than that of cisplatin [2, 58]. Similarly, a high-zinc diet would not protect against a possible testicular toxicity of carboplatin [43] such as that described for cisplatin [59].

The second limitation is that tumor-induced changes in the blood-brain barrier might affect the induction of MT in the brain. Although MT mRNA was not increased in 9L gliosarcomas in rats that were fed the high-zinc diet, this does not exclude the possibility that it could be increased in other types of brain tumors. However, the blood-brain barrier is intact in the zone surrounding the tumor [51], the most frequent site of recurrence, where cytotoxic drugs must be active to improve the therapeutic efficacy; conceptually, the intracerebral but extratumoral cancer cells would not be protected from carboplatin's toxicity by our therapeutic strategy. Finally, since MT is inducible in brain cells that are directly exposed to zinc [14], it is conceivable that a high-zinc diet would also induce MT in extracerebral brain tumor cells and confer resistance on metastatic brain tumor cells; however, this risk is minimal because brain tumors seldom metastasize [38].

Conclusions

In the present study, a high-zinc diet decreased the hematotoxicity of carboplatin without reducing its activity against the 9L brain tumor model. This protective effect may be due to the induction of MT synthesis in bone marrow cells. Optimizing the induction of MT might improve this dose advantage. Furthermore, the high-zinc diet allowed the administration of higher doses of carboplatin in mice without increasing its hematotoxicity in vivo. Finally, the high-zinc diet induced MT mRNA in the kidney, which may decrease the renal toxicity of increased doses of carboplatin. Because MT synthesis is not induced intracerebrally by dietary modifications of zinc, and if this is confirmed in other types of brain tumors, our experimental

strategy suggests a mechanism for increasing the therapeutic index of carboplatin in the chemotherapy of brain tumors. Because the toxicity of platinum compounds and alkylating agents is decreased by MT induction [15, 16, 26, 35], the use of this approach might be extended to these two groups of cytotoxic drugs, both of which are extremely important in brain tumor therapy.

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