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Effects of dietary zinc restriction on bismuth induction of rat kidney metallothionein

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Bismuth-stimulated elevation of metallothionein levels is proposed to inhibit side effects of chemotherapy drugs such as cisplatin and adriamycin. In the present study, low zinc intake by rats limited bismuth-induced accumulation of renal metallothionein protein, and metallothionein-associated zinc and bismuth. In contrast, bismuth strongly elevated metallothionein mRNA in rats fed low zinc. Thus, bismuth does not appear to require zinc to stimulate high metallothionein mRNA accumulation, but seems to need this metal to accumulate metallothionein protein. Therefore, the efficacy of bismuth in restricting chemotherapy drug side effects in humans may depend on the zinc status of the people under treatment. (J. Nutr. Biochem. 7:196–199, 1996.)

Keywords: metallothionein; bismuth; zinc; rats; kidney

Introduction

Kidney and liver metallothionein (MT) levels are increased by administration of a number of different metals.[1–3] There is likely more than one mechanism responsible for the increases. Zinc, cadmium, and copper, which all associate with MT, are thought to bind to proteins that activate metal regulatory elements of the promoter region of the MT gene.[2] Some metals that do not accumulate in MT protein, may also be capable of binding to this element.[3] Other metals, such as iron, could increase MT levels indirectly through general stress.[4] Some metals may also raise MT mRNA levels using zinc displaced from MT protein.[2] This present study examined the last possibility for bismuth (Bi).

There is clinical importance in determining if zinc is needed for Bi to raise MT levels. Elevated MT levels appear to protect cells against the toxicity of some chemotherapy drugs.[5–7] Thus, it could be desirable to maintain low MT levels in tumor cells, while raising levels in healthy cells vulnerable to chemotherapy drug toxicity. In mice, Bi raises MT levels in kidney and heart, but not in certain types of implanted tumors.[6,7] This action could protect humans against renal and cardiac toxicity of chemotherapy drugs such as cisplatin and adriamycin. However, if zinc is needed for the Bi effect on MT levels, then clinical use of Bi may be abolished or limited by poor zinc status in cancer patients. This is an important issue because cancer patients can often be somewhat zinc deficient.[8,9]

In theory, if Bi raises MT mRNA through zinc displaced from preexisting MT protein, the depletion of existing MT protein should inhibit this effect. Dietary zinc restriction provides a means of accomplishing this depletion.[10] The present study used this approach to provide initial insights into the mechanisms by which Bi elevates kidney MT concentrations.

Methods and materials

Male, Sprague Dawley rats, weighing about 150 g, were obtained from Harlan Sprague Dawley (Indianapolis, IN, USA), and fed the zinc-deficient diet sold by United States Biochemicals (Cleveland, OH, USA), plus or minus added $ZnCl_2$ at 30 ppm zinc. Baseline zinc content of the diet was about 1 mg/kg. All rats were initially given the zinc-adequate diet and deionized water ad libitum. After 5 days, rats were either switched to the low-zinc diet, given the zinc-adequate diet pair-fed to the low-zinc group, or continued with the adequate diet ad libitum. For the main experiment, rats were injected sc four times with Bi nitrate (14 mg/kg body weight) or solvent (water plus glycerol, 1 ml: 11 drops) starting on day 7 after dividing the rats by feeding regimen. The first three injections

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Table 1 Effects of diet on Zn status

Diet	Body Weight g	Serum Zn μM
Adequate Zn	229 ± 11[a]	22 ± 2[a]
Pair-fed	198 ± 10[b]	21 ± 1[a]
Low Zn	169 ± 9[c]	7 ± 1[b]

Values are the means ± SEM from five rats. Different superscripts in the same column denote significant differences ($P < 0.01$, two-way ANOVA + LSD).

were given 24 hr apart, the last one 16 hours later, 6 hours before sacrifice. In preliminary work, kidney MT protein contents were about the same at each of four sacrifice time points from 4 to 24 hours. In one experiment, general stress was produced by inflammation due turpentine (0.1 ml, im, leg, 2 days before sacrifice).

MT protein was measured by a silver binding assay.[11] This assay is accurate for MT containing a variety of metals, including copper, which binds more tightly to MT than other metals commonly associated with this protein.[11] Patterns of MT-bound metals were examined in kidney extracts derived from several similarly treated rats. Kidneys were homogenized in phosphate-buffered saline (1.5 ml/g), mixed with B-mercaptoethanol (10 μl/ml), placed in a boiling water bath for 3 min and centrifuged at 15,600 × g for 5 min. The supernatant was analyzed by gel filtration (Sephadex G-75, 75 × 2.5 cm column) followed by atomic absorbance spectrometry. MT mRNA was evaluated by Northern blot hybridization to an oligonucleotide probe as described by Cousins et al.[12] For comparison, RNA was also hybridized to a rat actin oligonucleotide probe (Clontech Laboratories Inc., Palo Alto, CA, USA). Ceruloplasmin activity was assayed by oxidation of p-phenylenediamine.[13] Serum zinc was determined by atomic absorbance spectrometry.

Table 2 Effects of Bi injection and diet on MT protein levels

Diet	MT Protein (μg/g kidney) Control	+ Bi
Zinc-adequate, ad libitum	26 ± 1[c]	118 ± 2[f]
Zinc-adequate, pair-fed	22 ± 2[b]	100 ± 3[e]
Zinc-deficient	11 ± 3[a]	38 ± 8[d]

Rats were treated with Bi or solvent as described in the text. Values are the means ± SD from five rats. As indicated by different superscripts, all values were significantly different from the others ($P < 0.05$, ANOVA + LSD).

Results

As indicated in *Table 1*, rats fed low zinc were deficient based on body weight and serum zinc. *Table 2* summarizes the effects of zinc intake and Bi injection on renal MT protein concentrations. The same patterns as those seen in this table were found in three other trials, though the absolute values for MT varied (data not shown). Rats fed low zinc showed low kidney MT contents. Values were higher for pair fed rats, though still somewhat below normal. Bi nitrate strongly elevated renal MT levels in rats consuming the zinc-adequate diet regardless of whether rats were given feed ad libitum or by pair-feeding. Low-zinc-intake-impaired Bi stimulation of MT protein accumulation. Na nitrate had no effect on kidney MT in rats consuming adequate zinc (data not shown).

In rats fed adequate zinc, Bi elevated the amounts of Bi, zinc and copper associated with MT (*Figures 1* and *2*), but

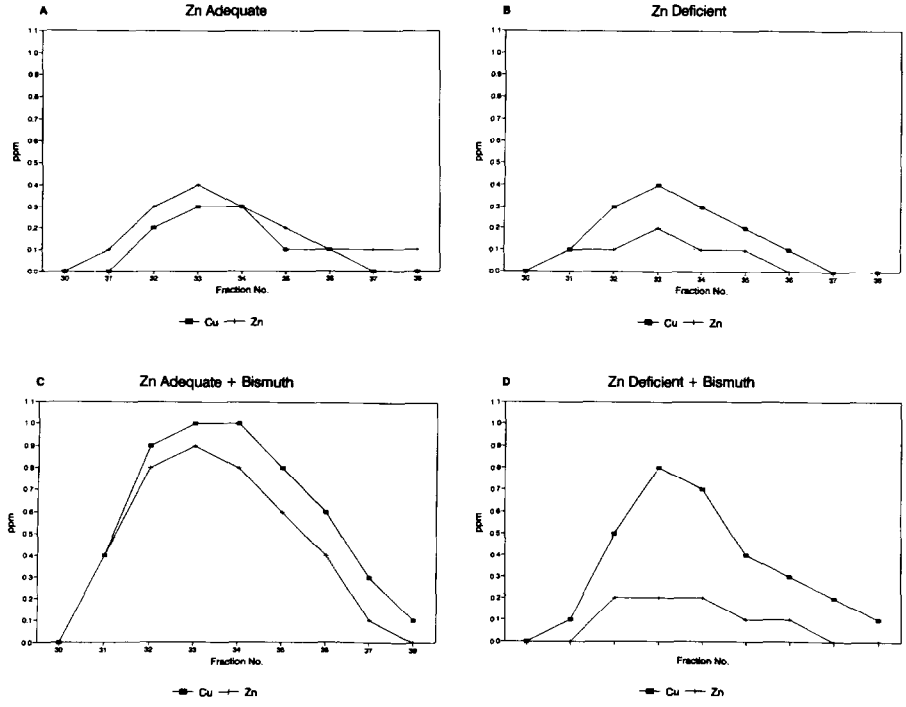

Figure 1 Zinc and copper accumulation in rat kidney MT. Heat-treated kidney extracts were separated by gel filtration on Sephadex G-75 as described in the text. Virtually all detectable Cu and Zn was found in the fractions shown (6 ml each). Purified metallothionein eluted in the same fractions.

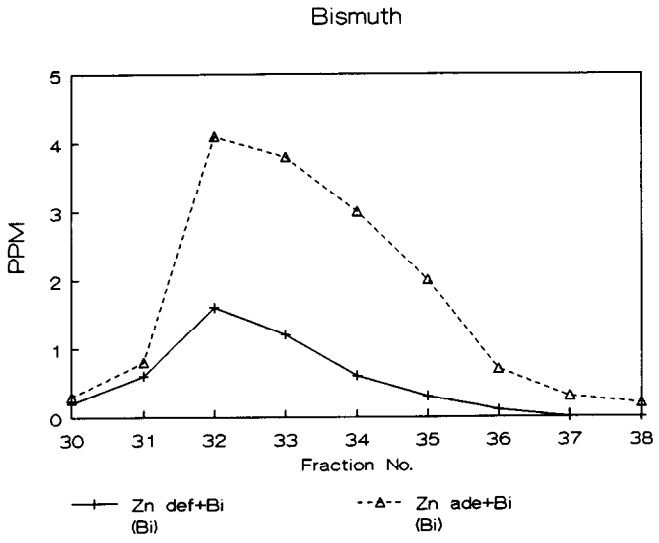

Figure 2 Bi accumulation in rat kidney MT. Kidney extracts were separated by gel filtration as noted for *Figure 1*. Virtually all detectable Bi was found in the fractions shown. These fractions were the same ones that contained the copper and zinc peak (*Figure 1*).

the rise in Bi and zinc was limited severely by low zinc intake. In contrast, Bi produced a strong increase in MT mRNA in rats fed low zinc (*Figure 3*). Densitometry analysis of the bands in *Figure 3* showed that the peak height for zinc deficient plus Bi was nearly 80% of the peak height for zinc adequate plus Bi. Analysis of RNA extracted from a different set of rats, treated the same as those of *Figure 3*, gave a blot pattern resembling that of *Figure 3* (data not shown).

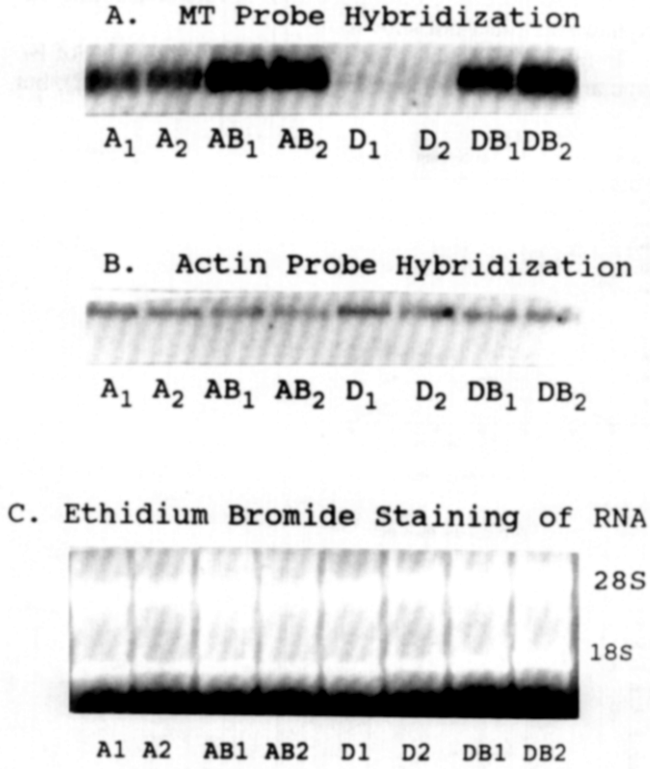

Figure 3 Effects of Bi and zinc intake on MT mRNA. Northern blot analysis was carried out on 5 µg renal RNA as described in text. Analysis of higher and lower amounts of RNA yielded patterns similar to that shown in this figure. A_1 and A_2 represent kidney RNA samples from two rats fed the adequate zinc diet, D_1 and D_2 represent RNA from two rats fed the zinc deficient diet. AB and DB are RNA from Bi-treated rats fed either adequate or deficient diet.

The differing effects of Bi treatment plus low zinc intake on renal MT protein and mRNA resembled those of a general inflammatory stress.[10] However, Bi, unlike turpentine-induced inflammation, did not raise serum ceruloplasmin activities, a parameter elevated by many stress states,[1] nor raise liver MT levels (*Table 3*).

Discussion

This study suggested that Bi does not elevate kidney MT mRNA through zinc displaced from preexisting MT. If this were the case, then depletion of MT protein through dietary zinc restriction should have limited Bi induction of MT mRNA. In contrast, MT mRNA accumulation was strongly elevated by Bi in rats fed the low zinc diet. This effect is consistent with the notion that Bi, like zinc, copper and cadmium, binds to proteins that activate the metal regulatory elements of the promoter region of the MT gene.[2] This idea has not yet been directly tested. Bi did not seem to elevate MT mRNA through general stress effects. In the present study, Bi did not produce two classic signs of general stress, namely, elevated liver MT and serum ceruloplasmin.[1]

This study raised the possibility that zinc is needed to prevent rapid degradation of MT protein after Bi treatment. Metal saturation of MT is thought to stabilize this protein against degradation.[1] MT protein in Bi treated, zinc adequate rats contained about equal molar concentrations of Bi, copper, and zinc (*Figures 1* and *2*). Thus, Bi may stimulate synthesis of more MT than it can saturate. Possibly, zinc fills the remaining binding sites, unless the rats are fed low zinc. Theoretically, copper could be substituted, but this did not happen (*Figure 1*). Body copper may not be able to move freely into the MT pool. Hence, nascent MT in Bi treated rats fed low Zn might be undersaturated with metals and degrade rapidly. Alternatively, zinc could affect translation of MT mRNA produced in response to Bi. However,

Table 3 Effects of Bi and inflammation on liver MT protein and serum ceruloplasmin activity levels

Treatment	MT Protein (µg/g liver)	Ceruloplasmin (Units/dl)
Zinc-adequate		
Control	43 ± 7[a]	104 ± 7[a]
+ Bi	59 ± 9[a]	117 ± 17[a]
+Inflammation	157 ± 13[b]	318 ± 19[b]

Rats consuming adequate zinc diet, ad libitum were treated with Bi (four injections), solvent (four injections), or the inflammatory agent turpentine (one injection) as described in the text. Values are means ± SD for five rats. Ceruloplasmin units were arbitrarily defined as change in absorbance at 540 nm per 15 min. Different superscripts within a column denote significant differences ($P < 0.01$, two-way ANOVA + LSD).

no current evidence suggests MT mRNA translation rates are regulated.[2]

The finding that zinc status can affect the ability of Bi to raise kidney MT protein levels has clinical implications. Bi induction of MT has been proposed as a possible means of limiting renal and cardiac toxicity of the chemotherapy drugs cisplatin and adriamycin.[6,7] The present study suggests that the usefulness of such Bi therapy could depend on zinc status.

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