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In Vivo Inhibition of Lysyl Oxidase by High Dose of Zinc (40836)

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Experimental evidence supports the view that lysyl oxidase is a copper containing enzyme. Pinnell and Martin (1) showed that apoenzyme can be activated mainly be cupric ions. In purified enzyme its activity coincides with peak of Cu^{64} on chromatograms (2). In states of copper deficiency the enzyme activity is inhibited and supplementation of copper restores lysyl oxidase activity (3).

An antagonistic relationship between copper and zinc has been established by several authors (4–7). In principle, zinc administration decreases the level of copper in serum and in tissues either by interference with enzymes transporting both metals from intestinal mucosa (8) or by competing with copper for ligand consisting of N–S (9). The effect of supplementary dietary zinc on copper level is striking (10). Zinc administration almost completely inhibits the activity of ceruloplasmin; administration of copper or iron restores its function (11).

On the basis of these and our own experiments on zinc–copper interaction (12, 13), we felt that zinc may interfere with copper at the active site of lysyl oxidase if this metal is linked to the apoenzyme by N–S ligand. Preliminary notes testing this assumption were published (14, 15).

It was the aim of this study to test if dietary or parenterally administered zinc interferes with the activity of lysyl oxidase in various tissues of the rat.

Materials and methods. This study reports the results of three experiments:

In the first experiment, Sprague–Dawley male rats, 80 and 250 g initial weight, were fed, for 8 days, special diets differing in zinc oxide content. Zinc-deficient diet purchased from Nutritional Biochemicals (Ohio) contained 0.5 ppm zinc, control diet contained 40 ppm zinc, and diet with high zinc content had 2000 ppm zinc. After 8 days, each animal was implanted with two 0.7 × 0.7 × 2.5-cm prisms of polyvinyl alcohol sponges (Ivalon, Unipoint Labs, N.C.), symmetrical to the backbone in the back region, and the dietary regimens were continued for another 10 days. There were eight rats in each group (Table I).

In the second experiment, male rats, 80 g body weight, fed a standard diet, were injected intraperitoneally for 5 days with 0.75 mg zinc sulfate · $7H_2O$/100 g body wt/12 hr. Then, Ivalon sponges were implanted and the injections continued for another 10 days. Control rats received saline (Table II).

In the third experiment, adult female Sprague–Dawley rats, 220 g body weight, were fed a diet containing 40 ppm zinc and injected intraperitoneally with saline (controls) or fed 2000 ppm zinc diet and injected in addition with 3 mg $ZnSO_4$ · $7H_2O$/100 g body wt every 12 hr for a total of 12 days. On the second day of the treatment, Ivalon sponges were implanted.

Sampling of tissues was done in all animals 10 days after the implantation of sponges. In the second experiment lungs and kidney were dissected in addition to the granuloma tissue.

Zinc, copper, and calcium content were measured by atomic absorption spectrometry using appropriate lamp and Perkin–Elmer Model 305 after digestion of the granuloma tissue in nitric acid and hydrogen peroxide. Determination of zinc in the serum was done directly after fivefold diluting the serum with deionized double-distilled water.

Assay of lysyl oxidase was that as described originally by Pinnell and Martin (1).

Total collagen content in the granuloma tissue is expressed by the amount of hydroxyproline of collagen, extractable into hot 0.3 *M* trichloroacetic acid. Hydroxyproline was measured by the automated Technicon Autoanalyzer procedure. *The rate of collagen synthesis* was determined in slices of granuloma tissue incubated in a

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medium as developed by Uitto (16) in the presence of [C^{14}]proline. Radioactive [C^{14}]hydroxyproline was isolated from hydrolyzed collagen by the procedure of Juva and Prockop (17). The *rate of the synthesis of noncollagenous proteins* was measured in slices, incubated with [C^{14}]proline and refers to the [C^{14}]proline activity in cold 5% trichloroacetic acid protein precipitate.

The amount of *collagen extractable* into acid or neutral medium was ascertained by threefold extraction of the homogenized granuloma tissue into 0.5 *M* acetic acid or 0.45 *M* NaCl at 4°C under continuous shaking for a total of 24 hr. The data on extractable collagen are presented in percentage of total collagen present in the granuloma tissue.

The content of *protein* in tissue extracts with lysyl oxidase was measured by Lowry *et al.* procedure (18). The content of *DNA* in granuloma tissue was determined by the procedure of Burton (19).

Results. After treating the young and adult rats for 18 days with three different zinc diets, several differences were noted between these age groups (Table I). Serum zinc levels in young rats significantly changed in relation to the zinc content in the diet. In adult rats kept on a zinc-deficient diet serum zinc was not reduced; supplementation of high zinc (2000 ppm) was, however, reflected in increased serum zinc. Zinc content in the granuloma tissue increased somewhat with increasing zinc content of the diet in young rats but not in the adults. In adult rats, the same zinc content in the granuloma tissue was found in all three dietary groups. The content of copper in the granuloma tissue was the same in both age groups irrespective of the dietary regimen. Calcium content was, however, significantly reduced with a diet high in zinc content.

Feeding a zinc-deficient diet significantly reduced the activity of lysyl oxidase in the granuloma tissue in both young and adult rats. The supplementation of a high zinc diet inhibited the enzyme activity in young rats only.

In the next experiment we administered zinc parenterally to young rats twice per day. We sacrificed the rat 12 hr after the

TABLE I. EFFECT OF DIETS WITH VARIOUS CONTENTS OF ZINC ON ZINC CONTENT IN THE SERUM AND GRANULOMA TISSUE AND ON LYSYL OXIDASE ACTIVITY IN GRANULOMA TISSUE IN YOUNG AND ADULT RATS[a]

	Young (80 g)						Adult (250 g)					
Parameter studied[b]	0.5		40		2000 ppm Zn		0.5		40		2000 ppm Zn	
Zinc												
Serum (g/100 ml)	88 ± 6.3	***[c]	125 ± 8.5	**	171 ± 9.8	***[d]	67 ± 2.5		73 ± 2.0	***	119 ± 4.8	***
Granuloma tissue (μg/g DW)	29.7 ± 0.92	**	36.8 ± 1.8	*	42.2 ± 1.8	***	37 ± 0.9		33 ± 2.3		36 ± 1.9	
Copper granuloma tissue (μg/g DW)	9.1 ± 1.6		7.6 ± 1.1		9.5 ± 1.0		9.8 ± 2.1		9.6 ± 1.3		10.2 ± 1.9	
Calcium granuloma tissue (mg/g DW)	27.3 ± 2.9		32.8 ± 2.2	**	22.8 ± 2.2		—		—		—	
Lysyl oxidase 10^3 × cpm/mg DNA	9.7 ± 1.8	***	18.9 ± 1.3	***	8.5 ± 1.3		13.4 ± 1.5	*	18.1 ± 1.5		15.5 ± 0.85	

[a] Rats were fed appropriate diet for 8 days, then sponges were implanted and the feeding continued for another 10 days.
[b] Variability is given as $\bar{X}$ ± SEM, DW = dry weight.
[c] Asterisks refer to *P* values, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The placement of asterisks indicates the two groups compared.
[d] Compares 2000 ppm with 0.5 ppm Zn group.

last zinc injection. Only the content of zinc in the serum was significantly increased by zinc supplementation. No effect of the zinc content in the granuloma tissue, lung, or kidney was found. Lysyl oxidase activity was significantly inhibited only in the granuloma tissue and in the lung, not in the kidney (Table II).

A similar experiment with parenteral administration of zinc was repeated in adult rats, 220 g body wt, and with the dose of zinc amounting to 3 mg zinc sulfate/100 g body wt/12 hr. In addition, the rats were fed a diet with high content of zinc. Thus, the dose was relatively high, at least four times higher than in the previous experiment (Table III). Inspite of such an excessive supplementation of zinc, the content of the metal in the granuloma tissue was increased only at 0.05 level of significance; no change in copper content in the granuloma tissue was noticed. The highly significant increase in the rate of the synthesis of collagen and activity of lysyl oxidase in the granuloma tissue of zinc-supplemented rats indicated the nonspecific toxic effect of the metal.

At the same time we noticed morphological and biochemical evidence of injury to the liver (not presented). It is worth mentioning that the increase in the activity of lysyl oxidase is not reflected in the extractability of collagenous proteins into neutral or acid media.

Discussion. In the introduction we presented the theoretical reasons why supplementation of high dose of zinc should interfere with the activity of lysyl oxidase. Indeed, the results of this study show that under certain situations both zinc-supplemented and deficient animals had lower activity of the enzyme as assumed. It was, however, rather difficult to develop a definite dietary or injection regimen with a zinc dose which would reproducibly result in the inhibition of the enzyme. Inhibition of lysyl oxidase in the granuloma tissue was seen only in young animals, not in adult rats. It has been our experience that adult or old animals do not respond to zinc-deficient or high zinc-containing diet with appropriate changes in serum zinc. This finding corresponds with similar observations of others (20, 21) who found alteration of tissue zinc only after weeks of feeding high zinc diet to rats.

Our data indicate that the actual content of zinc in a certain tissue does not correspond with the activity of lysyl oxidase or with copper content. It may be that the determination of zinc or copper in the whole tissue does not reflect the interaction of these metals occurring at the molecule level in specific tissue compartments. The reduction of calcium content in the granuloma tissue after supplementing with high zinc diet is of interest. The antagonistic relationship of Zn and Ca was reported (22) and may be of importance in the pathogenesis of some diseases (23).

It has already been shown by several authors that, in nutritionally balanced animals as well as in man, the administration of zinc does not enhance wound healing. Inhibition of wound healing in zinc-deficient states is commonly known. We believe that the lower activity of lysyl

TABLE II. EFFECT OF ZINC SUPPLEMENTATION ON THE CONTENT OF ZINC AND THE ACTIVITY OF LYSYL OXIDASE IN VARIOUS TISSUES OF YOUNG RATS[a]

	Lysyl oxidase activity[b] (cpm/mg protein)			Zinc[b] (μg/g DW)	
Tissue	Control		+ Zn[c]	Control	+ Zn
Lung	143 ± 20	*	94 ± 8	105 ± 18	104 ± 6
Kidney	48 ± 21		51 ± 27	118 ± 6	131 ± 6
Granuloma tissue	475 ± 52	*	312 ± 49	68 ± 3	75 ± 3

[a] Eight male Sprague–Dawley rats, 80 ± 10 g body wt, in each group.
[b] Variability is given as $\bar{X}$ ± SEM.
[c] A diet containing 2000 ppm zinc was fed *ad libitum* for 8 days before and 8 days after subcutaneous implantation of Ivalon sponges.

TABLE III. EFFECT OF EXCESSIVE ZINC DOSE ON SPONGE GRANULOMA TISSUE CHEMISTRY IN RATS[a]

Parameter studied	Control		+ Zinc
Lysyl oxidase (cpm/mg protein)	418 ± 49[b]	***	798 ± 56
Collagen synthesis			
[C^{14}]hyp dpm/μmole	7999 ± 681	***	14189 ± 751
Collagen content			
mg hyp/g wet wt	1.57 ± 0.12		1.17 ± 0.30
Noncollagenous protein synthesis			
[C^{14}]pro 10^3 × dpm/mg protein	15.6 ± 0.22		15.1 ± 1.0
Collagen extractability			
NSC (in % total)	6.12 ± 0.24	*	5.14 ± 0.22
ASC (in % total)	9.46 ± 0.98		9.58 ± 0.72
Zinc (μg/g dry wt)	46.6 ± 1.54	*	60.0 ± 4.49
Copper (μg/g dry wt)	11.2 ± 0.72		12.4 ± 1.05

[a] Sprague–Dawley female rats, five in each group, were treated either with saline (control) or fed a diet high in zinc content (2000 ppm) and injected with $ZnSO_4$ solutions for a total of 12 days. Injections of 3 mg $ZnSO_4 \cdot 7H_2O$/100 g body wt were administered every 12 ± 2 hr.

[b] Variability is given as $\bar{X}$ ± SEM.

oxidase in rats fed zinc-deficient diet is a part of a general picture of slower formation of inflammatory reactive granuloma tissue.

Our finding of a significant increase of lysyl oxidase activity, which coincides with increased synthesis of collagen after injection of excessive dose of zinc, should be considered as a nonspecific toxic reaction, as this dose was hepatotoxic and the rats showed changes in behavior typical for sick animals. We assume that in this case either humoral factors or other mechanisms, activated by topical cytotoxicity of high zinc, stimulate the function of fibrogenic cells in the granuloma tissue, as evidenced by increased rate of collagen synthesis and lysyl oxidase activity.

Summary. Young and adult rats implanted subcutaneously with polyvinyl alcohol sponges were fed diets containing 0.5, 40 (control), and 2000 ppm zinc. Diets with low and high zinc content inhibited lysyl oxidase activity in the granuloma tissue only in young rats. The content of zinc in the serum and in the granuloma tissue followed the zinc dietary regimen only in young rats. No effect on copper content in the granuloma tissue was found. High zinc diet significantly reduced calcium content in the tissue.

Parenteral administration of small dose of zinc (0.75 mg $ZnSO_4$/100 g/12 hr) also inhibited the activity of the enzyme in the granuloma tissue and the lung tissue without affecting the content of zinc in these structures.

Excessive supplementation of zinc (3 mg $ZnSO_4$/100 g/12 hr) appeared to be toxic and the activity of lysyl oxidase as well as rate of collagen synthesis in the granuloma tissue was significantly elevated.

We conclude that zinc supplementation in high dose to rats tends to inhibit lysyl oxidase activity in young animals. The method is, however, impractical due to the age limitation and unpredictable control of zinc content in the granuloma tissue.

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