Expression of Metallothionein in the Liver of Rats Fed Copper-Deficient AIN-93G Diet

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Copper-loading induces metallothionein (MT) synthesis in the liver of rats. Copper-MT from the liver of copper-injected rats has been isolated and analyzed. The copper-induced MT synthesis is characterized by increased incorporation of [35S]cysteine into the protein and enhanced production of the MT mRNA, suggesting that copper induction of MT occurs at the level of gene transcription. The mechanism by which copper induces MT synthesis, however, remains unsolved. It is also unknown whether copper is essential for MT production under a diversity of physiological and pathological conditions. The purpose of this study was thus to examine the effect of dietary copper deficiency on MT induction in rats.

A copper-deficient diet was formulated by replacing copper with the corresponding weight of corn starch in the AIN-93G diet. Diet analysis for copper yielded values of 5.7 mg Cu/kg diet for the copper-adequate diet and 0.4 mg Cu/kg diet for the copper-deficient diet. Male, weanling Sprague-Dawley rats (46–57 g; Sasco, Lincoln, NE, USA) were divided into two weight-matched groups having average weights of 52 g each; one had free access to the copper-adequate diet and the other to the copper-deficient diet. They also had free access to deionized water.

After 4 weeks on their respective diets and an overnight fast, each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg/kg body wt, Vet Labs, Lenexa, KS). Blood was withdrawn from the inferior vena cava for erythrocyte counting and plasma assays. The liver and heart were removed, flushed with cold 0.9% NaCl via their major vessels and divided for subsequent assays. Tissue samples including the liver, heart, and kidney were stored at −20°C for mineral assays and those for MT and mRNA assays were placed in liquid nitrogen, then stored at −80°C.

Hematocrit and hemoglobin content were determined on a cell counter. An automated analyzer was used to determine serum ceruloplasmin. Trace element contents of tissues were determined by inductively coupled argon plasma emission spectroscopy after lyophilization and digestion of the tissues with nitric acid and hydrogen peroxide.

A routine Northern blot assay was used to analyze MT-I mRNA. A probe corresponding to a 1185-base pair Hind III and Bgl II fragment of mouse MT-I cDNA was used to identify the MT transcript on the membrane. Autoradiographic images were scanned and analyzed by an imaging analyzing system. Densitometric values were then determined from digitized images of autoradiograms. MT concentrations were measured by the Cd/hemoglobin radiometric assay.

Characteristics of rats fed the copper-deficient diet were compared to those of rats fed the copper-adequate diet. Copper concentrations were significantly (p < 0.01) depressed in the liver, heart, and kidney of copper-deficient rats. Zinc concentrations were significantly (p < 0.01) decreased in the heart and kidney, but not significantly (p > 0.05) reduced in the liver. Iron concentrations were also significantly (p < 0.01) depressed in the heart and kidney, but significantly (p < 0.01) elevated in the liver. Other changes including reduced activity of ceruloplasmin in the plasma, depressed Cu,Zn-SOD in the tissues, and decreased hematocrit and hemoglobin concentrations in the blood were found in the rats fed the copper-deficient diet, typically indicative of severe copper deficiency.

The MT-I mRNA concentrations were elevated in the copper-deficient livers (n = 15) by 75.4 ± 3.7 fold (p < 0.01), but were not changed in the copper-deficient hearts (n = 12) or kidneys (n = 12). MT protein concentrations in the copper-deficient liver and heart did not change, but significantly (p < 0.01) decreased in the copper-deficient kidney.

It has been suggested that the enhanced synthesis of MT-I mRNA in the liver of brindled mutant mice results from stress (Mercer et al. 1991). Copper deficiency induces many biochemical changes and pathophysiological consequences in many organs including liver, heart, and kidney. Several studies suggest that oxidative stress is involved in the copper deficiency-induced pathological processes. Enhanced lipid peroxidation in copper-deficient tissues and inhibition of copper deficiency-induced defects by antioxidants were observed (Lynch and Strain 1989). Because MT is an important antioxidant participating in cellular protection against oxygen free radical-induced damage, up-regulation of MT synthesis may reflect a general adaption to the copper deficiency-induced oxidative stress. However, our previous studies (Chen et al. 1994) have shown that under the same experimental conditions, a higher degree of oxidative damage occurs in the heart than in the liver of copper-deficient rats. Therefore, the extent of oxidative stress alone cannot account for the elevated MT-I mRNA concentration in the liver. Mechanisms responsible for the selective response of liver MT gene transcription to copper deficiency need to be further investigated.

A corresponding increase in MT protein in the copper-deficient liver was not detected. MT is transported from the liver into the bile and blood (Bremner 1987). Accumulation of MT in the kidney would thus be expected if such a transport occurs. However, MT transport does not account for the undetected elevation of MT content corresponding to the increased MT mRNA, because the MT concentration in the kidney did not increase, but significantly decreased. Early studies on brindled mutant mice have shown that hepatic MT synthesis was reduced in the brindled neonate (Hunt and Port 1979), which has been attributed to the low hepatic copper concentration in the mutant (Piletz and Herschman 1983). This led to a conclusion that copper is the most likely regulator of hepatic MT synthesis in the neonatal mouse liver (Hunt and Port 1979, Piletz and Herschman 1983). These early studies, however, did not examine the concentration of MT mRNA. In contrast to the early conclusion, studies by Mercer et al. (1991) suggested that hepatic copper is not regulating MT mRNA production. In the present study, we measured both MT mRNA and protein concentrations. The results, together with previous studies (Mercer et al. 1991, Hunt and Port 1979, Piletz and Herschman 1983), suggest that copper is not essential for MT mRNA production, but it may be required for MT translation.

References

Discussion
Q1. John Beattie, Rowett Research Institute, Aberdeen, Scotland: Can your observations be explained by increased protein degradation or turnover?
A. I don’t know. I don’t think so, because I don’t know of any mechanism for Cu deficiency to increase degradation.
Q2. George Cherian, The University of Western Ontario, London, ON, Canada: Have you measured any cytokines in this model and could cytokines explain the induction in metallothionein?
A. That’s a very good question, but I don’t have an answer for you at this time. We will look at NF-kB involvement.
Q3. Harry McArdle, Rowett Research Institute, Aberdeen, Scotland: Some time ago we did some experiments where we were removing copper from hepatocytes in culture and looked at metallothionein message levels. We found an effect due to copper ions in the cells. In in vitro experiments, if Cu is chelated, then metallothionein decreases. Is it
possible that the changes in mRNA for liver metallothionein are some kind of secondary response? Why the difference in results between \textit{in vitro} and \textit{in vivo} systems?

A. I agree with the possibility of a secondary response but what is it? We should note that this response is observed only in the liver and not in the heart or kidney.

Q4. Ian Bremner, Rowett Research Institute, Aberdeen, Scotland: When measured by immunoassay, increased metallothionein in Cu deficient rats is associated by decreased feed intake as the stress. How was metallothionein protein measured in your study?

A. The Cd-affinity assay was used.