CHRONIC TREATMENT WITH DIMETHYL SULFOXIDE PROTECTS AGAINST CARDIOVASCULAR DEFECTS OF COPPER DEFICIENCY

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ABSTRACT. Published reports indicate that Cu deficiency reduces antioxidant defenses and may result in tissue peroxidation. Dimethyl sulfoxide (DMSO), a highly penetrant antiinflammatory agent and purported hydroxyl radical scavenger, was chronically fed to male, weanling Sprague-Dawley rats which were either supplemented with or deficient in Cu. DMSO was found to inhibit the cardiac hypertrophy, anemia and depression of heart Cu concentration which occurs with Cu deficiency. This suggests that the hydroxyl free radical may contribute to the cardiovascular defects caused by dietary Cu deficiency.

INTRODUCTION. Dietary Cu deficiency produces a variety of cardiovascular defects, including structural and functional deficits of the heart and blood vessels, anemia and hypercholesterolemia (1-5). That some of the cardiovascular defects may be the result of oxidative damage is supported by the fact that endogenous antioxidant enzymes, including superoxide dismutase, ceruloplasmin and catalase are reduced (6,7) and that enhanced tissue peroxidation occurs in Cu deficiency (8-10).

Dimethyl sulfoxide (DMSO) has long been known for its antiinflammatory and analgesic properties (11) as well as for a variety of other effects (see 12 for review). It also has high water and lipid solubility, penetrates tissues rapidly (13) and has a relatively low toxicity (14). More recently it has been utilized to scavenge hydroxyl free radicals and has been successfully used to counteract the deleterious effects of ischemia and subsequent reperfusion in various organs (15,16). DMSO was used in the present study to determine whether chronic application of a known antioxidant with a defined substrate specificity and high penetrant properties could prevent any of the more obvious symptoms of dietary Cu deficiency.

MATERIALS and METHODS. Forty, male weanling Sprague-Dawley rats[†] (Harlan Sprague-Dawley, Madison, WI) were fed, for four weeks, a purified diet (ad lib) which without supplementation was deficient in both Cu and Zn. The diet was based on 62% sucrose, 20% egg white and 10% corn oil (by weight) and contained all other nutrients known to be essential for rats (5). The animals' water (ad lib) was supplemented with 10 μg/ml Zn (as acetate) and either 2 μg/ml Cu (as sulfate, n=20) or no Cu (n=20). Ten of the Cu-supplemented rats and ten of the Cu-deficient rats received DMSO (4.75% by volume) in their water.

Final body weights were recorded just prior to sacrifice. The rats were anesthetized with Na pentobarbital (65 mg/kg, i.p.). Their chests were opened and blood was drawn by cardiac puncture. The heart and one lobe of the liver were dissected free, blotted, weighed and frozen for subsequent analysis.

Freeze dried tissues were acid digested and assayed for Cu, Fe and Zn by atomic absorption spectroscopy (17).

Statistical analysis employed one and two-way analysis of variance (See Table I) and Scheffe's test for comparison of means (18), with significance set at $p<.05$.

RESULTS. The results of this study are summarized in Table I.

There was a pronounced treatment effect of DMSO on several of the measured variables (CuS-DMSO), when compared to control values (CuS). These include lower body weights and heart weights, higher liver Cu and Zn concentrations and higher heart Cu concentrations.

Cu-deficient animals (CuD), when compared to controls (CuS), had lower body weights, higher heart weights and heart weight/body weight ratios (HW/BW), reduced hematocrits and reduced liver and heart Cu concentrations.

Cu-deficient rats treated with DMSO (CuD-DMSO), when compared to their CuD counterparts, showed further depressed body weights, a reduction in their heart size, as indicated by both heart weight and HW/BW, a

TABLE I. Comparison of variables[a] affected by Cu deficiency in the presence and absence of DMSO.

Variable	CuS	CuS-DMSO	CuD	CuD-DMSO
Body weight (g)	233 ± 5	179 ± 5[b]	189 ± 7[c]	161 ± 3[c,d]
Heart weight (mg)	967 ± 26	745 ± 19[b]	1405 ± 79[c]	877 ± 28[d]
HW/BW (mg/g)	4.16 ± 0.04	4.17 ± 0.08	7.49 ± 0.58[c]	5.45 ± 0.17[c,d]
Hematocrit (%)	41.8 ± 0.6	44.5 ± 0.6	23.4 ± 3.0[c]	32.4 ± 2.2[c,d]
Liver Cu (µg/g)	6.3 ± 0.7	12.0 ± 0.5[b]	0.85 ± 0.13[c]	0.93 ± 0.27[c]
Liver Fe (µg/g)	664 ± 56	837 ± 52	653 ± 45	689 ± 36
Liver Zn (µg/g)	80 ± 3	90 ± 4[b]	67 ± 3[c]	79 ± 3[d]
Heart Cu (µg/g)	7.4 ± 0.7	18.0 ± 1.6[b]	4.1 ± 0.2[c]	6.1 ± 0.4[d]
Heart Fe (µg/g)	276 ± 20	312 ± 21	225 ± 8	222 ± 15
Heart Zn (µg/g)	70 ± 5	75 ± 3	74 ± 2	71 ± 3

[a]Values are means ± SEM.
[b]Indicates significant difference from control (CuS) values by 2-way ANOVA and Scheffé contrast. The high liver and heart Cu values indicate a severe polydipsia leading to increased Cu intake. This treatment is therefore of limited value as a control and therefore subsequent ANOVA indicated below is a 1-way analysis comparing CuS, CuD and CuD-DMSO values only.
[c]Indicates significant difference from control (CuS) values by 1-way ANOVA and Scheffé contrast.
[d]Indicates significant difference from Cu-deficient group (CuD) values by 1-way ANOVA and Scheffé contrast.

rise in hematocrit and a rise in liver Zn and heart Cu concentrations. Liver Cu and Fe were unchanged by treatment of CuD animals with DMSO. From a purely subjective standpoint, the CuD-DMSO animals looked healthier and lacked the hair color change (red-brown tinge on the back) of CuD animals.

A comparison of CuD-DMSO treated rats to CuS rats indicated that DMSO moved HW/BW and hematocrit toward control values, and that liver Zn and heart Cu concentrations returned to control values.

DISCUSSION. This study indicates that DMSO significantly inhibits the appearance of three cardiovascular defects, cardiac hypertrophy, anemia and reduced heart Cu stores, which result from dietary copper deficiency.

In evaluating the results, the significant treatment effects of DMSO on several variables had to be dealt with. The severe growth retardation caused by DMSO has been observed previously and is most likely attributable to reduced food intake (12). The enhanced Cu concentrations in livers and hearts of CuS-DMSO animals are probably caused by polydipsia, observed qualitatively in this study and supported quantitatively by prior work (12). This effect makes the CuS-DMSO animals of limited value as controls for Cu status.

It is important to recognize, however, that the tendency of DMSO to increase Cu intake by polydipisia in CuS animals could not occur in CuD animals, which had no Cu in their water. For this reason it is reasonable that liver Cu in CuD animals was not altered by DMSO, indicating that the effects of DMSO are not due to a general alteration of Cu status.

Two control variables upon which DMSO had no treatment effect and which were therefore valid as controls are HW/BW and hematocrit. The constant HW/BW ratio with DMSO in CuS animals indicates that the reduced heart weight was simply a function of the reduced growth known to occur with DMSO (12). Cardiac enlargement with Cu deficiency is quite evident from both heart weight and HW/BW values. The lower heart weights caused by DMSO treatment of CuD animals is also clear, the lower HW/BW showing that heart weight reduction by DMSO was not just a consequence of reduced body weight. Likewise, for hematocrit, the lack of a DMSO treatment effect in control animals may be contrasted against the significant inhibition by DMSO of the anemia induced by Cu deficiency.

Because DMSO is a generalized antiinflammatory agent and powerful solvent, the mechanism of the inhibitory effect of DMSO on the cardiovascular defects of Cu deficiency cannot be stated with certainty. DMSO was used in this study because of its ability to scavenge hydroxyl radicals and at least one study relates this function to its antiinflammatory activity (19).

That the cardiac hypertrophy in Cu deficiency may be associated with direct oxidative damage is suggested by evidence of peroxidation in Cu-deficient hearts (8,10). If that damage is caused by hydroxyl radicals, DMSO could directly prevent it. Alternatively, the relative retention of heart Cu in CuD animals treated with DMSO may indirectly prevent oxidation by the associated retention of Cu-dependent antioxidant enzyme levels in the heart. Yet another proposal is that the cardiac hypertrophy may be secondary to Cu-dependent anemia (3).

Proposed mechanisms for the anemia of Cu deficiency include altered Fe metabolism (20) and oxidative damage to red cell membrane proteins (21). Because Fe status is not altered, this study supports the latter proposal most directly and implicates the hydroxyl radical as the culprit in the oxidative damage. However, because of the complex interaction among Cu, Fe, their dependent enzymes, as well as heme with production of oxygen-derived free radicals, a role of DMSO in the inhibition of an Fe-dependent component of anemia cannot be ruled out.

The rise in liver Zn concentration caused by DMSO (presumably because of polydipsia) can be ruled out as correcting the heart and hematocrit changes of Cu deficiency because increased Zn is known to depress Cu levels and tends to exacerbate effects of Cu deficiency (1).

In conclusion, the significance of this work lies in our ability to partially inhibit signs of severe Cu deficiency by chronic application (feeding) of a rather simple substance (DMSO) with a high degree of tissue penetration and known antiinflammatory activity. Because DMSO also has an antioxidant property, these findings support, although indirectly, evidence in the literature of oxidative damage in Cu deficiency.

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FOOTNOTES. [1]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

REFERENCES.

1. Klevay LM. The role of copper, zinc, and other chemical elements in ischemic heart disease. In: Rennert DM, ed., Metabolism of Trace Metals in Man. Boca Raton: CRC Press, Vol. 1:p. 129-157, 1984.
2. Kitano S. Membrane and contractile properties of rat vascular tissue in copper-deficient conditions. Circ Res 46:681-689, 1980.
3. Prohaska JR, Heller LJ. Mechanical properties of the copper-deficient rat heart. J Nutr 112:2142-2150, 1982.
4. Kopp SJ, Klevay LM, Feliksik JM. Physiologic and metabolic characterization of cardiomyopathy induced by chronic copper-deficiency. Am J Physiol 245:H855-H866, 1983.
5. Moore RJ, Klevay LM. Effect of copper deficiency on blood pressure and plasma and lung angiotensin-converting enzyme activity in rats. Nutr Res 8:489-497, 1988.
6. Owen CA. Biochemical Aspects of Copper. Copper Proteins, Ceruloplasmin, and Copper Protein Binding. Park Ridge, NJ, Noyes Publications, 1982.
7. Taylor CG, Bettger WJ, Bray TM. Effect of dietary zinc or copper deficiency on the primary free radical defense system in rats. J Nutr 118:613-621, 1988.
8. Paynter DI. The role of dietary copper, manganese, selenium, and vitamin E in lipid peroxidation in tissues of the rat. Biol Trace Elem Res 2:121-135, 1980.
9. Balevska PS, Russanov EM, Kassabova TA. Studies on lipid peroxidation in rat liver by copper deficiency. Int J Biochem 13:489-493, 1981.
10. Fields M, Ferretti RJ, Smith JC, Reiser S. Interaction between dietary carbohydrate and copper nutriture on lipid peroxidation in rat tissues. Biol Trace Elem Res 6:379-391, 1984.
11. Jacob SW, Bischel M, Herschler RJ. Dimethyl Sulfoxide (DMSO): A new concept in pharmacology. Curr Ther Res Clin Exp 6:134-135, 1964.
12. Debons AF, Fani K, Jimenez FA, Maayan ML. Inhibition of cholesterol-induced atherosclerosis in rabbits by dimethyl sulfoxide. J Pharmacol Exp Therap 243:745-757, 1987.
13. Jacob SW. Pharmacology of DMSO. In: Dimethyl Sulfoxide. New York:Marcel Dekker, Vol. 1:p. 99-112, 1971.
14. Brown VK, Robinson J, Stevenson DE. A note on the toxicity and solvent properties of dimethyl sulfoxide. J Pharm Pharmacol 15:688-692, 1963.
15. Ganote CE, Sims M, Safavi S. Effects of dimethyl sulfoxide (DMSO) on the oxygen paradox in perfused rat hearts. Am J Pathol 109:270-276, 1982.
16. Parks DA, Bulkley GB, Granger DN. Role of oxygen-derived free radicals in digestive tract diseases. Surgery 94:415-422, 1983.
17. Nielsen FH, Zimmerman TJ, Shuler TR. Interactions among nickel, copper and iron in rats. Liver and plasma content of lipids and trace elements. Biol Trace Elem Res 4:125-143, 1982.
18. Kleinbaum DG, Kupper LL. Applied Regression Analysis and Other Multivariable Methods. North Scituate, Mass.: Duxbury Press, 1978.
19. Repine JE, Eaton JW, Anders MW, Hoidal JR, Fox RB. Generation of hydroxyl radical by enzymes, chemicals, and human phagocytes in vitro. Detection with the anti-inflammatory agent, dimethyl sulfoxide. J Clin Invest 64:1642-1651, 1979.
20. Lee GR, Williams DM, Cartwright GE. Role of copper and iron in metabolism and heme synthesis. In: Prasad AS, Oberleas D, eds. Trace Elements of Health and Disease. New York:Academic Press, Vol 1, 1976.

21. Johnson WT, Kramer TR. Effect of copper deficiency on erythrocyte membrane proteins of rats. J Nutr 117:1085-1090, 1987.

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