Pediat. Res. *11*: 823–826 (1977)

Menkes' Kinky Hair Syndrome: Studies of Copper Metabolism and Long Term Copper Therapy

D. M. WILLIAMS,[20] C. L. ATKIN, D. B. FRENS, AND P. F. BRAY

Department of Internal Medicine and Division of Pediatric Neurology of the Departments of Pediatrics and Neurology, University of Utah College of Medicine, Salt Lake City, Utah, USA

Summary

A patient with Menkes' kinky hair syndrome was treated with oral $CuSO_4$. Plasma copper, ceruloplasmin, red cell copper, and 24-hr urine copper excretion remained essentially unchanged. Intravenous copper infusion resulted in a rise of serum ceruloplasmin. During administration of a test meal of $^{61}Cu(NO_3)_2$, the patient was given alternately a volume of 0.9% NaCl or an equal volume of plasma intravenously. Radioactivity found in the blood was unchanged after each infusion period. During 427 days of subcutaneous copper, plasma *p*-phenylenediamine oxidase activity and plasma copper rose toward normal. Subsequent balance studies showed that the patient was in negative copper balance because of large losses in feces. Scanning electron microscopy demonstrated persistent pili torti.

Speculation

Menkes' kinky hair syndrome is probably due to a generalized defect in a copper-binding protein that results in increased gastrointestinal copper loss and persistent abnormalities of hair in the copper-replete patient.

Menkes' kinky hair syndrome (MKHS) is an X-linked recessive disorder characterized by abnormaltities of hair (pili torti or twisted hairs), bones, and arteries, as well as by seizures, progresssive developmental deterioration, and death usually before the age of 2 years (11). The similarities between this clinical syndrome and the manifestations of nutritional copper deficiency in "sway-back" sheep led Danks and his coworkers (5) to their observations that copper metabolism was abnormal in patients with MKHS. This abnormality is most clearly reflected in very low levels of plasma copper and ceruloplasmin but is unlike that seen in experimental copper deficiency in that red cell copper is normal, and anemia and leukopenia are absent (4). Mucosal transport of copper appears to be defective in MKHS, and parenteral therapy can be used to circumvent this defect (1, 4). Intravenous copper therapy, although effective in raising the levels of plasma copper and ceruloplasmin, and reportedly of some clinical benefit, is cumbersome for long term use.

The purposes of these studies were to gain additional understanding of the nature of the defect in MKHS and to assess the effects of long term therapy with subcutaneous copper.

CASE REPORT

This male infant (*UUMC 30-58-82*) was the 1560 g (small-for-gestational age) product of a full term first pregnancy and uncomplicated delivery. Both parents were in good health without history of neurologic disease. At 4 months of age the patient began to have frequent seizures which were characterized by blinking of the eyelids, asynchronous jerking of the extremities, crying, and drooling. His height, weight, and head circumference were below the third percentile and developmental milestones were markedly delayed. His hair was light in color and very short. Visual fixation and response to light were absent. Muscle tone was universally decreased, but deep tendon reflexes were uniformly brisk, and sustained ankle clonus was present bilaterally. Serum electrolytes, pH, glucose, calcium, magnesium, zinc, hepatic and renal function tests, urine and serum amino acid chromatography, urinalysis, cerebrospinal fluid, and skull films were normal. Hematologic studies revealed volume of packed red cells, 36%; reticulocyte count, 4.0%; white blood cells, 7,200/mm^3; and platelets, 450,000/mm^3. Electroencephalograms demonstrated diffuse spike discharges. Minimal dilatation of both lateral ventricles was found by pneumonencephalogram, and marked tortuosity of all the intracranial branches of the right internal carotid artery was noted by arteriography. A possible fracture of the right eighth rib was seen on chest x-ray. Radiologic skeletal survey revealed multiple metaphyseal avulsion fractures. Microscopic examination of the child's hair showed pili torti. Bone marrow examination was normal for age. Distinctly reduced values for plasma copper and ceruloplasmin were found, but red cell copper and superoxide dismutase (SOD) and urine copper were not decreased (Table 1).

After partial seizure control was achieved with phenobarbital and diazepam, further investigative studies were undertaken following the approval of the Medical Isotopes and Human Research Committees and with the informed consent of the patient's parents.

MATERIALS AND METHODS

ANALYTIC METHODS

Blood for copper studies was drawn with heparinized plastic syringes. Hematologic determinations were carried out by standard methods (2). Bile was aspirated from a nasoduodenal tube placed under fluoroscopic observation. Saline-washed red cells, plasma, urine, and other fluids were stored in specially cleaned glassware. Plasma iron, red cell, urine, bile, and stool copper values were determined by flame atomic absorption spectrophotometry (9). Plasma Cu was determined in 5-μl aliquots in a Perkin-Elmer HGA-2000 graphite furnace. Plasma ceruloplasmin was measured by the *p*-phenylenediamine oxidase method of Ravin (13). Ceruloplasmin Cu was calculated assuming molecular weight as 134,000 and 6 Cu atoms/ceruloplasmin molecule (14). Superoxide dismutase was determined by the method of McCord and Fridovich (10).

When a radioactive test meal was given, blood samples were collected before and after the meal. Red cells were washed twice in cold 0.15 M NaCl and resuspended in 0.15 M NaCl. Aliquots of plasma and red cell suspension were counted in a Nuclear Chicago gamma well counter, model 8725. All counts were corrected for background and for isotope decay.

PREPARATION OF COPPER FOR INJECTION

Sterile $NaHCO_3$-neutralized solution was prepared to contain 23 mM $CuCl_2$ (1,450 μg Cu/ml), 57.5 mM L-histidine, and 0.9% NaCl. Amino acid mixtures were unsuitable chelators because some Cu(II)-amino acid complexes precipitated. Furthermore, commercial amino acids for parenteral administration contain HSO_3^- that was shown to give considerable reduction of Cu(II) to supposedly more toxic Cu(I).

Before use, all copper preparations were checked for sterility by culture and for absence of endotoxin by Limulus assays. Controls with added endotoxin showed that Cu(II)-histidine complexes did not affect the assay.

For study by scanning electron microscopy, hair was cut as close to the scalp as possible. The proximal end was oriented, and the samples were examined in a Cambridge Stereoscan, Mark 2A scanning electron microscope.

RESULTS

EFFECT OF NORMAL PLASMA IN COPPER ABSORPTION

Administration of oral copper supplementation failed to change baseline copper values, but intravenous copper given at two dose levels resulted in a rise of serum ceruloplasmin that was in good agreement with the observations of others (1). Therefore, to test the possibility that a missing plasma factor was required for copper absorption, additional studies were carried out using a test meal of $^{64}Cu(NO_3)_2$ (New England Nuclear) added to soybean milk substitute. The test meal was given through a nasoduodenal tube at a constant rate of 50 ml/hr for 4 hr. During each of two study periods, the patient received 100 μg Cu with 200 μCi ^{64}Cu. During the first 2 hr of the tube feeding, the patient was also given 0.9% NaCl intravenously at a rate of 20 ml/hr. Blood samples obtained at the end of the control infusion contained little radioactivity. Calculations based upon these levels, the patient's body weight volume of packed red cells (VPRC), and assumed blood volume of 0.75 dl/kg, could account for no more than 0.2% of the administered dose. During the second 2 hr of the feeding the patient was given a volume of his father's plasma intravenously at a rate of 20 ml/hr, estimated to represent about 20% of the patient's blood volume; VPRC fell from 36% to 32%. However, the radioactivity in the blood was unchanged from that observed during the saline infusion.

Table 1. *Copper and copper-containing proteins in patient with Menkes' kinky hair syndrome compared with normal values*

	Normal range	Patient
Plasma ceruloplasmin (mg/dl)	19–36	3
Plasma copper (μg/dl)	78–157	15
Red cell copper (μg/dl RBC)	63–107	75
Red cell superoxide dismutase (units/ml RBC)	314–636	857
Urinary copper excretion (μg/24 hr)	6–17	8
Bile copper (μg/dl)	35–208	28

EFFECT OF LONG TERM SUBCUTANEOUS COPPER TREATMENT

Daily injections of copper were administered subcutaneously at alternate sites on the inner aspects of both thighs. These injections were begun 226 days after the patient's birth. Injection sites were inspected daily, and no evidence of hemolysis was found in blood samples.

The patient's course during 427 days of subcutaneous copper treatment is shown in Figure 1. Throughout this time, the patient tolerated the injections well without local reaction or signs of copper toxicity. Both plasma ceruloplasmin and plasma copper rose progressively toward normal. However, normal levels of ceruloplasmin were not achieved until the patient had been treated for 65 days with quantities of copper some 2–7 times greater than the estimated normal requirement (16). During that time, the patient had received a total copper load of 30 mg or approximately 4.3 mg/kg. By comparison, copper concentration in the newborn has been estimated to be 4.7 mg/kg lean body mass (15). Urinary loss of copper increased during treatment. The loss of copper in urine when plasma ceruloplasmin levels were stable corresponds to only about one-fourth the administered copper. However, increased urinary copper losses persisted even after copper treatment was discontinued, and plasma copper dropped to 54 μg/dl.

Essentially all plasma copper could be accounted for by copper contained in ceruloplasmin except for two specimens received by mail (days 190, 240). Copper content of liver tissue obtained by percutaneous biopsy on day 209 was 38 μg Cu/g wet weight. This compares with the average liver copper of 24 μg Cu/g wet weight (range 6.9–57.6 μg/g) in 25 infants varying in age from 0–2 years of age (12).

COPPER BALANCE STUDIES

After the plasma ceruloplasmin had remained stable for approximately 2 months, the patient continued to receive daily injections of copper, 180 μg/day, and was fed weighed aliquots of a standard homogenized diet. Copper content of food, urine, and stool was determined.

The results of 13 days of dietary balance studies (days 202–215 in Fig. 1) are summarized in Table 2. The patient was clearly in negative copper balance, mainly because of large losses of

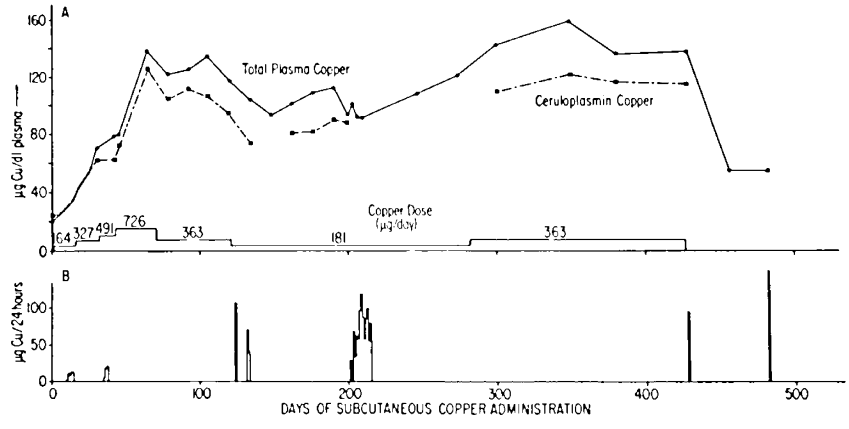

Fig. 1. *A:* the effect of subcutaneous copper administration on plasma copper (●——●) and plasma ceruloplasmin copper (□- - -□) during the course of 427 days. Ceruloplasmin copper was calculated from measured *p*-phenylenediamine oxidase activity, the assumption of 6 atoms Cu/molecule, and ceruloplasmin molecular weight of 134,000. Some samples received by mail could not be adequately assayed for enzyme activity. Changes in dose levels of copper are noted across the bottom. *B:* the effect of subcutaneous copper administration on urinary copper excretion. Vertical bars represent total urinary copper excreted during 24-hr intervals.

Table 2. *Copper balance studies during parenteral copper treatment*

	μg Cu/day (mean of 13 days)
Intake	
Oral	331
Subcutaneous	180
Total	511
Output	
Urine	69
Stool	898
Total	967

copper in feces. During the study, ceruloplasmin varied between 23.4 mg/dl and 18.6 mg/dl and plasma copper varied 90–105 μg/dl. No bile was obtained.

CLINICAL COURSE

During the prolonged period of subcutaneous copper therapy no changes occurred in the baby's somatic growth retardation, his clinical neurologic status, his EEG or in ultramicroscopic appearance of his hair deformity (Fig. 2). The only evidence of clinical improvement was the radiographic disappearance of the metaphyseal avulsion fractures.

DISCUSSION

Our patient demonstrated the typical clinical and laboratory features of Menkes' kinky hair syndrome. These findings have been remarkably constant in over 40 cases which have so far been described (1, 4, 7, 8, 11). These changes have been interpreted to suggest that the manifestations of MKHS are the result of copper deficiency secondary to an absorptive defect. In our patient, we were unable to overcome this defect with oral copper supplementation.

One possible explanation for the decreased absorption of copper is an abnormality in or a decreased amount of a plasma acceptor substance. Albumin is the primary copper transport protein and its copper-binding sites have been characterized in several species (6). We were unable to demonstrate abnormalities of the patient's albumin by several electrophoretic techniques. Also, infusion of the father's putatively normal plasma did not influence the absorption of radioactive copper. Nonetheless, these studies do not exclude the possibility that a plasma factor is missing in MKHS.

Our studies support the observations that the apparent mucosal block can be circumvented by the parenteral administration of copper. Intravenous infusion of copper at two dose levels resulted in a rise in plasma ceruloplasmin without evidence of acute toxicity. Intravenous copper treatment can be used to replete copper stores as shown by Grover and Scrutton (8). In our patient, copper replacement by the subcutaneous route was managed easily, but amounts of copper several times greater than estimated normal requirements (16) were needed to maintain normal levels of plasma copper and ceruloplasmin. In part, this appears to be due to increased copper excretion. Bile copper concentration was not increased during oral feeding of copper supplement or in the time immediately after intravenous infusion of copper, but at a time when liver copper was within the normal range and plasma copper and ceruloplasmin appeared to be stable, copper losses, especially in the stool, exceeded copper intake. It seems reasonable to propose that in addition to an absorptive defect, MKHS results in excessive copper loss due to a block in the reabsorption by the gastrointestinal mucosa of copper excreted in the bile. Alternatively, enteric secretion of copper occurs in normal humans (3), and this copper secretion may be increased or at least not reabsorbed in MKHS. Our data do not permit a resolution of these possibilities.

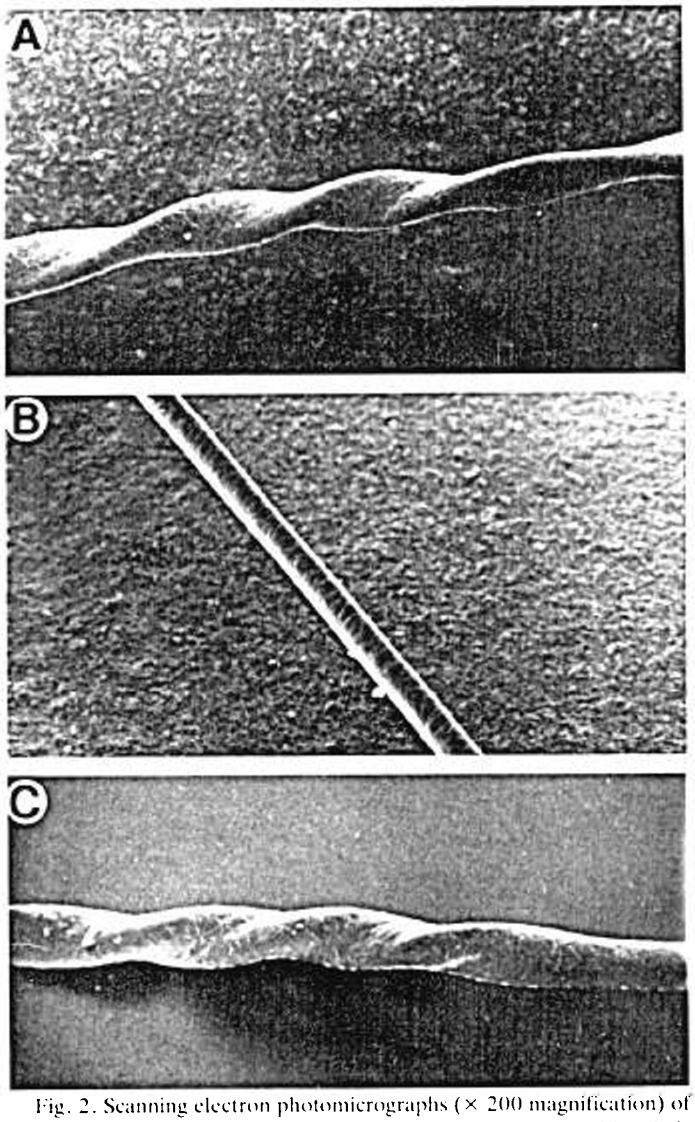

Fig. 2. Scanning electron photomicrographs (× 200 magnification) of patient's hair before copper administration (*A*) compared with a hair of a male infant of similar age (*B*) and the patient's hair after 5 months of subcutaneous copper administration (*C*). Scale-like plates or epicuticles are observed and aid in orientation of hair shaft direction. Flattening of the patient's hair shaft is noted as well as twisting of the hair around its central axis.

In contrast to the suggested improvement in the case of Grover and Scrutton (8), parenteral copper treatment did not benefit our patient clinically. The characteristic changes of the hair were present in our patient after 12 months of therapy whereas the changes seen in the wool of "sway-back" lambs are promptly reversed by copper therapy (15). Study of the possible role of special carrier proteins, such as metallothionein (7) in copper metabolism seems warranted in patients with MKHS, and the careful measurement of copper-dependent enzymes in different organs before and after treatment should be carried out.

CONCLUSION

Copper metabolism was studied in a 4-month-old patient with the clinical manifestations of Menkes' kinky hair syndrome. As in other reported cases, the patient's plasma copper and ceruloplasmin levels were decreased. Oral copper did not alter levels of red cell copper, plasma copper, or ceruloplasmin. Intravenous infusion of normal plasma did not enhance absorption of copper

from a radiolabeled test meal. However, increased ceruloplasmin was observed when copper was administered either intravenously or subcutaneously as Cu(II)-L-histidine complex. Normal levels of plasma copper and ceruloplasmin were achieved after about 2 months of subcutaneous copper treatment, but no measurable improvement in the patient's clinical condition occurred. Also, characteristic hair abnormalities persisted after 12 months of treatment. Copper loss, primarily in the feces, exceeded copper intake at a time when plasma copper levels were stable. Plasma copper fell when subcutaneous copper was discontinued.

REFERENCES AND NOTES

1. Bucknall, W. E., Haslam, R. H. A., and Holtzman, N. A.: Kinky hair syndrome: Response to copper therapy. Pediatrics, *52:* 653 (1973).
2. Cartwright, G. E.: Diagnostic Laboratory Hematology, Ed. 4 (Grune & Stratton, New York, 1968).
3. Cartwright, G. E., and Wintrobe, M. M.: Copper metabolism in normal subjects. Amer. J. Clin. Nutr., *14:* 224 (1964).
4. Danks, D. M., Campbell, P. E., Stevens, B. J., Mayne, V., Cartwright, E.: Menkes' kinky hair syndrome: An inherited defect in copper absorption with widespread effects. Pediatrics, *50:* 188 (1972).
5. Danks, D. M., Stevens, B. J., Campbell, P. E., Gillespie, J. M., Walker-Smith, J., Blomfield, J., and Turner, B.: Menkes' kinky hair syndrome. Lancet, *1:* 1100 (1972).
6. Dixon, J. W., and Sarkar, B.: Isolation, amino acid sequence and copper (II)-binding properties of peptide (1-24) of dog serum albumin. J. Biol. Chem., *249:* 5872 (1974).
7. Garnica, A. D., Frios, J. L., Easley, J. F., and Rennert, O. M.: Menkes' net., *10:* 149 (1974).
8. Grover, W. D., and Scrutton, M. C.: Copper infusion therapy in trichopoliodystrophy. J. Pediat., *86:* 216 (1975).
9. Gubler, C. J., Lahey, M. E., Ashenbrucker, H., Cartwright, G. E., and Wintrobe, M. M.: Studies in copper metabolism. I. A method of the determination of copper in whole blood, red blood cells, and plasma. J. Biol. Chem., *196:* 209 (1952).
10. McCord, J. M., and Fridovich, I.: The reduction of cytochrome *c* by milk xanthine oxidase. J. Biol. Chem., *243:* 5753 (1968).
11. Menkes, J. H., Alter, M., Steigleder, G. K., Weakley, D. R., and Sung, J. H.: A sex-linked recessive disorder with retardation of growth, peculiar hair, and focal cerebral and cerebellar degeneration. Pediatrics, *29:* 764 (1962).
12. Morrison, D. B. and Nash, T. P., Jr.: The copper content of infant livers. J. Biol. Chem., *88:* 479 (1930).
13. Ravin, H. A.: An improved colorimetric enzymatic assay of ceruloplasmin. J. Lab. Clin. Med., *58:* 161 (1961).
14. Rydén, L., and Björk, I.: A reinvestigation of some physico-chemical and chemical properties of human ceruloplasmin (ferroxidase). Biochemistry, *15:* 3411 (1976).
15. Underwood, E. J.: Trace Elements in Human and Animal Nutrition, Ed. 3, p. 57 (Academic Press, New York, 1971).
16. Wilson, J. F. and Lahey, M. E.: Failure to induce dietary deficiency of copper in premature infants. Pediatrics, *25:* 40 (1960).

17. We wish to thank Dr. Garth Meyers who referred the patient and Dr. Eleanor Sutherland of the Wyoming State Training School, Lander, Wyoming, who has so kindly followed the patient in her institution and has provided us with additional study samples. We also acknowledge the technical assistance of Miss Jacqueline Thomas, Mrs. Alice Tustison, and Mr. Bruce Geller and the nursing care provided by the staff of the Clinical Research Center.
18. Dr. Frens is the recipient of National Institutes of Health Fellowship 5F11-NS2579.
19. These studies were supported by the Eleanor Roosevelt Cancer Fund of the United States Department of Health, Education and Welfare, Washington, D.C., and by Clinical Research Center Grant RR-00064, Training Grant 5T1-NS-5503, and Research Grants 5T1-NS-5309 and M-04495 of the National Institutes of Health, Bethesda, Maryland.

20. Requests for reprints should be addressed to: D. M. Williams, M. D., Department of Medicine, University of Utah, 50 North Medical Drive, Salt Lake City, Utah 84132 (USA).

21. Received for publication July 25, 1976.
22. Accepted for publication November 18, 1976.

Printed in U.S.A.