Treatment of Wilson and Menkes Diseases

Bibudhendra Sarkar

Department of Structural Biology and Biochemistry, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada, and Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada

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I. Introduction

Copper is an essential trace element which forms an integral component of many enzymes.[1] While trace amounts of copper are needed to sustain life, excess copper is extremely toxic. Although various aspects of copper transport and metabolism have been investigated in the past,[2−6] very little is known about the specifics of intracellular copper transport. The cloning of the genes responsible for the two major genetic disorders of copper metabolism in humans, Menkes[7−9] and Wilson[10−13] diseases, is a major breakthrough in our understanding of intracellular copper transport. Both genes have been predicted to encode putative copper-transporting P-type ATPase similar to other cation-transporting P-type ATPases. Many laboratories have been involved in studying the intricate and specific mechanisms by which copper is made available to perform its essential function in cells and whereby the accumulation of excessive

* Correspondence should be addressed to Dr. Bibudhendra Sarkar, Head, Department of Structural Biology and Biochemistry, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario M5G 1X8, Canada. Phone: (416) 813-5921. Fax: (416) 813-5379. E-mail: bsarkar@sickkids.on.ca.

Bibudhendra Sarkar was born in India and had his early education in India. In 1959 he came to study in the United States. He graduated with his Ph.D. degree in Biochemistry in 1964 from the University of Southern California in Los Angeles. In the same year, soon after his graduation, he was recruited as Senior Staff in the Research Institute of The Hospital for Sick Children in Toronto, Canada, with a cross-appointment at the University of Toronto as Assistant Professor. In 1965 he became the first recipient of MRC Scholar Award at The Hospital for Sick Children. He quickly rose to the ranks of Associate Professor and Full Professor in the University of Toronto. He served as Visiting Scholar at Cambridge University in 1977. He also served as Visiting Professor at The University of Paris in 1976 and 1984. He was appointed as Head of the Department of Biochemistry Research at the Hospital in 1990. As of January 1, 1998, he has been Head of the Department of Structural Biology and Biochemistry in The Research Institute. Dr. Sarkar is an international authority on metal-caused diseases. He developed the copper–histidine treatment for Menkes disease, a devastating neurodegenerative disease in children caused by a genetic defect of copper transport which causes children to die before the age of three. The oldest patient treated by copper–histidine is now a 22-year old man. Dr. Sarkar received the Nuffield Foundation Award of England for his work in Cambridge. In 1996 he was named Distinguished Visiting Professor at the University of Manitoba, Winnipeg. Dr. Sarkar has been a member of many National and International Advisory Panels and Agencies including the National Institutes of Health, USA, and United Nations Programs. He was a Councilor of the Chemical Institute of Canada (1975–1978) and a Member of the Advisory Committee of its Scientific Affairs and became the Chairman of the Biological Chemistry Division of the Institute. He was elected a Fellow of the Institute (FCIC) in 1986. He is on the Editorial Board of several journals. Dr. Sarkar has organized 20 International Symposia. Annualy he delivers about 10–12 invited lectures internationally. He has edited four books and published over 200 scientific articles. He leads an international laboratory where over 60 postdoctoral fellows and graduate students from all over the world were trained by him.

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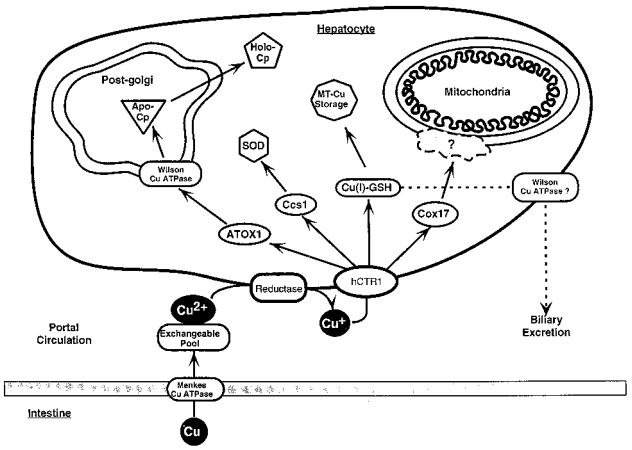

Figure 1. Normal copper-transport pathways.

amounts of copper becomes toxic. There are excellent reviews written in recent years which address various aspects of copper metabolism.[1−6] An attempt is made in this review to present the current understanding of the normal transport of copper in relation to the absorption, intracellular transport, toxicity, deficiency, and treatments of two copper-related inherited diseases: Wilson and Menkes diseases.

II. Normal Copper Transport

Copper is absorbed from the gastrointestinal tract and enters the exchangeable pool (Figure 1). This pool consists of copper bound to albumin and low molecular weight amino acid complexes in the Cu(II) form.[14−16] During uptake, copper is reduced to Cu(I) by a hypothetical membrane reductase and is absorbed by the cell via a transporter, hCTR.[17] Upon entering the cytoplasm, copper may be complexed to a variety of ligands. However, it is thought that the majority of cytoplasmic copper is complexed to glutathione (GSH) as Cu(I).[18] The Cu(I)−GSH complex can then donate copper to various intracellular proteins such as metallothionein.[19] There are copper chaperone proteins ATOX1, Cox17, and Ccs1 which have been suggested to deliver copper to copper-transporting ATPases,[20] cytochrome oxidase[21] and superoxide dismutase,[22] respectively. Menkes copper−ATPase is situated in the intestinal mucosal cell membrane, and Menkes gene mutation disrupts the intestinal absorption of copper. The Wilson copper−ATPase, which is positioned in the trans-golgi network, is responsible for providing copper to proteins such as ceruloplasmin (Cp). This ATPase may also be involved in moving copper across the canilicular membrane and into the bile. Copper is distributed in all cellular organelles including the nucleus, mitochondria, lysosomes, endoplasmic reticulum, and cytosol.[23] Copper proteins are located in all cellular compartments: superoxide dismutase in the cytosol and possibly in peroxisomes,[24] cytochrome oxidase in the mitochondria,[25] lysyl oxidase in the golgi and secretory organelles,[26] and metallothionein in the cytosol, nucleus, and lysosome.[27,28] Thus, intracellular copper trafficking is vital to the delivery of copper to copper-containing proteins and enzymes (Table 1).

III. Wilson Disease

Wilson disease is an autosomal recessive disorder of copper transport involving accumulation of copper in the liver and brain of affected individuals.[29] Patients with Wilson disease can be broadly divided into three groups: those displaying hepatic symptoms, those displaying neurologic symptoms, and those displaying both hepatic and neurologic symptoms. Wilson disease displays extensive clinical heterogeneity, with symptoms that are largely nonspecific, making the diagnosis of the disease difficult for all except the most experienced physicians.

The age of onset for Wilson disease extends from the age of 5 or 6 years to the mid-50s. Copper accumulates in the cytosol of hepatocytes, leading to hepatic necrosis and the release of large amounts of copper into the blood stream, causing damage to erythrocyte membranes, leading to hemolytic anemia.[30,31] Copper finally accumulates in other organs such as the brain, kidneys, and cornea. Defective biliary excretion of copper may be the single most important cause of copper accumulation in Wilson disease.[32]

The Wilson disease gene was initially mapped to the q14.3 region of chromosome 13. Using this information as a road map, three groups independently isolated the gene responsible for Wilson disease.[10−13] The Wilson disease gene (ATP7B) has been shown to span at least 80 kb of genomic DNA and is composed of 21 exons. Sequence analysis of the cDNA indicates that it encodes a 1411 amino acid P-type ATPase involved in the transport of copper (Figure 2). Northern blot analysis has revealed that the Wilson disease gene is expressed predominantly in the liver, kidney, and placenta.[10,11] The finding complements the biochemical and clinical evidence which indicates the involvement of these organs in the disease process. Recently, the N-terminal domain of the copper-transporting ATPase has been expressed and purified, which shows the protein to bind six copper atoms in Cu(I) state.[33−35]

IV. Menkes Disease

Menkes disease is a fatal X-linked disorder characterized by a widespread defect in intracellular copper transport.[36] Progressive neurodegeneration associated with psychomotoric retardation and connective tissue abnormalities are the main clinical features. Children usually die before the age of three. The basic biochemical defect is impairment of the intestinal absorption of copper.[37,38] The lack of available copper in affected individuals leads to decreased levels of developmentally important copper enzymes.[1] Deficiencies in enzymes such as lysyl oxidase, tyrosinase, cytochrome oxidase, dopamine β-hydroxylase, superoxide dismutase, and amine oxidase are thought to account for the major clinical features of the disease.[6,39]

The Menkes disease gene (ATP7A) had previously been mapped to the Xq13 region of the X-chromosome. Using this information as a guide, the gene responsible for Menkes disease was isolated independently by three groups.[7−9] Analysis of the cDNA

Table 1. Copper Enzymes in Humans

oxidoreductases	reaction catalyzed
Cu, Zn superoxide dismutase (EC 1.15.1.1)	$O^{\bullet}_2^- + O^{\bullet}_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2$
lysyl oxidase (EC 1.4.3.13)	Peptidyl-L-lysyl-Peptide + $H_2O + O_2 \rightarrow$ Peptidyl-allysyl-Peptide + $NH_3 + H_2O_2$
amine oxidase (EC 1.4.3.6)	$RCH_2NH_2 + H_2O \rightarrow RCHO + NH_3 + H_2O_2$
cytochrome *c* oxidase (EC 1.9.3.1)	4 Ferrocytochrome $c + O_2 \rightarrow$ 4 Ferricytochrome c + 2 H_2O
ceruloplasmin (EC 1.16.3.1)	4 Fe(II) + 4 $H^+ + O_2 \rightarrow$ 4 Fe(III) + 2 H_2O

monooxygenases	reaction catalyzed
tyrosinase (EC 1.14.18.1)	L-tyrosine + L-Dopa + $O_2 \rightarrow$ L-Dopa + dopaquinone + H_2O
dopamine β-hydroxylase (EC 1.14.17.1)	dopamine + ascorbate + $O_2 \rightarrow$ noradrenaline + dehydroascorbate + H_2O
peptidylglycine monooxygenase (EC 1.14.17.3)	peptidylglycine + ascorbate + $O_2 \rightarrow$ peptidyl(2-hydroxyglycine) + dehydroascorbate + H_2O

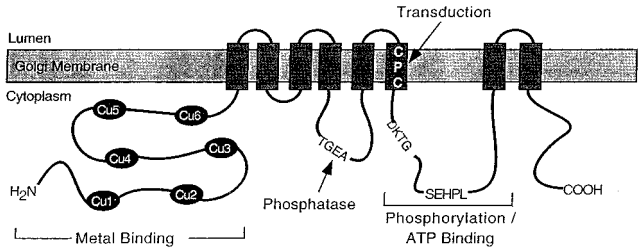

Figure 2. Predicted structure of the Wilson/Menkes disease copper-transporting P-type ATPase. The predicted structure contains domains which are common to other cation transporting P-type ATPases. In addition, the N-terminus contains six repeats of a 30 amino acid putative copper binding motif. The gray transmembrane segment contains a conserved Cys−Pro−Cys motif which is present in the transduction domain of other bacterial heavy metal ATPases.

sequence revealed that it codes for a 1500 amino acid protein, predicted to be a P-type ATPase responsible for the translocation of copper across membranes. The predicted protein contains domains which are homologous to those found in other cation transporting ATPases. In addition, the N-terminus contains six metal-binding motifs similar to that in Wilson disease ATPase (Figure 2). Northern blot analysis has indicated that the Menkes disease gene is expressed in all tissues except the liver.[7−9] The pattern of expression is consistent with clinical and biochemical features of the Menkes phenotype.

V. Treatment of Wilson Disease

The damage to vital organs (liver, brain, kidney) in Wilson disease is caused by the toxic action exerted by excessive amounts of copper deposited primarily in these organs. Treatment methods which can result in the production of a negative overall copper balance will lead to clinical improvement in symptomatic patients and most importantly will also prevent the development of signs and symptoms of the disease in those patients who are fortunate enough to be diagnosed in the presymptomatic phase of the disease. There are multiple pharmacologic regimens and therapeutic modalities available for the treatment of Wilson disease (Table 2). These are described below.

a. BAL (2,3-Dimercaptopropanol)

It was originally used for the purpose of treatment of Wilson disease as a decoppering agent.[40,41] Since this agent must be administered by intramuscular injection, its use was all but abandoned in favor of orally available chelating agents.

b. D-Pencillamine

D-Pencillamine was first introduced in the treatment of Wilson disease by Walsh.[42] It can be administered orally in capsules, at the usual recommended dose of 1 g/day. D-Pencillamine acts by reductive chelation.[43] It reduces copper bound to proteins, which causes the reduction of affinity of the protein for copper and allows D-pencillamine to bind the copper. For this reason, D-pencillamine is so much more effective as a decoppering agent. The immediate and most dramatic effect of the administration of D-pencillamine is a marked increase in urinary copper excretion. In patients with Wilson disease, in response to D-pencillamine, urinary copper excretion at the start of the treatment is usually more than 1000 μg/24 h and after as high as 4000−5000 μg/24 h. With continuation of the treatment, the 24 h urinary excretion gradually decreases so that after 3−4 months of treatment, the excretion is often 1000 μg/day. At this point, the patient has reached the maintenance phase of therapy. Removal of copper from the liver is incomplete, and hepatic copper levels may remain elevated even after years of therapy.[44] A major problem with D-pencillamine is its high level of toxicity. Approximately 30% of patients are hypersensitive to the drug, which may mandate its discontinuation.[45] Almost one-half of the patients who have the neurologic aspect of the disease may become significantly worse after they are started on a regimen of D-pencillamine.[46] Most of the long-term side effects involve either the immune system or connective tissues. D-Pencillamine may have deleterious effects on connective tissues as a direct reaction with D-pencillamine itself. It could also result from an interaction with the enzyme lysyl oxidase, which requires copper and is responsible for the cross-linking of collagen. Many of the side effects of

Table 2. Agents for the Treatment of Wilson Disease

agent	mechanism of action	daily adult dosage
D-penicillamine[a]	reduction and chelation of copper; urinary excretion of copper by mobilizing copper from organs	1−2 g orally in divided doses
triethylenetetramine (Trien)	copper chelator and urinary excretion	0.75−1.5 g orally in divided doses
zinc salts	inhibits intestinal absorption of copper by induction of intestinal cell metallothionein; may also induce hepatic metallothionein	150−200 mg orally in divided doses
british anti-Lewisite (BAL)	copper chelator	3 mL of 10% BAL in peanut oil im
tetrathiomolybdate[b]	blocking the intestinal absorption of copper and a copper chelator	Up to 2 mg/kg orally in divided doses

[a] Administered with supplementation of 25 mg of pyridoxine orally daily. [b] Experimental.

D-pencillamine occur late in the course of the disease treatment.

c. Trien

Trien (triethylenetetramine) was used by Walsh[47] to treat Wilson disease as an alternative to D-pencillamine. It is a chelator for copper and enhances urinary excretion of copper. There is less experience with trien than D-pencillamine, and its toxicity is relatively unexplored. Initial treatment in newly diagnosed patients will result in a large cupriuresis but the rate of cupriuresis, diminishes more rapidly than with D-pencillamine. Trien appears to compete effectively for copper bound to serum proteins but is unable to do so in the liver.[48]

d. Zinc

As early as 1946, zinc has been shown to produce copper deficiency in experimental animals.[49] The first report of zinc treatment of Wilson disease was published in 1979.[50] Extensive trials began using zinc as a therapeutic agent for the treatment of Wilson disease since 1983.[51] The mechanism of action of zinc involves the induction of metallothionein in the intestinal cells,[52] which binds copper with a high affinity and holds it until the intestinal cells are sloughed off. Thus, zinc inhibits absorption of copper from the intestine and increases the fecal excretion of copper. Zinc not only inhibits the absorption of copper from food, it also blocks the reabsorption of endogenously secreted copper from saliva and gastric juice. A major advantage of zinc treatment is its very low toxicity.

e. Tetrathiomolybdate

Molybdate is known to induce copper deficiency in ruminants[53] but not in monogastric animals. The difference is that in ruminants, molybdates are converted to the tetrathio derivative by bacterial action in the rumen, which does not occur in nonruminants. It was found that the administration of molybdenum compounds, particularly with added sulfate, impaired copper metabolism in ruminants.[54] Tetrathiomolybdate has been used to treat patients who were intolerant to D-penicillamine, trien, and zinc sulfate.[55] These patients were in better health upon treatment than at anytime since their disease was diagnosed. Tetrathiomolybdate seems to act both by blocking the intestinal absorption of copper and keeping the absorbed metal in a metabolically inert chelated form which is not taken up by the liver. It induces a very modest cupriuresis.[56] However, in view of the known toxic effects of tetrathiomolybdate on the skeletal system of growing animals,[57] one should be careful in administering this compound. In cases where patients have been intolerant of more conventional therapy, it may have a place in the management of Wilson disease. However, it should be considered as an experimental drug.

VI. Treatment of Menkes Disease

Menkes disease is a fatal genetic disorder with a widespread defect in intracellular copper transport. There is clinical heterogeneity in Menkes disease, and even though there are mild forms such as the occipital horn syndrome, most patients (90−95%) have severe classic form and usually die by 3 years of age.[58] Parenteral therapy with various copper salts, commencing either before or after neurologic impairment, has never been successful in resisting the progressive neurodegeneration caused by the disease.[59] The identification of copper−histidine in normal human serum and the knowledge gained from the studies of its chemistry and physiological significance led to the treatment of Menkes disease by copper−histidine as described below.

a. Isolation of Copper–Histidine Complex from Human Serum and Speciation Analysis

Most of the copper in normal human serum is bound to ceruloplasmin. Copper in this form is not exchangeable. The exchangeable form of copper is bound to albumin and amino acids. Copper−histidine complex was identified as the main copper−amino acid complex in human serum (Figure 3).[14] Subsequently, it was shown that human albumin forms a ternary complex with copper−histidine such as albumin−copper−histidine.[15,16] It was postulated that the ternary complex may play an important role in the regulation and control of copper transport across the cell membrane.[16,60] Detailed studies were carried out in this laboratory with the speciation analyses, stability constant measurements, and structural studies of copper−histidine, albumin−copper, and albumin−copper−histidine complexes.

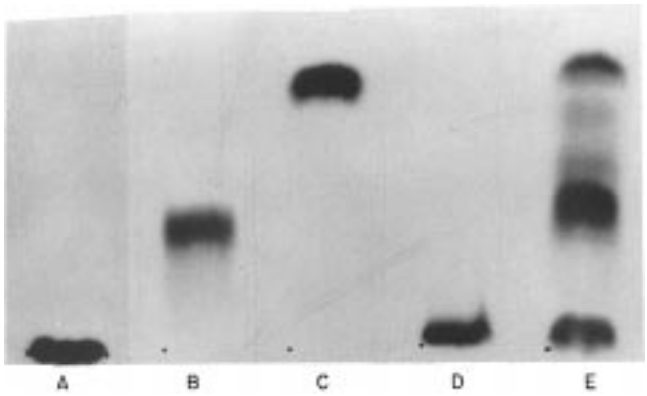

Figure 3. Identification of copper−histidine complex in human serum by thin-layer chromatography. Autoradiographic picture of thin-layer chromatograms (A) $^{64}Cu(OH)_2$; (B) ^{64}Cu−histidine; (C) ^{64}Cu−threonine; (D) ^{64}Cu + ultrafiltrate from dialyzed human serum; (E) ^{64}Cu + desalted serum ultrafiltrate. Analysis of the intense band in E having a similar R_f value as that of control ^{64}Cu−histidine in B showed a Cu:histidine molar ratio of 1:2. (Reprinted with permission from ref 14. Copyright 1966 Academic Press.)

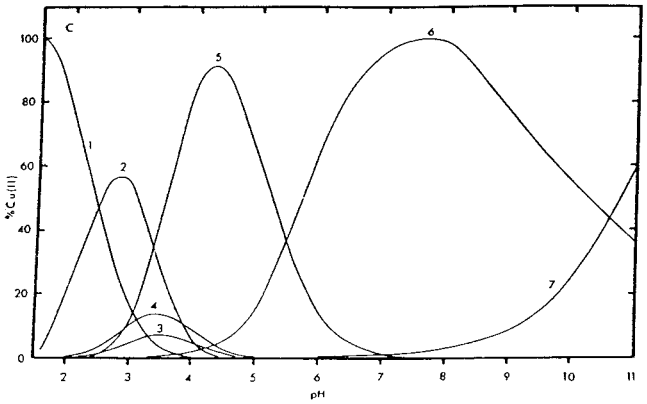

Figure 4. Species distribution in copper−histidine system as a function of pH: (1) unbound copper; (2) MHA; (3) MA; (4) MH_2A_2; (5) MHA_2; (6) MA_2; (7) $MH_{-1}A_2$. (Reprinted with permission from ref 61. Copyright 1973.)

The species detected in the copper−histidine system were MHA, MA, MH_2A_2, MHA_2, MA_2, $MH_{-1}A_2$, $MH_{-1}A$, and $M_2H_{-2}A_2$.[61] The species distribution is shown in Figure 4. At physiological pH, copper−histidine has one major species MA_2 with the highest stability constant ($\log \beta_{pqr} = 18.453$) compared to any other copper−amino acid complexes. The structure of the MA_2 complex in solution is either pentacoordinated or hexacoordinated or a mixture of both forms, as shown in Figure 5.[62]

b. Kinetics of Copper Transfer between Human Albumin and Histidine

Elucidation of the structure of the copper-transport site of human albumin[63] enabled a complete kinetic analysis of the copper-transfer reaction between human albumin and histidine. Studies utilizing the native albumin−copper transport site showed the importance of the formation of the ternary complex: albumin−copper−histidine for the copper-transfer reaction. The combined results of the equilibrium and kinetic analyses demonstrated the mechanism and rate controlling role played by the ternary complex.[64,65] The ternary complex formed by protonation or deprotonation is a kinetically and thermodynamically important intermediate in the copper-transfer reaction between albumin and histidine. A reaction scheme is derived in Figure 6 taking into account the kinetic effect of pH, histidine, and copper−histidine and the distribution of chemical species in the ternary system.

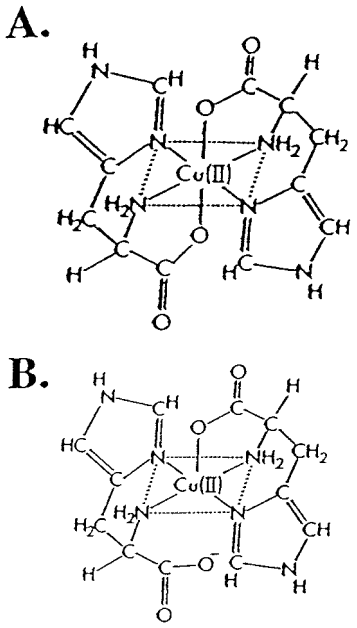

Figure 5. Proposed structures of MA_2 species in copper−histidine system: (A) pentacoordinated structure; (B) hexacoordinated structure. It is possible that both structures are present in solution in an equilibrium mixture. (Reprinted with permission from ref 62. Copyright 1973.)

c. Physiological Significance of Copper–Histidine in the Transport of Copper and the Basis for Its Use in the Treatment of Menkes Disease

Studies described above indicated that albumin has a high binding affinity for copper and acts as a buffer for copper while histidine competes with albumin for copper through the formation of a ternary complex. Experiments with hepatic tissues demonstrated that histidine enhances the uptake of copper in hepatic cells.[66] Similar results were obtained for the uptake of copper by placental cells using copper−histidine complex.[67] On the contrary, albumin was shown to inhibit the uptake of copper into the cell. Thus, it became clear that copper−histidine played a pivotal role in the transportation of copper into the cell. All these results were a key component and crucial to the development of copper−histidine as a therapeutic agent for the treatment of Menkes disease.[68−71] At present, this is the only treatment available for Menkes disease.

Two important factors are considered when using copper−histidine to treat Menkes patients. First, the dosage of copper−histidine to be administered should allow maintaining normal levels of copper and ceruloplasmin in serum. Second, copper−histidine has to be administered subcutaneously in order to bypass the intestine because the intestinal absorption of copper is defective in Menkes disease.

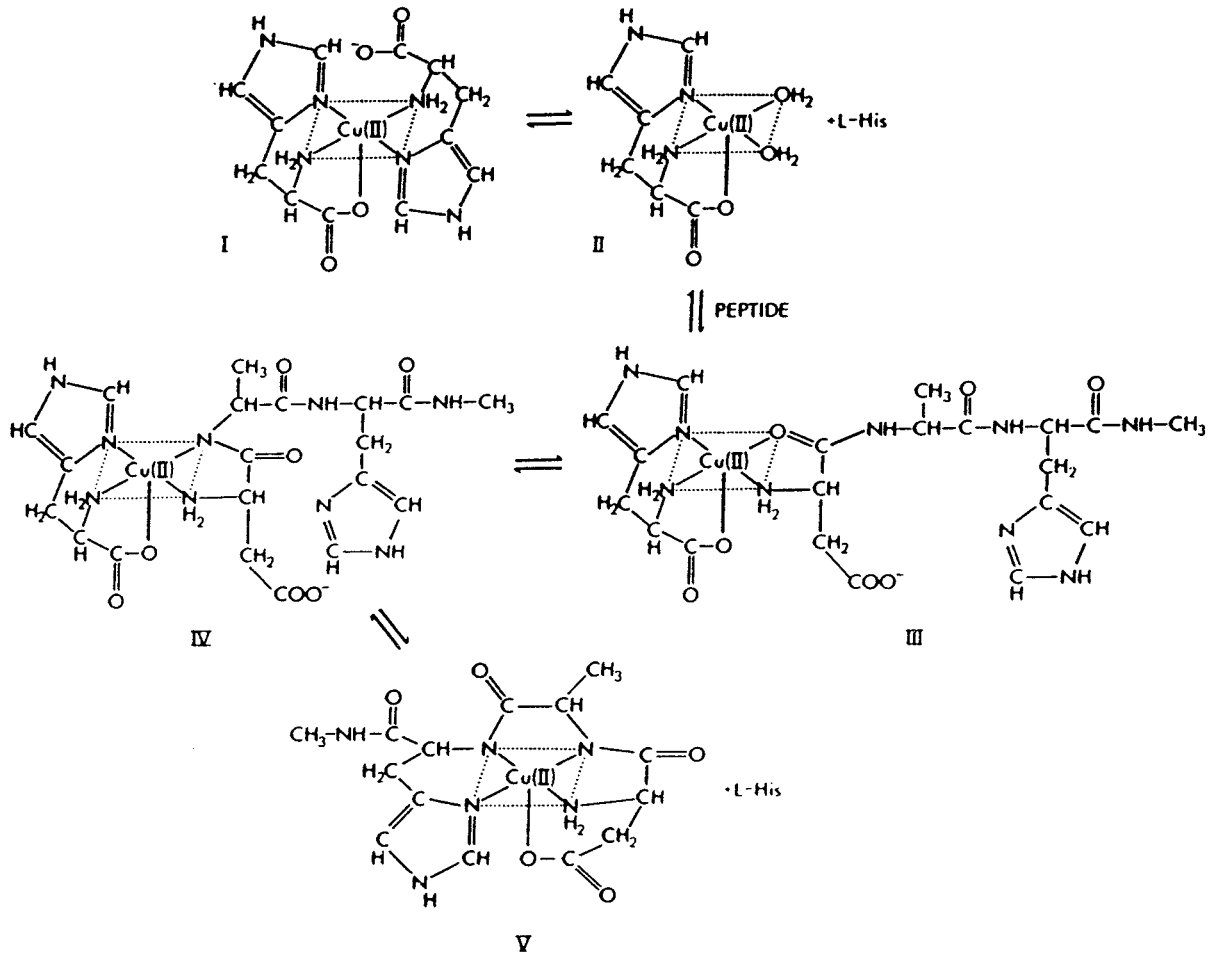

Figure 6. Proposed reaction mechanism for the copper transfer involving copper, native albumin-copper transport site (A), and histidine (B): (I) CuB_2; (II) CuB; (III) CuAB; (IV) CuH_{-1} AB; (V) $CuH_{-2}A$. (Reprinted with permission from ref 65. Copyright 1985.)

d. Copper–Histidine Formulation for the Treatment of Menkes Disease

Copper−histidine solution is prepared using full aseptic technique in a laminar air flow hood. The light in the laminar air flow hood must be turned off during copper−histidine preparation as the compound is light-sensitive. Copper chloride and histidine are dissolved in 0.9% sodium chloride for injection. The product is sensitive to oxygen, and vigorous stirring should be avoided. The solution is adjusted to pH 7.4, and final adjustment of the volume is made with sodium chloride 0.9% injection. The solution is drawn up into a sterile disposable plastic syringe and the desired aliquot filtered through a 0.22 μm filter into sterile glass vials. Each batch is tested for sterility, copper analysis, and pyrogen. Samples are stored and refrigerated in brown UV-light resistant bags.[71] It should be pointed out that copper−histidine is a relatively unstable inorganic complex and freeze-drying of the sample is avoided. Also, addition of potentially reactive excipient or diluent should be avoided since it may interfere with the copper−histidine equilibrium, allowing very little copper to remain in its transportable form as copper−histidine.[72−74]

e. Treatment Outcomes

The following is a summary of the long-term clinical outcomes of four unrelated Menkes disease patients treated with parenteral copper−histidine preparation soon after birth (Table 3).[75]

1. Patient 1 (M.F.)

M.F. is the fourth child of a nonconsanguineous marriage.[70] One of his brothers died at 3 1/2 years with classic Menkes disease following a severe neurodegenerative course. M.F. was born by spontaneous delivery at 37 weeks gestation. He was unusual looking, puckered lips and sparse hair. Initially he was treated with daily intravenous infusions of copper chloride at a dose of 150 μg/kg/day. From 3 months of age, treatment was changed to daily subcutaneous injections of copper−histidine with a starting dose of 600 μg/day. There was a good biochemical response to this treatment as judged by weekly plasma total copper and ceruloplasmin quantitation. He sat unaided at 10 months, walked at 2 years and was riding a tricycle by 3 1/2 years. He uttered his first intelligible words at 2 years of age. During the first few years of life, fine motor and cognitive abilities were better than gross motor and verbal skills. He has never had seizures. Formal assessment at 9 1/2 years of age showed a full-scale

Table 3. Summary of Clinical and Biochemical Features in the Menkes Disease Patients Treated with Copper−Histidine (Reprinted with Permission from Ref 75)[a]

	1 (MF)	2 (TL)	3 (RH)	4 (GV)
affected relative	brother	maternal uncle	none	maternal half-brother
Gestation at delivery	37 weeks	40 weeks	35 weeks	35 weeks (induced)
pretreatment features	facies, hypothermia	none	hypothermia, failure to thrive, bone changes, vascular abnormalities, abnormal hair	facies
		Treatment		
copper started (form)	4 weeks (iv $CuCl_2$ daily)	7 weeks (iv Cu acetate, then im Cu EDTA and D-penicillamine).	4 weeks (Cu−his)	2 days (Cu−his)
Cu−his started	3 months	18 months	4 weeks	2 days
plasma copper levels on treatment (μmol/L)	4−10.5	10−20	10−20	10−25
liver copper	NE	Decreased	NE	Normal
		Outcome		
present age (years)	20	17	died 10.25	10
school performance	age-appropriate	4 years behind	age-appropriate	1 year behind
neurologic deficits	nil	ataxia, apraxia (cerebellar vermis hypoplasia)	Nil	Nil
occipital horns	+	−	−*	+
other skeletal abnormalities	++	nil	+	++
bladder diverticulae	−	−	++	+
abnormal hair	+	initially present, now normal	no	++
postural hypotension	+++	+	+	++
chronic diarrhea	++	−	++	++

[a] Abbreviations: NE, not estimated; Cu−his, copper−histidine; EDTA, ethylenediaminetetraacetate. Asterisk (*) indicates palpable exostoses in the occipital region.

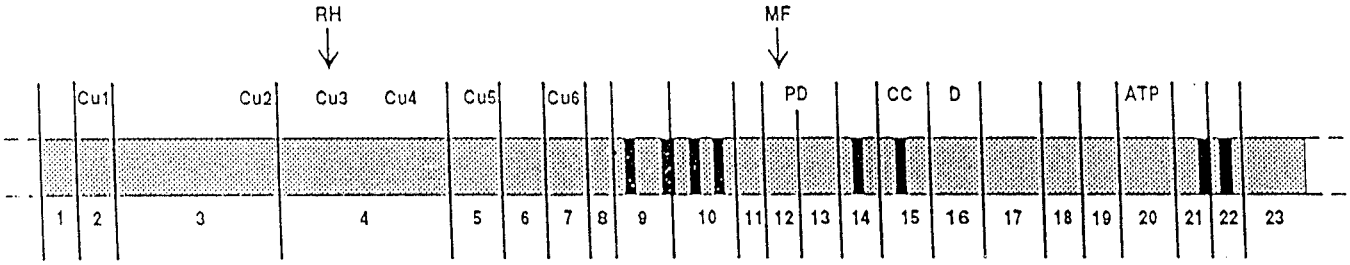

Figure 7. Localization of the mutations in the Menkes gene of the copper−histidine-treated Menkes disease patients (MF and RH) and the corresponding protein domains. Vertical lines indicate the position of the introns. The exons are numbered. The predicted copper-binding domains are indicated with Cu 1−6 and the transmembrane domains with a vertical bar: PD, phosphatase domain; CC, cation channel; D, phosphyrylation domain; ATP, ATP-bindign domain. (Reprinted with permission from ref 58. Copyright 1996.)

IQ of 87 (Wechsler Intelligence Scale for children−Revised). However, a progressive deterioration in exercise tolerance has been noted. He felt most comfortable in a squatting position. By 14 years, he could only walk short distances with a very stooped posture, spending most of his time in a wheel chair. Physical examination at 14 1/2 years revealed an elongated face with a paucity of facial movements and generalized hypotonia with a stooped posture. He had dental malocclusion and cutis laxa. He had a pale complexion with dry skin. At 20 years, he has been functioning well, apart from avoiding sporting activities.

The underlying genetic defect in this patient has been identified.[58] Using a combination of single-strand conformation analysis and direct sequencing of amplified exons, a single base pair deletion in exon 12 was detected. The mutation leads to a frame shift and creates a premature termination codon within the same exon. Further analysis suggested that the mutations do not lead to exon skipping and should result in a severely truncated protein. The mutation in this patient resides in exon 12 at codon 836, producing a termination codon upstream of the phosphatase domain (Figure 7). This protein product is predicted to lack the ATPase "core" except for the first four transmembrane domains.

2. Patient 2 (T.L.)

This patient had a maternal uncle who died at age 8 years 9 months of Menkes disease with severe, progressive mental retardation, typical facial appearances, and hair changes associated with low levels of liver copper, high levels of copper in intestinal mucus, and typical abnormalities of copper transport in cultured skin fibroblasts.[76,77] The diagnosis was made at 6 weeks of age when his plasma copper and ceruloplasmin levels were shown not to be rising in the manner expected in normal babies during the first 6 weeks of life. Copper treatment was started at 7 weeks with copper acetate followed by copper−EDTA and then copper−histidine. On this treatment, the plasma copper increased to above 10 μmol/L and was maintained in the range of 10−12 μmol/L thereafter.[78] Clinically, there was no neurological abnormalities at the time of the treatment. He began taking steps on his own by 18 months. However, when he began walking a few months later, he showed marked ataxia and was only able to walk unaided at 5 1/2 years of age. He is now 17 years

old. His height, weight, and head circumference are close to the 50th percentile. He can walk short distances without a stick, but he prefers to use a special bicycle. There has been no chronic diarrhea, urinary tract infections, or ultrasound evidence of renal tract abnormalities.

The underlying genetic defect in this patient is a missense mutation (Pro 852 Leu) in exon 12 coding for the phosphatase domain of ATP7A. This nucleotide change replaces the structurally important nonpolar proline residue, which is conserved in several copper-transporting ATPases, with the nonpolar but structurally different leucine residue. It is thus conceivable that this amino acid substitution affects the function of the protein, resulting in the expression of Menkes disease (Personal communication, Z. Tümer).

3. Patient 3 (R.H.)

This patient was born at 35 weeks gestation to healthy nonconsanguineous parents. Immediately after birth, he exhibited marked hypotonia, temperature lability with a tendency to hypothermia, feeding difficulty, and transient hypoglycemia. On the basis of his marked hypothermia, failure to thrive, temperature instability, skeletal abnormalities, tortuous arteries seen on angiography, and abnormal hair, a diagnosis of Menkes disease was considered and subsequently confirmed by the demonstration of abnormally low plasma copper and ceruloplasmin levels.[70] At 1 month of age he was started on treatment with subcutaneous injections of copper−histidine, 500 μg daily. After 1 month of treatment, the plasma copper and the ceruloplasmin levels became within the normal range. An EEG performed at 11 months of age was normal. Developmentally he did very well. He experienced moderate gross motor delay and some mild delay in verbal and fine motor/adaptive spheres; however, his socialization has been age appropriate. At the age of 10 years, he was in school in an age-appropriate grade. At this age, he enjoyed excellent general health and was active, sociable, and intellectually normal. He had dry skin, being particularly thickened and course on the extensor aspects of his knees and elbows. He also had mild cutis laxa. He had no seizure or episodes of syncope. He died suddenly of peritonitis at 10 years and 3 months.

The genetic defect in this patient has been characterized using a combination of single-strand conformation analysis and direct sequencing of amplified exons.[58] A single base pair deletion was detected in exon 4, leading to a frame shift and creating a premature termination codon within the same exon. Results suggested that the mutation in this patient does not lead to exon skipping and predicted to result in a severely truncated protein. The mutation lies in exon 4 at codon 297, introducing a termination codon within the third metal-binding domain (Figure 7). The resulting polypeptide would lack the entire ATPase "core".

4. Patient 4 (G.V.)

This patient was diagnosed as suffering from Menkes disease prenatally at 21 weeks of gestation by copper studies performed on cultured aminocytes samples at 16 weeks.[75] A brother died at the age of 14 months because of classic Menkes disease. The parents decided to continue the pregnancy despite the diagnosis. Labor was induced medically at 35 weeks with the intention of starting treatment with copper−histidine immediately after birth. A liver biopsy was performed in day 2 of life to provide a baseline for treatment and to confirm the diagnosis. Treatment was initiated on day 3 with 100 μg of elemental copper daily in the form of copper−histidine. The only significant health problem during infancy was a tendency to develop diarrhea with every intercurrent infection. He walked unaided at 16 months and began to talk at 2 years. Problems with motor coordination have been less significant since 3 years of age. At 7 years of age, he was much more energetic and physically active and no longer suffered from episodes of fainting. His chronic diarrhea was resolved. He now walks and runs. At 10 years of age, his postural hypotension and diarrhea is still well controlled by L-DOPS and his activity and school performance have improved greatly.

The genetic defect in this patient has recently been characterized. The patient has an insertion of a single nucleotide in exon 21 which leads to misplicing and predicted premature termination of the protein in transmembrane 7. Western blot analysis of fibroblast extracts shows the presence of a reduced amount (about 25%) of protein compared to normal. No data are available regarding activity of the predicted truncated protein (Personal communication, L.Ambrosini and J. Mercer).

Patients discussed above are the oldest patients described in the literature who were treated with parenteral copper−histidine preparation. One of the copper−histidine-treated patients is shown in Figure 8. They all have a severe classic form of the disease, three of whom are still living and are well at the ages of 20, 17, and 10 years. Patients 1, 2, and 4 had relatives severely affected with Menkes disease. Patient 3, although there was no family history, the classic form of the disease was suggested by characterizing the underlining genetic defect. The outcome of the patients differed markedly from those in their respective affected relatives who were not treated by copper−histidine. Other studies have also shown that the copper−histidine treatment normalized the plasma copper, ceruloplasmin, and CSF copper within 6 weeks with marked reduction of epileptic discharges, improved muscular tone, and increased motor activity.[79] After 3 months of treatment in these studies, the excessive CSF dopamine was normalized. Similar clinical benefits have not been observed in patients when copper−histidine treatment begun after the onset of severe neurologic symptoms. The improved outcomes, especially their relatively good neurologic outcome, can be attributed to early commencement of parenteral copper−histidine therapy. It should be pointed out, however, that the residual abnormalities can be substantial and some of the connective tissue abnormalities may become more severe later in life. It is important that more Menkes patients are assessed for the efficacy of early copper−histidine

Figure 8. A 9-year old Menkes patient treated with copper−histidine. (Reprinted with permission from ref 58. Copyright 1996.)

treatment and a complete documentation be made in the literature because it is a severe and a fatal disease in children in their early childhood.

VII. Conclusion

The isolation of the genes for both Menkes and Wilson diseases will allow the studies of intracellular transport and homeostatic control of copper. These studies will be critical in determining how the transport of copper is interrupted by mutations in the ATPase gene. Studies of kinetics and mechanism of copper transport through these ATPases will be key to the development of new therapeutic strategies. Among all the available therapeutic agents to treat Wilson disease, zinc appears to be the least toxic; however, most data accumulated so far are on maintenance treatment. It would be important to collect data on zinc when it is used as an initial treatment. While the available therapeutic modalities for Wilson disease continue to be used, newer therapeutic approaches should be actively sought. The copper−histidine therapy in Menkes disease appears to be effective in preventing the severe neurodegenerative problems when treatment is initiated very early in life. The mechanism of the efficacy of copper−histidine treatment in Menkes disease is not clear. Copper−histidine may bypass the defective efflux mechanism and avoid trapping in the cell. Alternatively, it may overcome cellular defect in the incorporation of copper into copper enzymes. Despite significant improvements in the early copper−histidine treatment of Menkes disease patients, connective tissue disorders continue to persist, indicating that lysyl oxidase levels are not restored by copper−histidine injection. It is possible that copper presented in this form is unavailable for incorporation into lysyl oxidase. The oxidation state of copper may affect its mobility into certain intracellular compartments. Further research in this area should explore the treatment with both Cu(I) and Cu(II) which may be more effective than either alone.

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