

PREFACE

Copper (Cu), an essential trace element, is required for the survival of organisms ranging from bacteria to mammals. Because Cu ions can adopt distinct redox states (oxidized Cu[II] or reduced Cu[I]), they play a pivotal role in cell physiology as catalytic cofactors in the redox chemistry of enzymes involved in a broad spectrum of biological activities. For example, copper is an important cofactor in mitochondrial respiration, iron absorption, collagen and elastin crosslinking, and free radical scavenging.

Copper balance studies in volunteer human subjects have indicated a minimum requirement of 1.5–2.0 mg per diem. Therefore, the RDA (Recommended Dietary Allowances) has been set at 2–3 mg per diem. In the United States, the average daily intake of Cu is approx 1 mg and diet is the primary source. The bioavailability of Cu from the diet is about 65–70% depending on a variety of factors including its chemical form and interaction with other metals and dietary components. Although ingested Cu is readily absorbed, little excess is stored. Therefore, it is both noteworthy and puzzling that symptoms of Cu deficiency have not been identified in the general population. However, the biological half-life of dietary Cu is 13–33 d with biliary excretion being the major route of elimination. In healthy persons, serum Cu concentrations range up to approx 1.5 mg/L. Gastrointestinal symptoms occur at whole blood concentrations near 3.0 mg Cu/L.

It is well known that Cu plays a fundamental role in the biochemistry of the human nervous system. The dramatic neurodegenerative phenotypes of Menkes and Wilson diseases underscore the essential nature of this metal in nervous system development and the consequences of perturbation of neuronal Cu homeostasis. In addition, inherited loss of ceruloplasmin, an essential ferroxidase contains 95% of the Cu found in human plasma, is associated with progressive neurodegeneration of the retina and basal ganglia. Recent studies also have implicated Cu in the pathogenesis of neuronal injury in Alzheimer's disease and the prion-mediated encephalopathies, suggesting that further elucidation of the mechanisms of Cu trafficking and metabolism within the nervous system will be of direct relevance to understanding the pathophysiology and treatment of some neurodegenerative diseases.

Free radical damage has been implicated in several pathological conditions of the central nervous system (CNS) and multiple lines of evidence implicate redox-active transition metals as the mediators of the oxidative stress associated with these disorders. Free radicals produce tissue damage through a variety of mechanisms, including excitotoxicity, metabolic dysfunction, and disturbance of intracellular calcium homeostasis. Considerable research data implicate oxidative stress in ischemia/reperfusion injury and chronic neurodegenerative disorders such as familial amyotrophic lateral sclerosis (ALS) and Parkinson's disease. Gain-of-function missense mutations in the cytosolic Cu/Zn enzyme, superoxide dismutase, are associated with the motor neuron degeneration of ALS and current evidence suggests a direct pathogenic role for Cu in this process. It would appear then that therapeutic approaches focused on limiting oxidative stress may be useful in ameliorating such conditions.

The precise distribution of Cu in the cell occurs through diverse pathways. For example, the delivery of Cu to Cu/Zn superoxide dismutase (SOD1) is mediated through a soluble factor identified as *Saccharomyces cerevisiae* LYS7 and human CCS (Cu chaperone

for SOD). This factor is specific for SOD1 and does not deliver Cu to proteins in the mitochondria, nucleus, or secretory pathway. Yeast cells containing a *lys7*Delta null mutation have normal levels of SOD1 protein but fail to incorporate Cu into this enzyme which is, therefore, devoid of superoxide scavenging activity. *LYS7* and *CCS* specifically restore the biosynthesis of holoSOD1 in vivo. Elucidation of the *CCS* Cu delivery pathway may aid development of novel therapeutic approaches to human diseases that involve SOD1 such as ALS.

Recently, components of the Cu homeostasis system of humans have been characterized at the molecular level. These include Cu-transporting P-type ATPases, Menkes and Wilson proteins, and Cu chaperones. The findings have contributed to a better understanding of the physiology of both Cu deficiency and toxicity. For example, because Cu is highly toxic, cellular uptake and intracellular distribution must be precisely orchestrated processes. Thus, Cu homeostasis is maintained by the coordinated activity of a number of proteins that results in its delivery to specific subcellular compartments and, subsequently, Cu-requiring proteins without release of free Cu ions that could damage cellular components. Genetic studies in prokaryotic organisms and yeast have identified membrane-associated proteins that mediate the uptake or export of Cu from cells. Within cells, small cytosolic proteins, the Cu chaperones, bind Cu ions and deliver them to specific compartments and Cu-requiring proteins. The identification of mammalian homologs of these proteins supports a structural and functional conservation of Cu utilization across the evolutionary spectrum from bacteria to yeast to mammals. Furthermore, studies of the function and localization of the products of the Menkes and Wilson's disease genes, which are defective in patients afflicted with these diseases, have provided valuable insight into the mechanisms of Cu balance and their role in maintaining appropriate Cu distribution in mammalian cells and tissues.

In Wilson's disease, a Cu toxicosis condition, and Menkes disease (including mild Menkes disease and occipital horn syndrome [OHS]), a Cu deficiency disorder, Cu homeostasis is perturbed by genetic mutation. Wilson's disease is an autosomal recessive inherited disorder of Cu metabolism resulting in pathological accumulation of Cu in many tissues and organs. The Menkes disease complex of related disorders of Cu transport are responsible for abnormal neurodevelopment and connective tissue pathology that can precipitate premature death. In addition, excessive intake of Cu can result in early childhood cirrhosis (ECC, Indian Childhood Cirrhosis [ICC] or, when found outside India, Idiopathic Cu Toxicosis [ICT]).

Menkes disease is a recessive, X-linked neurodegenerative disease that occurs in approx 1 in 200,000 live births. The affected males manifest a systemic Cu deficiency due to malabsorption caused by a defect in the Menkes (*ATP7A*) gene, designated MNK, which encodes a transmembrane Cu-transporting P-type ATPase that functions to export dietary Cu from the gastrointestinal tract. Based on homology to known P-type ATPases, the MNK gene product is highly evolutionarily conserved. Copper export from the gastrointestinal tract is activated upon the binding of Cu(I) to the six metal-binding repeats in the amino-terminal domain of the Menkes protein. Each of the Menkes protein amino-terminal repeats contains a conserved -X-Met-X-Cys-X-X-Cys- motif (where X is any amino acid). Such metal-binding repeats are conserved in other cation-exporting ATPases involved in metal metabolism and in proteins, such as metallothionein, involved in cellular defense against heavy metals intoxication. Owing to reduction/loss of Menkes protein activity and dietary intake, Cu accumulates in the cytoplasm of cells of the intestine bound to metallothionein resulting, ultimately in the Cu deficiency syndrome pathogno-

monic of Menkes disease. In addition to neurological perturbation, characteristic features of the disease include arterial degeneration and hair abnormalities that can be explained by the decrease in the activity of cuproenzymes.

Mild Menkes disease and OHS (a mild Menkes disease variant) also have been identified as genetic disorders resulting from mutations within the Menkes disease gene. Because the clinical spectrum of Menkes disease is broad, males with mental retardation and connective tissue abnormalities should be screened for biochemical evidence of defective Cu transport. The Menkes/OHS gene normally is expressed in nearly all human tissues and its Cu-transporting P-type ATPase product localizes to the trans-Golgi network. Mutations of the Menkes gene show great variety, including missense, nonsense, deletion, and insertion mutations. In over 70% of the Menkes and OHS patients studied, expression of the gene is abnormal. Major gene deletions, detectable by Southern blotting, account for 15–20% of Menkes/OHS patients. The central region of the gene appears particularly prone to mutation and mutations affecting RNA processing appear to be relatively common. Mutations in the Menkes gene in patients with mild Menkes disease or OHS indicate these diseases to be allelic variants of Menkes disease. Improved understanding of the molecular and cell biological mechanisms involved in normal Cu transport ultimately may yield new and better approaches to the management of these disorders. Of interest in this regard are mutations in the mottled gene, the murine homolog of the Menkes gene. Mutations of this gene have been demonstrated in mottled mutant mice that display biochemical and phenotypic abnormalities similar to those observed in patients with Menkes disease.

The objective in treatment of Menkes disease and OHS is to deliver Cu to the intracellular compartments where cuproenzymes are synthesized. Currently, the treatment of choice is parenteral Cu administration. Unfortunately, in patients with classical Menkes disease, treatment started after the age of 2 mo does not prevent the characteristic neurological degeneration. Even when treatment is initiated in newborns, neurological degeneration is prevented only in some cases. Moreover, early treatment cannot improve non-neurological problems such as perturbed connective tissue development.

The Wilson's disease gene encodes a Cu-transporting P-type ATPase, *ATP7B*. In humans, it is localized on chromosome 13. Approximately 100 mutations of the gene have been documented. They occur throughout the gene. The most common is the His1069Gln point mutation. Wilson's disease includes a variety of clinical conditions, the most common of which are liver disease (ranging from acute hepatitis to fulminant hepatic failure and chronic hepatitis to cirrhosis), hemolytic anemia and neuropsychiatric disturbances. The diagnosis of Wilson's disease usually is made on the basis of clinical findings (Kayser–Fleischer rings, typical neurologic symptoms) and abnormal clinical laboratory values (e.g., low serum ceruloplasmin, increased hepatic Cu content). Lifelong treatment with chelating agents (D-penicillamine, trientine) or zinc usually is sufficient to stabilize the patient and to achieve clinical remission in most.

Liver diseases of infancy and childhood generally are rare and, within the spectrum of these disorders, only a few subtypes are related to abnormal hepatic Cu accumulation. Idiopathic Cu toxicosis has been defined as such a subtype. Although this disease is characterized by distinct clinical and pathologic features, its exact etiology is controversial. It has been hypothesized that idiopathic Cu toxicosis is caused by synergistic interaction between an autosomal recessive inherited defect in Cu metabolism and excess dietary Cu. In this regard, numerous cases of infantile cirrhosis originating in several families in the

Austrian province of the Tyrol have been investigated. Although termed Endemic Tyrolean Infantile Cirrhosis (ETIC), this disorder is indistinguishable from Indian Childhood Cirrhosis (ICC) and Idiopathic Cu Toxicosis (ICT) and resembles the early form of Wilson's disease (WND). It was suggested that ETIC might be the manifestation of an allelic variant of the WND gene, which codes for the ATP7B Cu-transporting P-type ATPase. Assuming that the incidence of ETIC is the result of a founder effect, the possible role for *ATP7B* in ETIC was investigated by association studies and haplotype sharing. Because of its lethality, the mapping of ETIC had to focus on obligate gene carriers, the parents of the patients. The data obtained indicated that ETIC is a genetic entity separate and distinct from WND. Cases of Cu-associated Early Childhood Cirrhosis (ECC) have been reported from Austria, Australia, Germany, Ireland, and the United States. Cases occurring in India are designated Indian Childhood Cirrhosis (ICC) while cases occurring outside of India are designated Non-Indian Childhood Cirrhosis (NICC) or Idiopathic Cu Toxicosis (ICT). It is of interest to note that eight cases of infantile liver cirrhosis, classified as ICT, were reported in five families in Emsland, a predominantly rural area in Northern Germany. In two of these cases, although the children had been exposed to increased levels of dietary Cu, a diagnosis of ICT could not be confirmed. However, in the remaining six cases, clinical presentation and liver pathology were consistent with a diagnosis of ICT. Analysis of the pedigrees of the affected families revealed complex relationships and occasional consanguinity among the parents suggestive of an autosomal recessive mode of inheritance. Furthermore, the households were served by private wells delivering water of low pH through Cu pipes. Thus, chronic alimentary exposure to increased levels of Cu may have precipitated the condition. The findings of this investigation support the hypothesis that ICT develops in genetically predisposed infants who are exposed to increased levels of dietary Cu. It should be emphasized that, although reducing dietary Cu intake cannot prevent the development of Wilson's disease, it can alleviate the symptoms of ICT.

The gene associated with Wilson's disease (*ATP7B*) as well as the Cu-transport genes *hCTRI*, *hCTR2*, and *ATOX1* have been excluded as etiologic agents both in NICC and Cu toxicosis in Bedlington terriers (which is phenotypically similar to Wilson's disease and ICC). A genome-wide screen is being carried out to localize the NICC gene. If the NICC and Bedlington terrier Cu toxicosis genes are homologous, the canine mutation should be of great utility in defining the molecular pathology of NICC. If there is no homology, the genes will still represent an important addition to the list of genes associated with mammalian diseases of Cu metabolism.

It has been suggested that elevated Cu concentrations in wheat and maize from an area of China (Linzhou) at high risk area of esophageal cancer may be related to the etiology of this cancer. Unfortunately, there is little information on the possible association of excess dietary Cu and cancer. Indeed, there is little information on the relation (if any) of Cu status and most diseases afflicting humans in particular and animals in general. This situation is even less clear with regard to the plant kingdom and the so-called "lower organisms." Obviously, there is much work to be done. With this in mind, the *Handbook of Copper Pharmacology and Toxicology* has been developed to provide researchers and students with a view of the current status of research in selected areas of Cu pharmacology and toxicology and to stimulate research in these areas. If the *Handbook* proves useful, updated versions will be forthcoming. Therefore, we invite your comments and suggestions.

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