Manifestations of copper excess

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ABSTRACT Although copper is an essential micronutrient normally subject to effective homeostatic control, excess dietary intakes can in some circumstances be toxic. Susceptibility to copper toxicity depends, however, on many factors, including species, genetics, age, and diet. This appears to reflect not only variations in the efficiency of the absorption and excretion of copper but also differences in the intake of other hepatotoxic or protective factors, differences in the cellular distribution of copper, and differences in the expression of specific copper transport and storage proteins. Many of the toxic effects of copper, such as increased lipid peroxidation in cell membranes and DNA damage, are related to its role in the generation of oxygen free radicals.

KEY WORDS Copper, copper excess, copper toxicity, hepatotoxicity, lipid peroxidation, rats, sheep, humans, pigs, Wilson disease

INTRODUCTION

Although certain regulatory authorities have been reassessing safe amounts of copper in food and drinking water, the incidence of copper toxicosis in the general population is remarkably low. There have been occasional reports of acute copper poisoning, such as through contamination of beverages from copper-containing vessels or dispensers, with symptoms including metallic taste, nausea, diarrhea, jaundice, hemoglobinuria, hematuria, anuria, and oliguria (1). Indeed, copper has been used in suicide attempts although the lethal dose is ≈1000 times normal dietary intakes. Chronic copper toxicosis is likely to be of greater concern than the acute syndrome and is characterized by a gradual hepatic accumulation of copper that results eventually in liver damage, and in extreme cases, in death.

The low incidence of chronic copper toxicosis, despite considerable variation in copper intakes, reflects the efficiency of the homeostatic control mechanisms that operate at the levels of both intestinal absorption and biliary excretion to keep tissue copper concentrations within a narrow range. Nevertheless, copper poisoning can develop under certain conditions, depending on factors such as species, genetics, age, and diet (2). For example, sheep, which do not appear to be able to increase biliary copper excretion in response to increased intakes, are particularly susceptible to copper toxicity, whereas pigs are so tolerant of copper that they used to be given diets containing 250 mg Cu/kg as a growth stimulant. Neonatal and milk-fed animals are more susceptible to copper poisoning than their adult counterparts, probably because of the high efficiency of copper absorption and the immaturity of biliary excretory mechanisms. This may explain why reports of copper-induced cirrhosis in humans, such as Indian childhood cirrhosis, are restricted mainly to young children (3). There is also a genetic component to copper toxicosis, as exemplified by the occurrence of Wilson disease in humans and the increased susceptibility of Long Evans Cinnamon (LEC) rats to copper poisoning. This is now known to reflect the failure to express a specific copper transporter protein, particularly in the liver (4, 5). However, there are also differences between breeds or strains of animal in copper tolerance, as has been shown in sheep (6); as yet, these have not been traced to variations in the expression of particular copper-binding proteins. This raises the possibility that certain individuals may be particularly prone to copper toxicosis.

As indicated above, this variable incidence of copper toxicosis can be partly attributed to differences in the efficiency of absorption and excretion of copper but other factors are also involved. Thus, the presence of dietary antagonists of copper metabolism, such as sulfur and other trace metals and of other hepatotoxins or protective factors, is also important. For example, dietary zinc supplements can be used to prevent copper toxicosis in sheep, as well as in patients with Wilson disease, by restricting the accumulation and modifying the distribution of copper in the liver (2). Increased intakes of hepatotoxic pyrrolizidine alkaloids, which compromise liver function, decrease the tolerance for copper in sheep (7), whereas increased intakes of antioxidant vitamins like α-tocopherol protect against copper toxicity in rats (8). Other important factors include the distribution and speciation of copper in the liver, particularly with regard to lysosomal accumulation and the occurrence of metallothionein (9), and the expression of copper transporters, as in Wilson disease and LEC rats (4, 5).

CLINICAL FEATURES OF COPPER TOXICOSIS

The symptoms of chronic copper toxicosis are not constant and vary between species. In pigs, which are relatively tolerant of...
copper, excessive copper intakes eventually give rise to dullness, weakness, respiratory distress, anemia, and jaundice, with pulmonary edema and ulceration of the esophageal region of the stomach (10). Rats are also relatively tolerant of copper but at high intakes, >500 mg/kg diet, growth is impaired and extensive necrosis of hepatocytes develops. There is also widespread necrosis of proximal convoluted tubule epithelial cells of the kidney. However, rats can adapt to prolonged exposure to copper, and liver copper concentrations eventually decrease in association with liver regeneration; this is accompanied by extrusion of apoptotic bodies and persistence of Mallory-like structures (11, 12).

In contrast, copper toxicosis in sheep develops as a two-stage process, even at relatively low dietary copper intakes of ~25 mg/kg (2). In the first phase there are no overt signs of toxicity and both food intake and growth rate are generally (but not invariably) normal. Blood copper concentrations are either unchanged or only slightly elevated but there are elevated concentrations of liver-specific enzymes in plasma. This is a reflection of the gradual but substantial accumulation of copper in the liver, with concentrations increasing to ~1000 μg/g dry matter. This leads to necrosis of isolated parenchymal cells and of swollen copper-containing Kupffer cells, rich in acid phosphatase (13, 14).

The second phase of the disease occurs very suddenly and is commonly referred to as a hemolytic crisis. The main clinical features include jaundice, anorexia, excessive thirst, and hemoglobinuria (13, 14). Dramatic reductions in blood hemoglobin and glutathione concentrations occur within a few days, with a transient increase in blood methemoglobin concentrations. Animals usually die within a few days although some can survive. The onset of these symptoms is associated with liberation of stored copper from the liver and a massive increase in blood copper concentrations. The activities of the liver-specific enzymes in the plasma increase further, consistent with extensive liver degeneration and the occurrence of focal necrosis, inflammatory cells, bile plugs, and large periodic acid–Schiff (PAS)-positive Kupffer cells in liver samples. There is also considerable kidney damage, with necrosis and loss of mitochondrial enzyme activity from the proximal convoluted tubules. Kidney copper concentrations increase dramatically at this time, probably as a result of enhanced tubular reabsorption of circulating copper.

Ultrastructural examination of the livers of copper-poisoned animals during the prehemolytic phase of the disease revealed extensive hypertrophy of the smooth endoplasmic reticulum and a gradual increase in the occurrence of autophagic vacuoles in parenchymal cells (15). The vacuoles originally contained nondegraded cellular components but, as they matured and lysosomal degradation progressed, their contents acquired the characteristics of electron-dense residual bodies containing granular material, lipid droplets, and copper. There was also an increased incidence of cell deletion and of the formation of apoptotic bodies as liver copper concentrations increased.

HEPATIC DISTRIBUTION OF COPPER

A key feature of the development of copper toxicosis is clearly the hepatic accumulation of copper. However, the distribution of copper within the liver is also important, both in terms of its subcellular distribution and its speciation. For example, the progression of Wilson disease is associated with changes in the distribution of copper, from a cytosolic to a lysosomal preponderance, and with reduced severity of the hepatotoxic lesions. That hepatic accumulation of copper per se is not always a cause of liver damage is evident from the absence of liver problems in newborns, even at very high liver copper concentrations. The distribution of copper in the liver and its association with specific binding proteins depends on factors such as species, age, genetics, and duration of exposure to copper (9).

Under normal circumstances, much of the copper in the liver occurs in the cytosol but as copper accumulates an increasing proportion occurs in particulate fractions, principally the nucleus and lysosomes. The lysosomal accumulation is probably linked to copper-induced autophagy and has generally been assumed to be part of a detoxification process and a prelude to biliary excretion of the metal (although there are also nonlysosomal pathways for biliary copper excretion). Lysosomal copper accumulation has been reported in neonates (16), Bedlington terriers (17), patients with Wilson disease (18), and copper-loaded rats (19). However, because of the difficulties in histochemical staining for copper and of separating copper-loaded lysosomes from nuclear fractions on differential centrifugation of liver homogenates, there is some doubt as to the reliability of some claims of lysosomal localization. For example, use of digitonin to lyse lysosomal membranes before density-gradient centrifugation showed that the copper in fetal deer liver was associated not with lysosomes but with the nuclear fractions (20). However, X-ray microanalysis of the livers of copper-loaded rats confirmed the presence of copper in both lysosomes and nuclei (19). Despite increased numbers and diversity of lysosomes, there was no evidence of lysosomal rupture or disintegration, contrary to other claims that copper damaged lysosomal membranes through lipid peroxidation. However, accumulation of copper in nuclei does destabilize DNA (21) and inhibit RNA polymerase activity in isolated nuclei (22). The cytotoxic effects of copper have therefore been attributed to nuclear disorganization, which leads to disposal of necrotic debris in the form of apoptotic bodies and their subsequent phagocytosis.

SPECIATION OF COPPER IN THE LIVER

Relatively little is known of the proteins involved in the hepatic accumulation of copper. Metallothionein plays an important role and its synthesis in liver can be induced by copper by a process involving increased gene transcription (9). Preinduction of metallothionein by zinc reduces the toxicity of copper in primary hepatocyte cultures (23) and hepatocytes from metallothionein-null mice are more sensitive to copper than hepatocytes from normal mice (JH Beattie, I Bremner, unpublished observations, 1996). Copper-resistant hepatoma cell lines are characterized by their high levels of metallothionein expression, which result from increases in both transcription rates and gene copy number (24). Much of the copper in these copper-resistant cells is associated with metallothionein. However, this is not always the case with the liver because the contribution of metallothionein to the binding of copper can vary from <5% to >80%. Thus, in copper-loaded pigs (25) and in Bedlington terriers (17), most of the hepatic copper is bound to metallothionein in both the cytosol and in particulate fractions, including lysosomes. However, if the pigs are zinc-deficient, very little of the copper is bound to metallothionein. This dependence on zinc status also applies in sheep and calves, because the amount of copper bound to metallothionein is a function of both liver zinc content and the copper-zinc ratio in the liver; as a rule only a small proportion of liver copper is bound to metallothionein (9). The situation in the
The generation of hydroxyl radicals has been confirmed by analysis of the products of DNA damage, by study of aromatic hydroxylation reactions, and by electron-spin resonance spectroscopy using specific spin traps. The latter technique has been used to detect hydroxyl radical formation in vivo in rats dosed with copper and ascorbic acid; this involved reaction of the hydroxyl radical with dimethyl sulfoxide to generate a methyl radical that was then detected as a spin trap adduct in bile (29).

Not unexpectedly, a common consequence of copper-induced production of reactive oxygen species is increased lipid peroxidation. This has been manifested as increased production of pentane and hepatic malondialdehyde and of thiobarbituric acid–reacting substances (TBARS) when liver homogenates or hepatocytes are reacted with ionic copper. Moreover, dietary copper overload in rats resulted in in vivo peroxidation of mitochondrial membrane lipids, as shown by increased concentrations of conjugated dienes and TBARS (8). This resulted in dilated, cystic cristae of inner mitochondrial membranes in the liver and other biochemical and histologic signs of hepatocyte injury. These effects were even more evident in copper-loaded rats that were deficient in vitamin E, supporting the view that copper was inducing oxidant injury to the hepatocytes. This is consistent with the detection of a radical adduct in bile of copper-loaded rats deficient in vitamin E and selenium (30). No such radical could be detected if rats were given supplements of either vitamin E or selenium. These results provide convincing evidence for the involvement of in vivo lipid peroxidation in the toxicity of copper.

Because no comparable effects occurred in microsomes, the mitochondria may be a specific initial target of copper-induced liver damage. This is consistent with subsequent findings that mitochondrial function is also impaired in copper-loaded rat livers, with decreases in state 3 respiration and the respiratory control ratio in the mitochondria when several electron donors were used (31). Analysis of the oxidoreductase activities of the mitochondrial electron transport protein complexes revealed a reduction in complex IV (cytochrome c oxidase) activity. Such changes in mitochondrial function could contribute to hepatocellular dysfunction by reducing cellular energy charge, increasing mitochondrial leakage of calcium into cytosol, or exposing the cell to increased amounts of superoxide generated by the disruption of normal electron flow.

Copper-catalyzed lipid peroxidation also appears to underlie the alterations in hepatocyte lysosomes in copper-loaded rats (32). Concentrations of TBARS in the isolated lysosomal membranes of these rats were doubled, with an increase in their fragility and decrease in their fluidity. There were also changes in the membrane content of selected fatty acids, with an increase in polyunsaturated fatty acids. As lysosomal pH also increased, these membrane alterations may have affected the function of the proton ATPase pump.

The occurrence of apoptotic bodies in the livers of copper-loaded animals is indicative of copper-induced damage to DNA (15). Even though copper catalyzes the production of hydroxyl radicals in vivo, it does not follow that they would necessarily damage DNA because they react indiscriminately at diffusion-controlled rates with many biomolecules and are likely to be scavenged by other cell constituents before they reach the DNA. However, copper binds readily to DNA to form adducts and is indeed involved in chromatin condensation (33). It is possible therefore that endogenous DNA-associated copper could promote local production of hydroxyl radicals and hence oxidative damage to DNA. The fact that copper accumulates within the nucleus in copper overload obviously enhances the likelihood of such reactions occurring.

The exposure of DNA to hydrogen peroxide in the presence of Cu(II) and ascorbic acid results in oxidative damage to the DNA in the form of strand breaks and base oxidation. It might be expected that glutathione, as another reducing agent that occurs within nuclei at relatively high concentrations, would also stimulate such changes but in fact was found to inhibit free radical formation (34). This was attributed to its stabilization of copper in the Cu(I) state and prevention of its ability to participate in free radical generation. Glutathione may therefore protect against copper-induced DNA damage. Similar protection against copper-induced fragmentation of DNA was afforded by zinc-metallothionein, apparently because it also sequestered the copper and prevented its participation in redox reactions (35). However, glutathione stimulated free radical formation and DNA damage in the presence of copper and 1,10-phenanthroline because it was then unable to remove Cu(I) from the 1,10-phenanthroline but could participate in redox-cycling of the reactive 1,10-phenanthroline–copper complex (34).

The DNA damage that occurs during oxidative stress could conceivably result from direct attack by the reactive oxygen species or from endonuclease activity (apoptosis). Incubation of HepG2 cells with the 1,10-phenanthroline–copper complex caused internucleosomal DNA fragmentation, which is a hallmark of apoptosis (36). However, because fragmentation did not
occur at low temperature and activity was restored by addition of ascorbic acid, the DNA fragmentation may indeed have resulted from direct attack of hydroxyl radicals on DNA. The hydroxyl radicals are produced from oxygen by the redox cycling of the phenanthroline-copper complex, which is supported by metabolic processes at normal temperatures. At low temperatures, ascorbic acid produces an artificial reducing environment, thereby restoring hydroxyl radical formation. Internucleosomal cleavage of DNA was also detected when isolated rat liver nuclei were incubated with 1,10-phenanthroline and was further enhanced in the presence of ascorbate and hydrogen peroxide (37). The DNA contained 8-hydroxydeoxyguanosine, indicative of oxidation by the hydroxyl radical. These results are consistent with phenanthroline being bound to copper, which exists normally in chromosomes, forming a complex that promotes hydroxyl radical–dependent DNA fragmentation.

Although these observations were based on studies in in vitro systems, increased concentrations of 8-hydroxydeoxyguanosine have been recorded in the liver and kidneys of rats continuously exposed to copper through implantation of osmotic minipumps (38). This supports the view that copper can induce oxidative damage to bases in DNA.

REFERENCES


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