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Mitochondrial involvement in genetically determined transition metal toxicity II. Copper toxicity

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Abstract

Copper, like iron, is an essential transition metal ion in which its redox reactivity, whilst essential for the activity of mitochondrial enzymes, can also be a source of harmful reactive oxygen species if not chelated to biomolecules. Therefore, both metals are sequestered by protein chaperones and moved across membranes by protein transporters with the excess held in storage proteins for future use. In the case of copper, the storage proteins in the mitochondria are a distinct ceruloplasmin and metallothionein (MT). If the cell accumulates too much copper or copper is needed by other cells, then copper can be chaperoned to the trans-Golgi secretory compartment where it is transported into the Golgi by ATP-dependent pumps ATP7A/B. In liver, the copper is then incorporated into ceruloplasmin in vesicles that travel to the plasma membrane and release ceruloplasmin into the plasma. This paper reviews the genetic basis for diseases associated with copper deficit or excess, particularly those attributed to defective ATP7A/B transporters, with special emphasis on pathologies related to a loss of mitochondrial function.

Keywords: Mitochondria; Copper; Cytotoxicity; Hepatotoxicity

1. Introduction

Copper is an essential element whose redox properties and coordination chemistry are useful for a number of catalytic and transport functions in living cells and their organelles, particularly the mitochondria [1]. Copper is essential for mitochondrial function, e.g., it is a prosthetic group for mitochondrial cytochrome oxidase. Copper is also a prosthetic group for other enzymes such as superoxide dismutase (SOD1), tyrosinase, lysyl oxidase, and dopamine-β-hydroxylase, and is also required by ceruloplasmin to function in iron utilization [2]. Liver copper content and erythrocyte SOD1 were decreased whereas the copper chaperone CCS was increased in rats fed a copper deficient diet [3]. This copper deficiency caused mitochondrial lipid peroxidation to double [4,5]. The increased CCS protein by copper deficiency was later shown to result from an inhibition of CCS degradation by the 26S proteosome and mRNA was unaffected [3]. Copper may therefore regulate CCS activity by this process. Heart mitochondrial cytochrome oxidase was also inhibited in rats fed a copper deficient diet [6] and was associated with cardiomyopathy [7]. Furthermore copper deficiency in cultured neuronal cells impaired

Abbreviations: AGE, advanced glycation end products; COX, cytochrome *c* oxidase; LEC, Long-Evans cinnamon rats; MT, metallothionein; ROS, reactive oxygen species; SOD, superoxide dismutase

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cellular function and induced apoptotic death through a mitochondrial signalling pathway [8]. Patients with copper deficiency as a result of Menkes disease developed neurodegenerative disorders [8]. Mild copper deficiency also results in anemia, neutropenia, osteoporosis, decreased glucose tolerance, hypercholesterolemia, arthritis, myocardial disease, cardiac arrhythmias, and neurological problems [8,9]. Copper deficiency occurs in malnourished children, patients receiving long-term total parenteral nutrition and patients receiving iron or copper chelating drugs.

However, excess copper in cells also causes toxicity as the redox properties essential for its function in cuproenzymes can also result in marked reactive oxygen species (ROS) formation that can damage lipids, nucleic acids, and proteins [10,11]. Thus, intracellular protein copper chaperones and cellular transporter mechanisms have evolved to allow the organism and cells to handle essential copper safely by escorting copper ion directly from the diet to the cuproenzymes so as to keep cells as free of unchelated copper as possible [12]. At the same time, genetic defects in these transporting mechanisms increase the risk of copper toxicity, which is manifest in many different ways. It has long been known that in disorders of copper overload, the mitochondrion is a major target for toxicity, with oxidative mitochondrial membrane damage and poisoning of enzymes of the tricarboxylic acid cycle and energy metabolism recognized as potential targets [13,14]. However, whether these effects are primary effects on mitochondria, or secondary to more general aspects of tissue damage has not always been clear. In the following paragraphs, we review the evidence for mitochondrial copper accumulation and direct toxicity, and then describe some genetic disorders that lead to mitochondrial copper overload or impaired mitochondrial copper homeostasis.

2. Mitochondrial copper homeostasis and accumulation

Dietary copper is absorbed in the stomach and small intestinal epithelial cells likely by a brush border membrane transporter (e.g., enterocyte CTR1 and possibly divalent metal transporter (DMT-1) [2]. The Menkes protein ATP7A is a Cu^{+} transporting P-type ATPase located in the intestinal epithelial cell that is responsible for copper uptake by the trans-Golgi network for packaging in vesicles. The vesicles are then sent to more peripheral membrane compartments including the basolateral plasma membrane [15]. To avoid copper toxicity, excess copper in the diet is trapped in the enterocyte by metallothionein (MT) and is excreted in the feces as a result of the rapid sloughing off of these cells. Metallothionein binds cuprous ions, which largely renders them redox-inactive.

The copper released from enterocytes is transported by the portal vein to the liver mostly complexed to albumin with some copper complexed to histidine. As portrayed in Fig. 1, the hepatocyte takes up portal vein copper using the hCtr1 and DMT-1 transporters [2]. The copper is then transferred to various chaperones, e.g., CCS (for Cu–Zn superoxide dismutase (SOD1)), Cox17 (for cytochrome oxidase) or HAH1/ATOX1 [16]. The function of HAH1 is to transfer Cu(I) to the ATP7A (cells other than liver) and ATP7B (liver) proteins. This activates Cu(I) translocation across the membrane and/or ATP7A/B relocalization from the trans-Golgi to the cytoplasmic membrane [17]. ATP7A and B transport the copper to ceruloplasmin, which is released into the plasma. At high liver copper levels, the ATP7B redistributes to pericanalicular vesicles that move the copper (likely chaperoned by MURR1) to the biliary epithelium for excretion into the bile. MURR1 is mutated in canine copper toxicosis and has similar phenotype to Wilson's disease in humans [12,18]. Inactivation of ATP7B in Wilson's disease prevents copper incorporation into ceruloplasmin and copper release into the bile. Low plasma ceruloplasmin can serve as a marker for Wilson's disease [19,20]. About 65% of plasma copper is bound to ceruloplasmin and about 30% is bound to the N-terminus of albumin, which is also synthesized and secreted by the liver. The remaining 5% is in low molecular weight complexes, mostly with histidine [21]. Plasma copper speciation changes in hypoalbuminemic or aceruloplasminic mice would be expected but has not been reported. However, no serious disorders of copper metabolism have been reported in aceruloplasminemia patients [20].

Diet-derived copper enters the liver in the Cu(I) form which reacts with hepatic GSH upon entering the hepatocyte to form GS-Cu(I) which enables Cu to bind to metallothionein or reconstitute apo SOD or apoceruloplasmin. GSH can also release copper from metallothionein. GSH can act as a copper donor but not as a chaperone [22]. However, SOD1 retains a certain degree of activity in cells that are null for the CCS chaperone and GSH is essential for this activity [23]. The GS-Cu(I) complex is unlikely to be excreted in the bile via cmoat/mrp2 as biliary secretion of endogenous copper was unaffected in cmoat/mrp2 deficient rats [21]. Cu excretion was also independent of bile GSH levels and the Cu(I) was excreted into the bile via ATP7B. On the other hand, an excess hepatic Cu(I) load following an iv

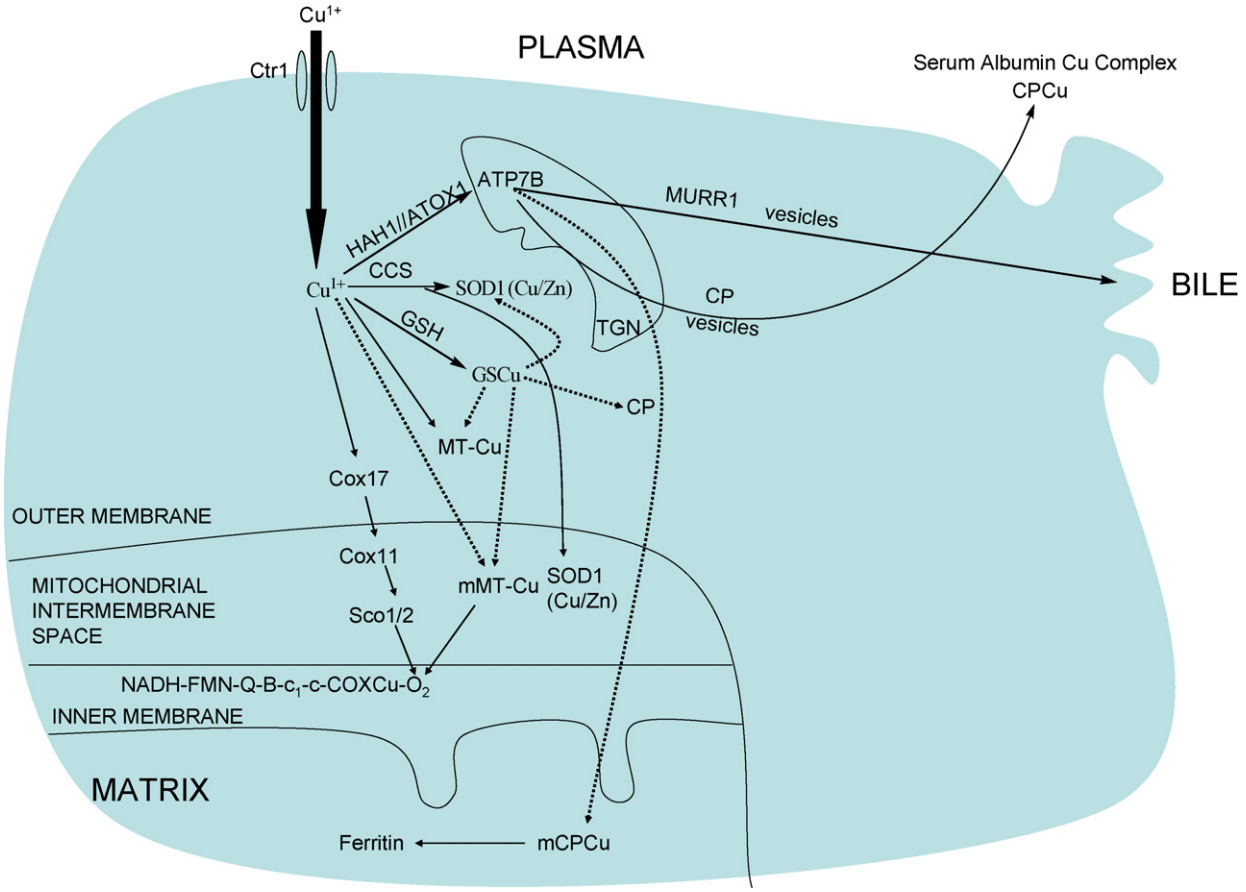

Fig. 1. Model of copper transport and chaperoning in hepatocytes. At the plasma membrane, copper is taken up by the transporter CTR1. Once in the hepatocyte, copper is distributed to individual chaperones. Chaperones HAH1/ATOX1 deliver excess copper to the trans-Golgi network (TGN) packaging and secretory compartments. In these compartments, copper is transported by ATP7B into vesicles, which is then incorporated into apoceruloplasmin. These vesicles move to the plasma membrane and the ceruloplasmin is discharged into the plasma. Alternatively, when cellular enzymes require copper, the copper is chaperoned, e.g., by CCS to superoxide dismutase (SOD1) of the cytosolic and mitochondrial intermembrane space. Chaperone Cox17 also delivers copper to the mitochondrial inner membrane cytochrome a/a_3 (COX) with the assistance of chaperones Cox11, Sco1, and Sco2. Mitochondrial (mMT) and cytosolic metallothionein (MT) store copper that is delivered via unidentified chaperones and possibly involving copper glutathione (GSH) complexes. Mitochondrial ceruloplasmin (mCP) catalyzes the oxidation of ferritin ferrous iron. — Proposed pathway; - - - speculative pathway.

administration of Cu(II) forms a GS–Cu(I) –SG complex with GSH which is excreted across the bile canalicular membrane via cmoat/mrp2 [24]. In the bile copper likely exchanges amongst a number of low molecular weight ligands such as glutathione or bilirubin in which cupric is reduced in the process [25]. Copper may also be chelated by bile salts, e.g., glycocholate > cholate [26].

Some cells including hepatocytes, retain copper more readily than other cells largely due to their ability to induce high levels of the high-affinity cytosolic copper-binding MT [27]. Plasma ceruloplasmin is synthesized by the liver and other cells (e.g., erythrocyte and cells of the heart, lung, kidney, and placenta) also have a ceruloplasmin receptor [28,28]. However, ceruloplasmin is not essential for systemic copper distribution as demonstrated by the normal copper homeostasis found in genetic aceruloplasminemia [29,20].

Copper is required for mitochondrial function as it is essential for the activity of the cytochrome *c* oxidase (COX) complex IV. COX is the terminal respiratory oxidase located in the inner mitochondrial membrane and COX and ceruloplasmin are the only known enzymes that can reduce oxygen to water with four electrons. Its action generates the proton gradient that drives ATP synthesis. Three copper ions arranged in CuA and CuB redox centres are required for its activity. There are 13 subunits in cytochrome oxidase with subunit 1 containing CuB and subunit 2 containing CuA sites, which are encoded by the mitochondrial genome. Therefore, copper ion insertion must occur within the mitochondrion.

As partly portrayed in Fig. 1, the delivery and insertion of copper ions into cytochrome *c* oxidase requires at least three proteins, Cox17, Sco1, and Cox11 [30,31]. Cox17 is a copper metallochaperone necessary for the phosphorylation and GTPase-regulated recruitment of copper ions from the intermembrane space and delivery to CuA. In Cox17, three out of six conserved cysteines are present in a CCxC sequence motif essential for binding three copper ions via thiolate ligation [16]. Cox17 likely chaperones these copper ions to the mitochondrion and transfers them to Sco1, an inner mitochondrial membrane protein required for cytochrome oxidase assembly. Cox11 seems to be responsible for copper delivery to CuB and COX assembly only, whilst Sco1 seems to act as a multifunctional linker connecting a variety of biological processes. Inherited mutations in these three proteins can cause severe pathology in human infants associated with cytochrome *c* oxidase deficiency [32]. Copper is also required for stimulating ferrochelatase required for heme synthesis, another mitochondrial role [30,33].

Most of the cellular Cu, Zn superoxide dismutase (SOD1) is located in the cytosol, but about 5% is located in the mitochondrial intermembrane space [34] and could remove superoxide radicals generated by the respiratory chain. CCS is believed to transfer copper to SOD1 in the mitochondria as shown in Fig. 1. [23] Approximately 20% of cases of familial amyotrophic lateral sclerosis have been associated with at least 100 point mutations in the SOD1 gene which, whilst not inactivating cytosolic and mitochondrial SOD1, increases their prooxidative activities such as peroxidase or superoxide reductase activities. Superoxide formation by partially reversing SOD could also result in toxic peroxynitrite formation and contribute to motor neuron and mitochondrial damage [35]. Mutant SOD1 and aberrant SOD1 macromolecular aggregates were also found to accumulate in the matrix of brain mitochondria isolated from transgenic mice. This suggests that mutant SOD1 is misfolded, prone to aggregation and could also contribute to selective neuronal degeneration [36].

Metallothionein is the major copper-binding protein in higher animal, and localizes in the cytosol and nucleus. Mitochondrial MT has also been localized in the intermembrane space of rat liver mitochondria (Fig. 1) but not in the matrix or membranes of mitochondria [37]. MTs are low M_r (ca. 6 kDa) proteins in the cytosol containing 61 amino acids of which 30% are cysteine. The high cysteine content may help to prevent oxidative stress and may chelate toxic metals or act as a store for essential metals (Zn and Cu). MTs are found in most tissues, and are generally highly inducible, especially in the liver and the kidney. They are generally more abundant in human tissue than rodent tissue [38]. MTs are induced by a number of factors, including ROS, metals, and cytokines [38], an exception being isoform MT-III, which is constitutively expressed in brain [39]. Induction of MT in cells increases their resistance to oxidative stress and metals, including metal-based anticancer drugs such as cisplatin [40]. Mitochondrial MT could assist in the mitochondrion's defense against cytosolic or respiratory chain ROS formation. This localization could also protect mitochondria from toxic metals and help in the insertion of copper into cytochrome oxidase. Whilst this has not been demonstrated yet for Cu-MT, incubating Zn_7-MT-2 with mitochondria caused the Zn_7-MT-2 to enter the mitochondrial intermembrane space and release Zn^{2+}, which then inhibited mitochondrial respiration. Zn^{2+} release may have resulted from the oxidation of MT by respiratory chain oxygen radicals and/or a more acidic pH [41,37]. Mitochondrial, but not cytosolic aconitase, was also shown to receive Zn^{2+} from MT in a cell free system, but not from $ZnCl_2$. This occurs through a direct protein–protein interaction as demonstrated by co-immunoprecipitation of MT and aconitase [42]. This suggests that MT could act as a Zn^{2+} chaperone and donor to Zn^{2+}-binding proteins. Releasing copper from MT would be even more effective in disrupting mitochondrial respiration as copper was 15-fold more effective than zinc at collapsing the hepatocyte mitochondrial membrane potential when added to hepatocytes [43].

In mammals nearly all of the copper in the blood is contained in plasma ceruloplasmin, a glycoprotein with 1040 amino acids, 6 copper ions and 1 adventitious copper ion whose role in copper transport if any is under debate [20]. Ceruloplasmin is synthesized and secreted by the liver [28]. Interestingly, a ceruloplasmin pseudogene has been identified in hepatocytes and non-hepatocyte cells. The mRNA coded by this gene is translated into a much smaller non-glycosylated ceruloplasmin polypeptide with a mass of 110 kDa that was located in the mitochondrial matrix and inner membrane of rat liver, brain, testes and mammary glands. This mitochondrial ceruloplasmin peptide (mCP) was shown to be synthesized in the cytosol and imported into the mitochondria by an energy- and cytosol factor-dependent process as shown in Fig. 1 [44]. It has been suggested that its ferroxidase activity plays a role in the synthesis of [Fe–S] clusters for the cell. The function of mitochondrial ceruloplasmin is proposed to be oxidation and detoxication of iron ions inserted into mitochondrial H-ferritin ferroxidase (Fig. 1). Mitochondrial ceruloplasmin may also bind nitric oxide and regulate NO levels in the mitochondria [44].

Table 1
Genetic disorders in copper homeostasis in humans and rodent models

Genetic disorder	Hepatic Cu deficiency	Hepatic Cu overload
(a) Human	Menkes disease	Wilson's disease
Changes in Cu levels	Decreased Cu liver Increased Cu serum, intestine/kidney	Increased Cu liver, urine Decreased Cu serum, bile
Defect	Decreased intestinal absorption	Decreased biliary excretion
Gene mutation	ATP7A transporter	ATP7B golgi network transporter
Gene location, Gender	Xq13.3, males	13q14.3, autosomal recessive
Symptoms	Kinky-hair, CNS degeneration, vascular complications	Liver cirrhosis, cornea Kayser-Fleischer rings, neurological
Subcellular	COX inhibited, ROS formation, mitochondrial toxicity	Mitochondrial lipid peroxidation
Incidence, onset/death age	1/300,000, birth, <3years	1/100,000, 8–55years
Therapy	Cu-histidine inj.	Cu-chelators oral
(b) Rat models		Long-Evans cinnamon model
Pathology target		Liver (chronic hepatitis/cancer)
Gene mutation		ATP7B
Molecular toxic mechanism		Cu-MT uptake into lysosomes causes lipid peroxidation, mitochondrial ROS toxicity
Symptoms		Hepatitis/jaundice at 3 m
Diagnosis		Low ceruloplasmin, high liver Cu
(c) Mouse models	Mottled 9H mutant model	Toxic milk mutant C57BL/6J model
Cu levels	Cu deficient embryo Cu accumulation in placenta	Born Cu deficient Increased Cu adult liver Cu-MT overload, Increased Cu kidney, brain, eye Decreased Cu in milk of homozygous dam
Pathology target	Ruptured blood vessels	Liver, neurological/psychiatric
Gene Mutation	ATP7A	ATP7B, mammary gland mislocalization
Molecular toxic mechanism	lysyl oxidase deficiency?	Cu-MT
Symptoms	Prenatal lethality	

3. Mitochondrial involvement in copper toxicity

3.1. Mitochondrial copper overload toxicity

As summarized in Table 1 various genetic disorders of copper transport in patients and rodent models can result in copper overload with liver toxicity and hepatitis. This hepatotoxicity has largely been attributed to oxidative stress caused by the Cu^{+} ion reacting with oxygen or H_2O_2 to form ROS. Copper is rapidly taken up by mitochondria by the Na^{+}/Ca^{2+} antiporter and at 5 μM stimulates respiration and collapses the membrane potential. At 10 μM, copper causes mitochondrial swelling, cytochrome inactivation, oxidative stress and Ca^{2+} efflux [45]. Mitochondrial oxidant injury, i.e., mild swelling and increased granularity with a few crystalline inclusions has been reported in the early stages of copper overload in rats. This resulted in decreased mitochondrial state 3 respiration, loss of respiratory control and decreased cytochrome oxidase activity [46]. Atomic absorption spectroscopy studies showed that rat liver Cu was bound to the protein located in the cytosol, and then the order for copper binding was mitochondria > microsomes > nuclei [47]. In an earlier study, loading rats daily with 1.2 mg copper/kg for 6 weeks increased hepatic copper 10-fold and the intracellular distribution showed mitochondrial copper (increased 2.4-fold to 60 μg/g wet weight of liver) was greater than nuclei > cytosol > microsomes. At much higher copper loading the mitochondria became saturated and nuclei became the major storage site [48]. Unfortunately lysosomes were not isolated separately and would be distributed in the mitochondrial and nuclear fractions.

Studies with copper-loaded sheep have also shown that whilst copper is mostly located in the mitochondrial fraction, it increased in the cytosol and lysosomes with copper dosing [49]. Semi-feral North Ronaldson sheep were particularly susceptible [50]. There was progression to mitochondrial ballooning, degeneration and rupture, with subsequent autophagic degradation or matrix

condensation (pyknosis). Evidence that this resulted from oxidative injury was the induction of cytosolic isocitrate dehydrogenase and thioredoxin-dependent peroxide reductase (antioxidant protein-1). Cathepsin-D precursor was up-regulated indicating increased lysosomal activity and activation of stellate cells. The latter formed modified extracellular matrix, leading to collagen fibril formation, proliferation, and fibrogenesis. Kupffer cell activation also contributed by releasing TGF-β1 [51].

The high hepatic copper level in Wilson disease is associated with frequent, diverse and early deletions of mitochondrial DNA, and therefore this can also be considered to be a mitochondrial disease involving premature oxidative aging of mitochondrial DNA [52]. Impaired cytochrome oxidase activity and inhibition of the tricarboxylic acid cycle as a result of aconitase inactivation was found in patients with Wilson disease, and was attributed to mitochondrial DNA mutations [53]. A similar mitochondrial oxidant injury was also reported in Long-Evans cinnamon (LEC) rats in which hepatic copper levels for 23 rats reached a mean of 930 μg/g dry weight [54]. In Wilson disease and LEC rats this oxidant injury progressed to mitochondrial condensation.

3.2. Mitochondrial copper imbalance in neurodegenerative diseases

Copper is essential for brain metabolism as a cofactor for cytochrome oxidase, dopamine-β-hydroxylase, amyloid precursor protein, and ceruloplasmin. A maternal copper deficiency during pregnancy can also result in copper deficiency and neurodegenerative disorders in the offspring [55]. Copper deficiency caused brain mitochondrial dysfunction due to a decreased cytochrome oxidase activity resulting in the triggering of apoptosis by reactive oxygen species [8]. A genetic defect in ATP7A located in the placenta as well as the blood brain barrier caused a profound copper deficiency in the fetuses and newborn infants resulting in profound neurodegeneration.

On the other hand, the genetic defect in ATP7B in Wilson disease leads to a failure of copper excretion in the bile giving rise to liver copper overload. Such an overload can also present itself as a psychiatric or neurodegenerative disease. Amyotrophic lateral sclerosis and Alzheimer, Parkinson, and prion diseases are associated with increased brain protein copper complexes and mitochondrial dysfunction [8]. Copper neurotoxicity has also been attributed to mitochondrial toxicity as copper added to neuroblastoma cells caused marked mitochondrial ROS formation, and inhibited pyruvate dehydrogenase in the tricarboxylic acid cycle and respiratory complex I [13]. Copper added to neuronal/glial cultures also collapsed the mitochondrial membrane potential and inhibited mitochondrial pyruvate and α-ketoglutarate dehydrogenases, which was attributed to ROS formation. Furthermore, the mitochondrial dehydrogenase cofactors lipoic acid and thiamine prevented copper cytotoxicity and dehydrogenase inhibition [14]. Thiamine in the drinking water also markedly extended the life span of LEC rats from 6 months to greater than 16 months [56].

3.3. Copper contribution to chronic diseases

Decreasing dietary copper is not only beneficial for the treatment of Wilson disease but may also be beneficial for patients suffering from angiogenesis, fibrosis, and inflammatory diseases [57]. Surprisingly, copper chelators have also been beneficial for treating rheumatoid arthritis, diabetic neuropathy, and diabetic heart disease, and have proven effective in animal models of cancer, retinopathy, fibrosis, and inflammation [57]. Tissue copper levels have also been shown to be increased in disease states other than Wilson disease, e.g., conditions associated with oxidative stress, inflammation and infection [58]. Copper is known to catalyse oxidative glycation and protein advanced glycation end product (AGE) formation, e.g., tendon collagen. AGEs accumulate in diabetics and are generally believed to contribute to the pathogenesis of the disease [59]. Plasma "exchangeable copper" and tendon-bound copper were increased 1.7-fold and 2.54-fold in diabetic rats; this was attributed to copper chelation by AGE or other glycation products to form glycochelates [59]. Copper-dependent proteins may also contribute to fibrosis or the inflammatory process.

4. Genetic disorders of mitochondrial and cellular copper transport

Excess hepatic intracellular copper is removed by human atox-1 homologue (HAH1) which traffics copper from the cytosol to the secretory compartment of the cell (the trans-Golgi network membrane) where it is incorporated into ceruloplasmin (Fig. 1). The Wilson disease 160 kDa P-type ATPase (ATP7B transporter) has 64% homology with ATP7A and is located on the hepatocyte Golgi network membrane where it pumps copper into the lumen for insertion into apoceruloplasmin. Ceruloplasmin is then secreted via vesicles, which travel to the plasma membrane and are released into the plasma.

On the other hand, excess hepatic copper is pumped by ATP7B into vesicles, which fuse with the membrane at the biliary canaliculus for release into the bile. Excess copper is likely transported out of human liver mitochondria by a unique 140 kD transporter that may be a protease product of the ATP7B transporter [60], as shown in Fig. 1.

As outlined in Table 1, when ATP7A is non-functional as a result of the Menkes mutation (Menkes disease) pathological copper deficiency ensues. Brain copper deficiency can also result from an inhibition of ATP7A in the blood brain barrier [61]. Because of the different tissue location of ATP7A and ATP7B, the intestinal ATP7A mutation in Menkes disease results in systemic copper deficiency and enterocyte copper overload. On the other hand, the liver ATP7B mutation in Wilson disease results in systemic copper overload. The largest increases in tissue copper occurs in the liver (25-fold) and brain (15-fold), which reached 341–430 and 74 μg/g dry weight, respectively, with urinary copper excretion reaching 1307 mg/day [62,63]. The in vivo liver mitochondrial copper concentration of LEC rats, an animal model of Wilson disease, was found on average to be about 930 μg/g dry weight [54].

Apoceruloplasmin patients have hepatic iron overload, increased plasma and brain lipid peroxidation and defective electron transfer in mitochondrial complexes I and IV [29].

5. Conclusions

There is now compelling evidence that both iron and copper, when present in excess, accumulate in mitochondria at levels significantly greater than in the cytosol. This evidence is perhaps strongest for hepatocytes, but the phenomenon seems to be general in mammalian cells. Accumulation of copper in mitochondria is a consequence of the special requirements of the organelle to utilize copper for the synthesis of enzymes such as copper cytochrome oxidase, and to regulate copper homeostasis at the level of biliary excretion. These requirements in turn dictate that specific proteins transport or chaperone copper following its cellular uptake and incorporation into cuproenzymes. These proteins include the CCS chaperone protein for copper superoxide dismutase and Cox17 chaperone protein for the mitochondrial respiratory chain copper cytochrome oxidase via the Cox17 chaperone. Excess intracellular copper is exported by the trans-Golgi network following delivery by chaperone Hah1/Atox 1. However, glutathione also plays a role in copper storage by metallothionein or copper release from metallothionein. Glutathione conjugate can also restore the activity of apoSOD1 or apoceruloplasmin. Our knowledge of these mechanisms is still incomplete. What we do know with more certainty is that the mitochondrial accumulation ofcopper has significant toxic consequences, both for the cell as a whole and for the mitochondrion itself. Whether targeting mitochondrial copper overload would be a useful strategy for preventing mitochondrial ageing diseases, e.g., neurodegenerative diseases remains to be seen.

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