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Is copper a new target to counteract the progression of chronic diseases?

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Altered regulation of copper (Cu) homeostasis may contribute to the development of many pathologies, such as metabolic, cardiovascular, neurodegenerative and cancerous diseases. Cu serum concentrations are strictly related to oxidative stress. During the past decade, it has been well demonstrated that even marginal deficits of this element contribute to development and progression of a number of chronic diseases. On the other hand, an excess of Cu may become a potent oxidant causing the generation of reactive oxygen species (ROS) and leading to the formation of macromolecules dangerous to health. In this review we will describe several chronic inflammatory diseases pathogenically related to the alteration of serum copper levels. Some considerations on present and future perspectives for use of natural antioxidants as effective drugs for the treatment of chronic diseases will be made to shed a glimmer of light on some of the mechanisms employed by natural antioxidants in controlling the distribution and concentration of biometals.

Introduction

Oxidative stress can be a physiological protective mechanism against different toxic stimuli, but if this process is not adequately controlled it may play a major part in the development of chronic and degenerative illnesses.

Cu is an essential trace metal. This ion is crucial due to its pleiotropic effects on biological systems. Cu is a key element for a wide range of physiological cellular functions: aerobic respiration, oxidative stress protection, cardiovascular functionality, neuropeptide biogenesis, lipid, iron and potassium metabolism, erythropoiesis and angiogenesis.^{1–6} Cu in metabolic processes is associated with its ability to fluctuate between oxidized (Cu^{2+}) and reduced (Cu^+) states, as a result of accepting and donating single electrons in oxido-reductive cellular reactions.⁷ Thus, Cu is essential as a structural component for different proteins, as well as a catalytic cofactor for the activation of several metallo-enzymes, such as superoxide dismutase (SOD) and cytochrome *c* oxidase (or mitochondrial complex IV),^{8,9} as seen in Fig. 1. Due to this, the intra- and extra-cellular levels of Cu undergo stringent regulation. The imbalance of Cu homeostasis, in fact, can induce oxidative damage. This contributes to the onset and progression of inflammation and oxidative stress in several pathologies.¹⁰ In particular, an increase in unbound Cu promotes pro-oxidant effects that, in turn, determine tissue damage. In fact, during the transition between the reduced and oxidized states, free Cu ions induce the overproduction of ROS, which is a common

feature of several pathologies.¹¹ This event stimulates the production of advanced glycation end-products (AGEs), advanced lipoxidation end-products (ALEs) and protein oxidation products (PrOPs).^{10,12} The latter molecules activate pro-inflammatory cascades, participating in tissue damage and inducing stress-related responses. Thus, the interplay between Cu, oxidative stress and inflammation represents a crucial regulatory mechanism to prevent or induce tissue damage during chronic inflammatory diseases,¹³ as seen in Fig. 2.

In this review, we will highlight the importance of a Cu imbalance in the pathogenesis of several chronic inflammatory diseases.

All of the information reported herein leads us to give a brief opinion on issues related to use of natural antioxidants that are able to target Cu. We are convinced that these drugs, if used properly, may give important opportunities in the future to develop innovative and effective personalized treatments.

Cu homeostasis

Cells have evolved specific and complex trafficking systems that participate in Cu binding and compartmentalization, to ensure both the delivery and detoxification of any Cu excess. The amount of this biometal in the human body may be influenced by dietary intake, efficiency of absorption and excretion, as well as by the presence of nutritional components that might promote or impair its absorption (*e.g.* high levels of zinc).¹⁴ The recommended daily Cu intake is between 1 and 3 mg, and the main sources of this micronutrient in food are grains, nuts, beans, seeds, shellfish and liver,¹⁵ as seen in Fig. 1.

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COPPER ABSORPTION AND BIOAVAILABILITY 110 mg Cu / total body

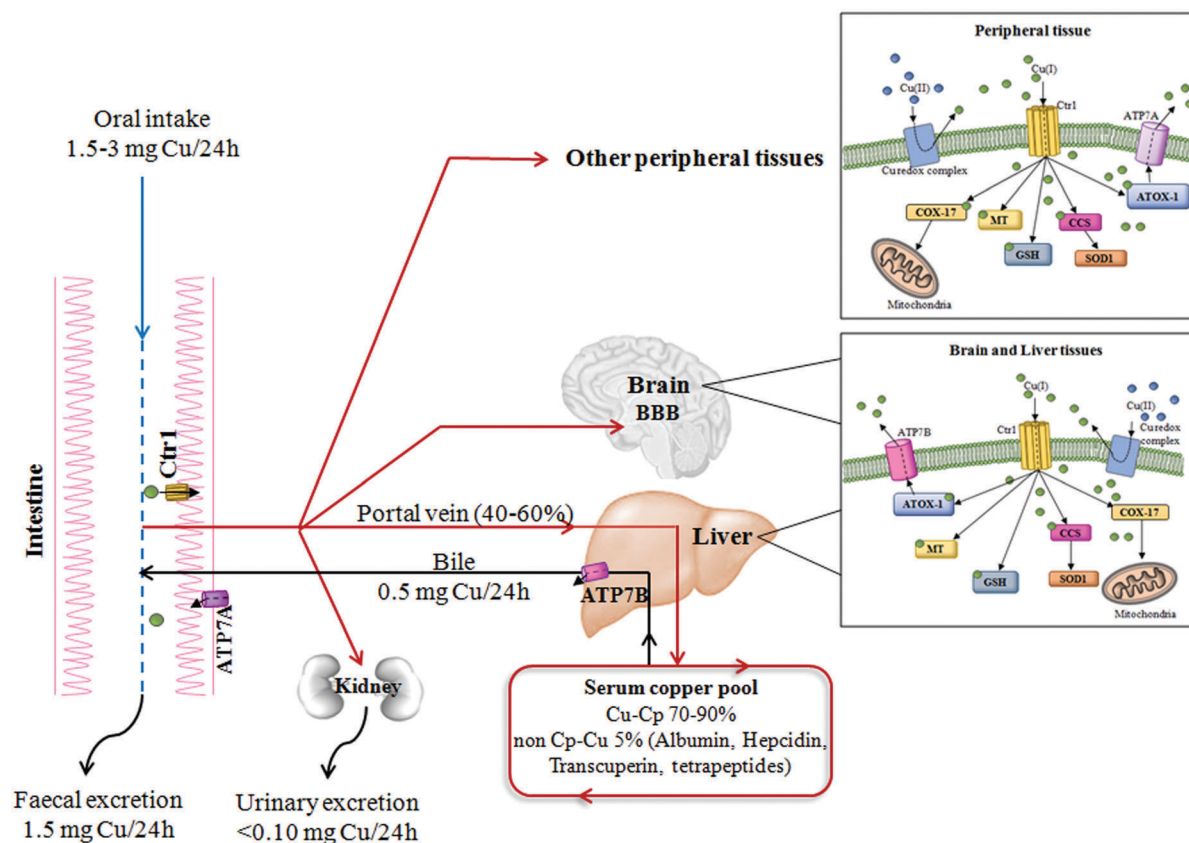


Fig. 1 Copper absorption and bioavailability. The absorption and distribution of copper from the human gastrointestinal system to peripheral tissues. The main proteins involved in copper homeostasis have been reported, as well as total body copper amount and specific distribution in different body compartments. Abbreviations: Cu, copper; Ctr1, high affinity copper uptake protein 1; ATP7A/B, copper-transporting ATPase (ATP7); Cp, ceruloplasmin; ATOX1, antioxidant 1 copper chaperone; MT, metallothionein; GSH, glutathione; SOD1, superoxide dismutase 1; CCS, copper chaperone for superoxide dismutase; COX-17, cytochrome c oxidase copper chaperone; and BBB, blood-brain barrier.

The majority of dietary Cu is absorbed in the duodenum through high-affinity Cu transporter 1 (CTR1), encoded by the *SLC31A1* gene. CTR1 mediates the influx of reduced Cu ions (Cu^+) into human cells.¹⁶ In intestinal epithelial cells, this metal is carried across the basolateral membrane by a Cu-transporting P-type ATPase ATP7A in the portal system up to the liver. From the liver, which is the principal organ for Cu storage, the ion is incorporated into ceruloplasmin (Cp-Cu, which is responsible for chelating between 70 and 90% of Cu in the blood) and is subsequently distributed to other peripheral tissues. Less than 5% of the total Cu concentration circulates as “free Cu” independent of binding with ceruloplasmin (non-Cp-Cu)¹⁷ and is the main factor in clinically significant oxidative stress. “Free Cu” binds to several proteins, such as albumin, hepcidin, transcuperin and low-molecular-weight complexes or tetrapeptides.^{18,19} The excess Cu returns to the liver to be excreted into the bile duct *via* Cu-transporting ATPase ATP7B. Moreover, ATP7A and B participate in intracellular routing of Cu between cytosol and cellular organelles¹⁶ (Fig. 1).

In the cytoplasm, Cu may be chelated by metallothionein for storage bound to Cu chaperones for delivery to specific proteins or to reduced glutathione. In the presence of an

excess of intracellular Cu, these proteins move to intracellular vesicles contributing to an export of Cu.²⁰ When extracellular Cu is increased, CTR1 undergoes Cu-dependent endocytosis, in peripheral tissues, to protect these tissues against an excess of Cu accumulation.²¹ Contrary to other tissues, in the liver, CTR1 expression is elevated under high Cu conditions to facilitate detoxification from circulation and to counteract Cu overload in other Cu-susceptible peripheral tissues.²²

Note that, in response to high levels of Cu concentrations, cell systems displace ATP7A to post-Golgi vesicles and to the plasma membrane and increase the expression of this protein in order to enhance the export of Cu to restore intracellular Cu homeostasis.²²

In our opinion, the crucial role of ATP7A in maintaining Cu homeostasis should be better studied, because its altered expression may even sensitize cells toward transformation.

Cu and human chronic diseases

Inflammation and oxidative stress are characteristic of chronic diseases. Excess non-Cp-Cu becomes a potent oxidant by



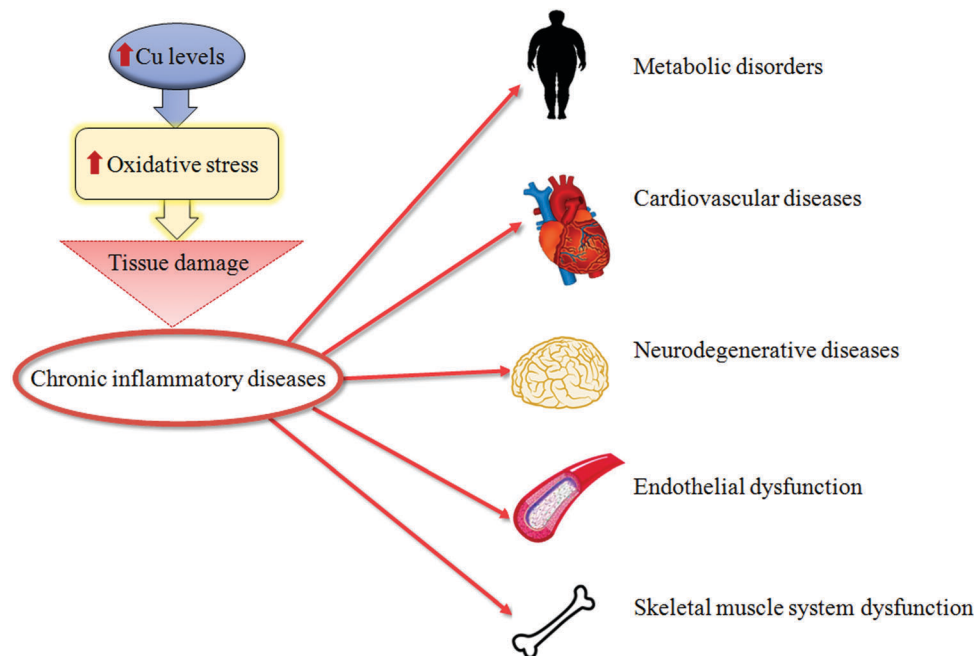


Fig. 2 Copper and tissue damage. A synthetic representation of how high copper (Cu) levels might cause tissue damage of different organs during chronic inflammatory diseases.

generation of ROS, implicated in the decay of biological macromolecules.¹¹ For the reasons described above, Cu levels are fine-tuned and Cu imbalance has important implications in the development and progression of several chronic inflammatory diseases, such as metabolic, cardiovascular, neurodegenerative and cancerous diseases.^{2,3,23,24} Thus, currently the main open issue of a correlation between altered copper regulation and chronic inflammatory diseases should be more deeply and better studied.

Cu and metabolic diseases

Recent results indicate that altered serum Cu homeostasis has an important role in diabetes, obesity, metabolic syndrome and non-alcoholic fatty-liver disease (NAFLD).^{25,26} In fact, diabetes mellitus and obesity are known to correlate with an imbalance of transition metals, including Cu.²⁷ Oxidative stress, insulin resistance, end-product molecules, adipokines, pro-inflammatory cytokines and mitochondrial dysfunction are all related to higher Cu serum levels and are implicated in the development and progression of several chronic inflammatory diseases.²⁸ Accordingly, down-regulation of CTR1, Cu-responsive transition-metal-binding metallothionein proteins (MT1/MT2), antioxidant-protein-1 (ATOX1) and ATP7B was observed in the left-ventricular myocardium of diabetic rats and has been associated with Cu deficiency.²⁹ Moreover, in obese ob/ob mice and in high-fat diet-induced (HFD) mice, pathogenic lipid accumulation promotes a hepatic marginal Cu deficiency, as well as changes in the mitochondrial morphology and function.^{30,31} Interestingly, our recent data highlighted a high Cu serum concentration in NAFLD-cirrhotic patients, which became even more evident in patients with NAFLD-related hepatocellular carcinoma (HCC).³²

All of the data reported above suggest that it will be an important agenda in research to elucidate details of the pathogenic role of altered Cu serum levels (especially “free Cu”) and the expression of the Cu-related proteins (*e.g.* CTR1, ATP7A, ATP7B, MT1, MT2, ceruloplasmin, albumin, transferrin, hepcidin, transcuperin, *etc.*) during chronic inflammatory diseases. In fact, despite studies on redox-active tracers being quite advanced, Cu-related pathological features in metabolic diseases have been poorly studied until now.

Cu and heart diseases

The heart has the highest resting energy expenditure compared to any other organ in the body and has a constant high demand for bioenergy to maintain its workload. Transition metals (*e.g.* Fe, Cu, Zn, *etc.*) are particularly important in heart mitochondrial respiration, where they play essential roles in the production of ATP and detoxification of ROS. Unfortunately, these transition metals can also promote the formation of dangerous radicals,^{33,34} and so meticulous control of their concentration and subcellular compartmentalization is needed.

Cu deficiency has been described in heart attacks and hypertension and causes a decrease in the activity of several important Cu-related enzymes, including Cu,Zn SOD, the potent cellular anti-oxidant enzyme that prevents the formation of free radicals and increases peroxidation of lipids and proteins.³⁵ The pathogenic role of Cu,Zn SOD seems to be mainly related to the over-production of hydrogen peroxide during the transfer of an electron from one molecule of a superoxide anion to another. Although hydrogen peroxide is not a ROS, it is a potent toxic oxidizing agent, playing, in this way, an important role in inducing oxygen toxicity.³⁶



In addition, human cardiac hypertrophy has been described as result of dietary Cu deficiency,³⁵ but, on the other hand, heart-specific deletion of CTR1 allowing increased serum Cu levels together with an increased expression of ATP7A, correlate with the appearance of cardiac hypertrophy.²² Thus, to date, the importance of dietary Cu intake on the onset of cardiac hypertrophy is a question that has yet to be answered. In fact, even if several Cu trafficking proteins have been reported to be implicated in cardiovascular diseases, the related-pathogenic mechanisms are not completely understood and should be better studied in the near future.

To give an example: ceruloplasmin is involved as ferroxidase in the conversion of iron from the ferrous state (Fe^{2+}) to the ferric state (Fe^{3+}) mediating transferrin iron uptake.³⁷ Thus the lack of the ferric state of iron can cause anemia that, in turn, is involved in cardiac hypertrophy and/or ischemia. Thus, oversimplification should be avoided and further studies should be dedicated to finely understanding metal ion regulation in cardiovascular diseases.

Cu and neurodegenerative diseases

After the liver, the brain is the organ containing the highest Cu concentration.³⁸ In a normal brain, Cu has a heterogeneous distribution with a higher concentration in grey matter (e.g. *Substantia nigra*, dentate nucleus and cortex and basal ganglia) rather than in white matter.³⁹ Currently, it is known that Cu is transported to the brain through the blood–brain barrier (BBB). The BBB regulates the influx of Cu into the brain, and together with the blood–cerebrospinal fluid contributes to the maintenance of Cu homeostasis in the brain's extracellular fluids by specific Cu transporters and chaperones.³⁸ One pathogenic mechanism seems to be related to the altered ability of Cu in preventing excessive activity of N-methyl-D-aspartate receptors (NMDARs), which determine synaptic plasticity and are excitatory neurotransmitter receptors.⁴⁰ Hypo- or hyper-activation of NMDARs seems to contribute to the onset and progression of depression, strokes and Alzheimer's disease.⁴¹

In genetic and neurodegenerative diseases, a Cu deficiency or overload may appear for ineffective Cu-related homeostatic mechanisms. Following on from this, anomalous Cu transport and aberrant Cu–protein interactions have been described in genetic diseases, as well as in numerous human neurological disorders, confirming the critical relevance of this trace metal for proper neuro-development and neurological functions.⁴²

Menkes' disease is an X-linked fatal disorder characterized by the accumulation of Cu in the intestine and kidney, while Cu is low in the liver, brain and other tissues, such as bones, skin and hair.⁴³ Patients with Menkes' disease lack functional ATP7A protein, causing a severe Cu deficiency that is often fatal in early childhood.⁴⁴ To date, the molecular mechanisms at the basis of the pathogenic effects of ATP7A inactivation are yet to be clarified, even if it is known that this condition leads to altered Cu concentrations in cytosol, nuclei and mitochondria.⁴⁵

Although most mutations in the *ATP7A* gene cause Menkes' disease, some missense mutations induce a weaker disease

called Occipital Horn Syndrome.^{46,47} Patients with Occipital Horn Syndrome suffer from connective tissue abnormalities as with Menkes' disease patients, but do not usually present neurological symptoms and have a considerably longer average lifespan.⁴⁷

Genetic Wilson's disease is an autosomal recessive disorder characterized by a lack of fully functional ATP7B protein, thus Cu accumulation in the liver and subsequently in the brain and kidney occurs. In addition, Wilson's disease patients typically develop severe liver disease and may present additional neurological symptoms as well.⁴⁴ In particular, the excess of Cu in the liver is caused by its defective excretion by bile, that, in turn, increases oxidative stress, leading cells to apoptosis and fat accumulation. At the same time, the incorporation of Cu into ceruloplasmin is impaired, thus elevated levels of free Cu are released into the bloodstream exerting extrahepatic Cu toxicity.⁴⁵

Interestingly enough, polymorphism in the *ATP7B* gene has also been associated with an increased risk of Alzheimer's disease (AD) and may be useful to stratify specific metabolic subtypes of AD.⁴⁸ AD is a late-onset multifactorial disease and is related to multiple risk factors. AD is the most common form of dementia worldwide and AD patients have higher Cu serum levels^{49,50} that seem to be involved in the formation of the β -sheet form of β -amyloid peptides.⁵¹ β -Amyloid in turn becomes redox-active in the presence of high levels of Cu.⁵² In particular, Cu, when complexed with β -amyloid, catalyzes the production of ROS. Among the different ROS generated by Cu and β -amyloid interactions, it is worth mentioning that hydroxyl radicals are able to induce oxidative damage, not only on the β -amyloid peptide, but also on other molecules, such as lipids and proteins.⁵³ Accordingly, it has been demonstrated that serum non-Cp-Cu ("free Cu") levels predict the progression of mild cognitive impairment during AD.^{48,49}

Besides AD, a large number of studies have described the dysregulation of Cu homeostasis in diseases of the central nervous system, such as Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral syndrome (ALS).^{38,42}

PD is the second most common neurodegenerative disease, and oxidative stress is one of the most common causes of its etiology and pathogenesis.⁵⁴ PD is characterized by gradual loss of dopaminergic neurons, and by the existence of aggregates of misfolded proteins (mainly α -synuclein), known as Lewy bodies.⁵⁴ Cu, as an oxidative stress promoter, plays a key role in the pathogenesis of PD by its ability to form a complex with α -synuclein, inducing the formation of neurotoxic masses.⁵⁵ Accordingly, the reduction of cellular Cu levels results in a significant decrease in aggregate formation.⁵⁶ On the other hand, it has been reported that the presence of Cu induces the formation of structurally different and less-damaging α -synuclein aggregates, suggesting that protein aggregation could not be a primary cause of cytotoxicity.⁵⁷ It has been reported that post-mortem *Substantia nigra* derived from PD patients shows increased levels of iron and decreased levels of Cu.⁵⁸ Moreover, in the *Substantia nigra* of a PD mouse model, the ceruloplasmin protein shows severely reduced ferroxidase activity.⁵⁹



even over-expression of SOD1 alone, still able to bind Cu, can function as an efficient trigger for increasing Cu spinal levels; this effect is probably due to the high Cu affinity of this enzyme.⁷⁰ To date, a consensus does not exist on which Cu-related proteins (SOD1, CCS, ATP7A, MT *etc.*) are really implicated in the pathogenesis of ALS, and how.

Given this, restoration of Cu homeostasis could be a good therapeutic approach for ALS. Accordingly, administration of Cu chelator drugs (*e.g.* ammonium tetrathiomolybdate, TM) has significantly improved and/or slowed down disease progression in severe phenotypes of ALS transgenic mice recently.⁷¹

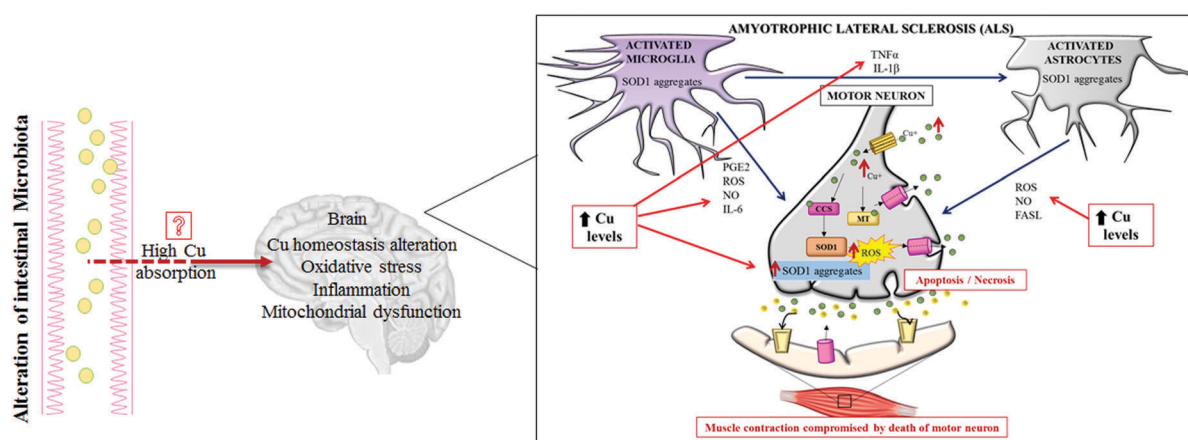
We think it should be found that the most efficient way to correct intracellular Cu homeostasis is by choosing drugs able to chelate and stabilize abnormal accumulation of Cu ions. However, to achieve this aim in ASL patients, we should beforehand finely characterize how, when and where Cu accumulates in the spinal cord.

ALS is a sporadic disease. Only 10% of this malignancy is familial with a known genetic background.^{65,66} ALS is a fatal neurodegenerative disorder affecting upper and lower motor pathways of the central nervous system, characterized by muscle atrophy, paralysis and death within a few years after disease onset.⁶⁷ Currently, the ALS pathogenic mechanisms are still unclear, but the involvement of several altered intracellular signaling pathways have been described, controlling Cu homeostasis,⁶⁸ oxidative stress, inflammation and dysfunction of mitochondrial respiration (Fig. 3). Among several genes responsible for familial ALS, mutations in the Cu,Zn SOD1 gene have been deeply studied and over 180 different mutations have been recognized as causes of ALS.⁶⁹ SOD1 is a major Cu-binding protein and regulates Cu homeostasis in cells; therefore, toxicity of mutant SOD1 could arise from the disruption of Cu homeostasis.⁴⁵

Interestingly, increased levels of Cu have been evidenced in the ventral areas of the spinal cords of sporadic ALS patients, as well as other metals (lead and zinc), but mutations in the SOD1 gene have not been highlighted in those cases. To note,

An important mechanism for the development of several cancers is the deregulation of oxidative stress that impairs cell DNA repair mechanisms due to the overproduction of ROS.⁷² Some lifestyle habits, such as smoking or drinking alcohol, may promote oxidative stress, whereas consumption of nutrients, rich in dietary antioxidants, may protect against oxidative stress.⁷³

High Cu levels, both in serum and in tumor tissue, have been reported in different tumor types, including cancers of the lung, liver, breast, gastrointestinal tract and gynecological malignancy.⁷⁴⁻⁷⁷ A persistent status of Cu-related oxidative stress contributes to tumor development and progression by inducing cell damage and impairing cellular repair processes.⁷⁸ Furthermore, it has been recently reported that a high Cu/Zn ratio is positively associated with colorectal cancer.⁷⁹ In addition, recently, it has been demonstrated that increased levels of



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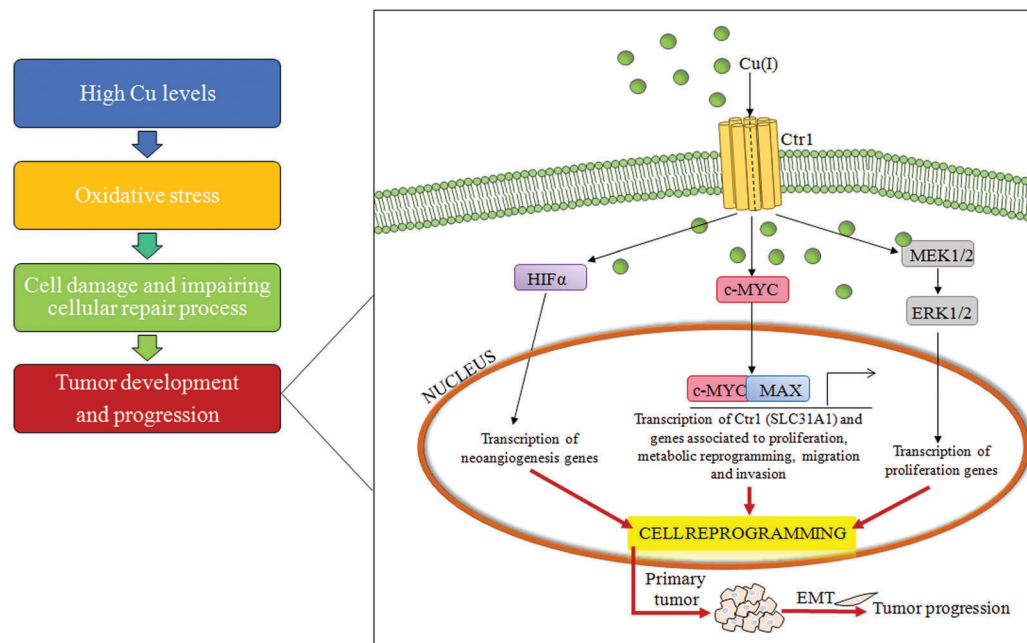


Fig. 4 Copper and cell transformation. A schematic representation of the copper-related pathogenic liver transformation. In particular, high serum copper levels have the ability to induce over-expression of the c-MYC oncogene, reprogramming differentiated cells. Abbreviations: Cu, copper; Ctr1, high affinity copper uptake protein 1; MAX, MYC associated factor X; HIF-1 α , hypoxia inducible factor 1 subunit alpha; MEK1/2, MAP kinase-ERK kinase; ERK1/2, extracellular regulated MAP kinase; and EMT, epithelial–mesenchymal transition.

exogenous Cu push hepatocytes towards transformation through the Cu-dependent over-expression of c-MYC oncogene,³² as seen in Fig. 4.

Moreover, several studies have highlighted the role of Cu in tumor growth and neo-angiogenesis, and so Cu seems to be a promising target for anticancer therapy.^{80,81} Accordingly, Cu chelators act as anti-angiogenic agents reducing the secretion of many positive regulators of angiogenesis, such as vascular endothelial growth factors (VEGFs) and microvascular density, in animal models.^{82–84} Cu stabilizes the transcription factor promoting tumor angiogenesis, hypoxia-inducible factor-alpha (HIF α), under normoxic conditions, inducing hypoxia-response element (HRE)-dependent reporter gene expression.^{80,85} Furthermore, Cu is required for oncogenic BRAF signaling and its related-tumorigenesis.⁸⁶ Mutated BRAF kinase (V600E) phosphorylates and activates MEK1 and MEK2 kinases, which in turn phosphorylate the ERK1 and ERK2 kinases, thus promoting cell transformation. If treated with TM, tumor-carrying mice, derived from mouse embryonic fibroblasts expressing mutated BRAFV600E (Val600 $\rightarrow$ Glu), have a significant decrease in tumor size.⁸⁷ In line with these findings, TM efficacy is increased by association with a low Cu diet.⁸⁸

Future perspectives for alternative treatment of chronic diseases

A good knowledge of Cu regulatory mechanisms and related redox reactions will give additional indications of how oxidative stress should be counteracted to prevent tissue damage during

chronic diseases. In this context, in our opinion, it is very important to better explore metal-binding specificity and selectivity of antioxidants. In fact, the excess of antioxidant activity could be, in particular conditions, even dangerous and not associated with an increased life span.

It has been reported that some enzymatic and non-enzymatic antioxidants (Fig. 5), such as oleuropein, epigallocatechin gallate, gallic acid and curcumin, display metal chelating activity, becoming interesting drugs to counteract the progression of several chronic diseases.^{89,90}

Taking into account the Cu-related pathogenic mechanisms described above, natural antioxidants, with an ability to chelate metals, could hopefully be an adjuvant therapeutic strategy for chronic inflammatory diseases. However, an antioxidant compound, to be safe, should have the ability, after scavenging the radical, to form a new radical that must be stable through intramolecular hydrogen bonding upon further oxidation.⁹¹ Therefore, it is clear that these drugs should be modified in order to create selective, efficient and safe molecules.

In this context, it is very important to point out again that natural antioxidants may not be effective in all patients even if they are affected by the same pathology, because often different pathogenic mechanisms underlie the onset and the progression of oxidative stress-related damage. Emblematic of this is a study on 38 772 post-menopausal women, who regularly supplemented their diet with vitamins and micronutrients such as Cu, Zn, Fe, Mg, folic acid, vitamin E *etc.*, to avoid chronic disease. These women presented an increased total mortality risk.⁹² This is sobering work, because it clearly highlights that antioxidants, in the form of vitamins or other



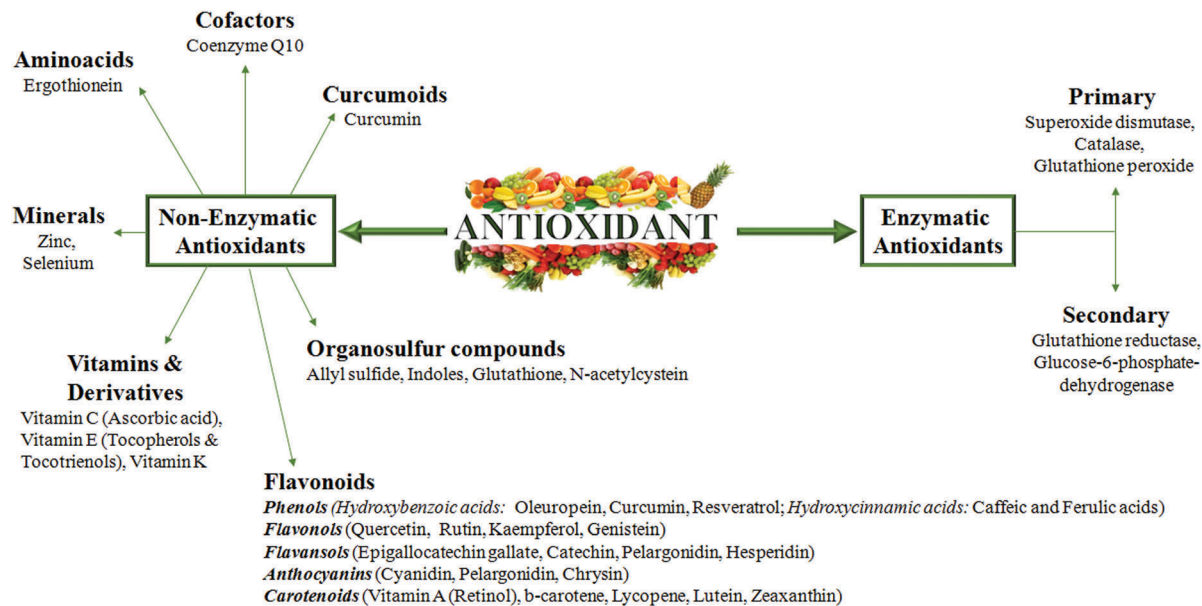


Fig. 5 Non enzymatic and enzymatic antioxidant compounds. The figure represents all of the antioxidant molecules reported to be able to counteract oxidative stress and inflammation in chronic diseases.

supplements, can sometimes cause damage and not benefits in healthy individuals.⁹³ Thus, it is urgent to improve our knowledge on the mechanisms of action of natural antioxidants for a conscious use of them.

Even if at present, most of our knowledge is limited to *in vitro* and preclinical studies, in the near future, we are convinced that ongoing clinical trials will yield very crucial indications to what is necessary to obtain positive effects by using antioxidants.

Conclusion

In the light of what has been said, metals need extensive screening, mainly for recent evidence of their physiological correlation to cardiovascular diseases, metabolic and degenerative diseases. Recent developments in imaging techniques (magnetic resonance imaging) for metal detection might elucidate the role of copper in the pathogenesis of chronic inflammatory diseases. Interestingly, advanced MRI techniques and susceptibility weighted neuroimaging can detect metal deposition in tissues with high sensitivity.^{94,95}

In this context, there is no doubt that Cu plays an important role in a huge number of chronic inflammatory diseases, thus it might represent a promising target for the treatment of these diseases and their complications.

In this review, we highlighted how a Cu imbalance may contribute to the clinical expression of chronic inflammatory diseases, although a full understanding of the biological basis of the imbalance of Cu homeostasis has yet to be achieved. In fact, the specific chemical details of Cu-shunting mechanisms, point by point from its cell entry to its functional targets, should evolve over the next few years through genetic, biochemical, structural and functional studies.

Major comprehension of complex mechanisms in the regulation of Cu signaling pathways in chronic inflammation may be useful in the rationale for developing new promising and personalized therapeutic strategies.

Conflicts of interest

The authors have no conflicts of interest to disclose.

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