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REVIEWS: CURRENT TOPICS

The significance of copper chelators in clinical and experimental application

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Abstract

The essentiality and redox-activity of copper make it indispensable in the mammalian system. However, a comprehensive understanding of copper metabolism and function has not been achieved. Copper chelators have been used as an approach to provide insights into copper acquisition, distribution, and disposition at both the cellular and organism level. Unfortunately, the understanding of effective copper chelators is predominantly based upon their chemical structures and their reactions with copper. The understanding of the efficacy of copper chelators in the biological system has been equivocal, thereby leading to under- or misleading-utilization of these agents in clinical and experimental approaches. Current use of copper chelators in vivo almost exclusively either limits the availability or focuses on the removal of copper in mammalian organ system. There are at least two aspects of copper chelators that are yet to be explored. First, copper chelators preferentially bind either cuprous or cupric. As a result, they potentially modulate copper redox-activity without removing copper from the system. Second, copper chelators are characterized as either membrane-permeable or -impermeable, thus would serve as an organ-selective copper delivery or deprivation system to manipulate the biological function of copper. Here we review clinically relevant copper chelators that have been experimentally or clinically studied for their role in manipulation of copper metabolism and function, paying critical attention to potentially more valuable usage of these agents.

Keywords: Copper; Copper transport; Cuprous; Cupric; Chelator; Homeostasis

1. Introduction

The essentiality of copper ions in biological systems has long been recognized [1]. Copper ions are integrated components of some critical protein structures, involved in catalytic activities and crucial for regulatory functions. The average content of copper is only about 100 mg in human body, but there is virtually no free copper in the cell [2]. By coordinating to proteins and obtaining assistance from chemical ligands such as sulfur, oxygen and nitrogen, copper participates in mitochondrial respiratory reaction and energy generation, regulation of iron acquisition, oxygen transport, cellular stress response, antioxidant defense and several other important processes [1]. By regulating the activities of several critical copper-binding proteins such as cytochrome *c* oxidase (CcO), copper-zinc superoxide dismutase (Cu,Zn-SOD), dopamine β-hydroxylase (DBH), prion protein (PrP), tyrosinase, X-linked inhibitor of apoptosis protein (XIAP), lysyl oxidase, metallothionein (MT), ceruloplasmin and various others, copper exhibits its extensive role in living organisms from microbes to plants and humans [3].

In biological systems, copper ions usually exist in two oxidation states: cuprous (Cu^{1+}, reduced) and cupric (Cu^{2+}, oxidized). This redox activity has been utilized for catalysis by a number of enzymes [1]. Proteins take advantage of the redox nature of copper to achieve facile electron transfer reactions and gain activities [1]. However, the chemical properties that grant copper biologically useful are also potentially toxic. Redox reactions in which copper takes part generate hydroxyl radical and potentially result in severe damage to lipids, proteins, and DNA [4]. Moreover, the imbalance of copper homeostasis in humans causes serious health problems including neurodegenerative symptoms [5,6], cardiovascular structural and functional defects [7,8], bone metabolism disorders [9,10], musculature diseases [11,12] and deregulation of inflammatory responses [12,13].

Integrated approaches are required to understand the plethora of biological activities of copper in organisms. Copper chelators have long been the primary selection to advance the study of copper-related biological functions [14]. These chelators have proven to be valuable in clinical approaches to manipulate disease conditions due to alterations in copper metabolism [15]. Copper chelators have been principally used in three aspects: (1) the understanding of the molecular basis for copper and copper-binding proteins in biological system, (2) the treatment for diseases due to alterations in copper metabolism and (3) the diagnostic application for copper metabolic disorders.

Many compounds, observed with appropriate chelate denticity, suitable donor binding groups, and matching cavity size of geometric

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conformation, are suggested to form stable complexes with copper ions. However, copper ions in different oxidation state prefer different donor binding groups. Cu^{1+} is the lowest oxidation state. It has a diamagnetic d^{10} configuration and forms complexes with flexibility in geometric arrangements [16]. This means that Cu^{1+} reasonably adopts tetrahedral, trigonal, or even linear geometries which are disfavored by other metals [16,17]. Chelate complexes of Cu^{1+} are constructively prepared using relatively soft polarizable ligands comprising of thioethers, nitriles, cyanide, iodide and thiolates. Cu^{2+} is the oxidized state of copper. It has a d^9 configuration, which favors amines, imines and oxygen donors to form square-planar, distorted square-planar, trigonal-pyramidal, and square-pyramidal geometric conformations [16]. Attributable to Jahn-Teller distortions, additional distorted octahedral may be observed as an axial elongation or a tetragonal compression in six-coordinate Cu^{2+} complexes [17]. The geometry of the ligand field influences the redox state of copper. For example, ligands that impose a tetrahedral arrangement, explicitly unfavorable for Cu^{2+} but reasonably favorable for Cu^{1+}, will destabilize the Cu^{2+} form, shifting the reduction potential more positive in support of Cu^{1+} [18,19]. The ligand-induced change in reduction potential allows to purposefully select a desired oxidation state in biochemical experiment. Numerous compounds were exploited as possible copper chelators and investigated for miscellaneous purposes. However, it is virtually an impossible mission to ascertain a perfect chelator for copper in any particular situation. Different chelators have different features that lead to their specific usage under certain circumstances.

The understanding above is based on the chemical structures of the compounds capable of chelating copper and the analysis of ligand-copper interactions. This understanding leads to clinical application of selected compounds for chelation therapy for copper overload or toxification. The goal is to remove excess copper from the organ system. However, an important factor, which has been not received equivalent attention in the design of clinical application of the chelate compounds, is the absence of free copper in mammalian cells [2]. The removal of Cu^{1+} versus Cu^{2+} from the organism leads to different consequences, which have not been fully understood or analyzed. On the other hand, the increase in total copper concentrations in the organism does not suggest an equal elevation of Cu^{1+} and Cu^{2+}. It is exceedingly complicated that copper is coordinated to proteins, indicating the elevation of copper concentrations is associated with alterations in cellular protein composition and protein-protein interactions.

Pertaining to the above scenarios, the affinity of copper for different proteins varies and insinuates the possibility that a chelate compound may deprive copper from one protein but transfer it to another protein, thereby leading to alterations in copper intracellular trafficking and inter-organ transport by the chelate compounds. Therefore, there are at least two aspects of copper chelators that have not been fully explored in either clinical application or experimental studies: (1) copper chelators may change the balance between Cu^{1+} and Cu^{2+} in organisms, which may or may not be associated with changes in total copper concentrations, and (2) copper chelators may redirect the intracellular trafficking and the inter-organ transport. These applications require more comprehensive understanding of the biological aspects of copper chelators and copper speciation in the biological system.

In this review, we focus on clinically relevant and commonly used copper chelators, paying attention to the biochemical aspects of the structure and ligand analysis. Our efforts will be devoted to summarize the significant characteristics of typical chelating compounds, consisting of chemical name, structure, stability constants of copper and other common transition metals, complex solubility, and cell membrane permeability. The information presented in Table 1 is a general summary of the discussion. The following sections highlight some of the chelate agents widely used in clinical and experimental applications. The development of comprehension for these copper chelators in biological experiment and clinic application based on the analysis of the chemical mechanism of these compounds will be discussed in the following sections.

2. Copper-chelating compounds

2.1. Polyaminocarboxylate chelators

Polyaminocarboxylates are organic chelating agents consisting of a basic ligand of amino oxalic acid [$-N(CH_2COOH)_2$] (Fig. 1). There are two donor groups in these chelators: amino nitrogen and carboxyl oxygen. The former favors copper, zinc, mercury and cobalt, and the latter is able to chelate almost all metal ions in oxidation state. Currently, dozens of polyaminocarboxylate chelators have been applied in various fields.

Ethylenediaminetetraacetic acid (EDTA) is a classic chelator, commonly used in molecular biology and biochemical studies. It has a high affinity for Cu^{2+} and the mechanism of transferring copper from chelation with peptides to EDTA has been studied as early as 1968 [14]. In the studies of physiological and pathological activity of copper-dependent compounds, EDTA was often used as a classic Cu^{2+} chelator in experiments to modulate copper concentrations in the cell, or to terminate biochemical process of copper-containing compounds [21–23]. EDTA binds to copper and prevents copper-dependent biological events when it is added into media containing copper before the latter binds to targeted proteins or peptides. However, once the linkage between copper and protein has been formed, the addition of EDTA fails to despoil copper from the protein bound complex but impedes further cupro-protein formation [24,25].

Diethylenetriaminepentaacetic acid (DTPA) is another typical chelator with amino oxalic acid, comprising of octa denticities in its structure. The mechanism of action in copper chelation of DTPA is similar to EDTA. Both EDTA and DTPA are Cu^{2+} chelators and both exhibit the ability to cross cell membrane. Without uncertainty, these two chelators are capable of inhibiting the Cu,Zn-SOD activity [26–28]. However, both EDTA and DTPA selectively inhibit nitric oxide (NO) transferase (S-nitrosoglutathione-reductase) activity of Cu,Zn-SOD but demonstrate no effect on the superoxide dismutase activity. It has been reported that EDTA or DTPA did not remove the bound copper in Cu,Zn-SOD, but EDTA or DTPA formed a complex with Cu,Zn-SOD in one chelator per homodimer [27]. Since the homodimer failed to bind two large ligands, a decreased access of large substrates such as glutathione and *s*-nitrosoglutathione at the catalytic site in the Cu,Zn-SOD-chelator complex was observed. Therefore, Cu,Zn-SOD may exhibit half-site reactivity, indicating both its free and chelator-bound forms possess the same SOD activity, but only the free form possesses s-nitrosoglutathione-reductase activity.

2.2. Acyclic amino chelators

The fundamental ligand of acyclic amino chelators is ethylene diamine [$-NHCH_2CH_2NH-$] (Fig. 2). Typical chelating agents in this class, for instance trientine and tetraethylenepentamine (TEPA), are homologues. Trientine includes four amino nitrogens in its chemical structure whereas TEPA includes five. Although the number of donor groups in trientine and TEPA is less than that in EDTA, their affinity for copper is significantly higher than EDTAs. The rationale for this preference resides in the greater stable formation of copper-chelate complex with a square-planar geometric conformation instead of other geometric conformations.

N,*N*,*N*′,*N*′-tetrakis (2-pyridyl-methyl)ethylenediamine (TPEN) is another chelator in this category. It is a tetrapyridylmethyl derivative

Table 1
Characteristics of copper chelating compounds

IUPAC name	Abbreviation	CAS registry number	Molecular formula	Stability constant (K)[a]				Ligand properties		
				Cu^{2+}	Cu^{1+}	Zn^{2+}	Fe^{3+}	Complex solubility	Membrane permeability	Chemical mechanism
2-[2-[*bis*(carboxymethyl)amino]ethyl-(carboxymethyl)amino]acetic acid	EDTA	60-00-4	$C_{10}H_{16}N_2O_8$	18.8		16.5	24.2	Hydrosoluble	Permeable	Cu^{2+} hexadentate ligand
2-[*bis*[2-[*bis*(carboxymethyl)amino]ethyl] amino] acetic acid	DTPA	67-43-6	$C_{14}H_{23}N_3O_{10}$	21.1			22.0	Hydrosoluble	Permeable	Cu^{2+} octadentate ligand
N′-(2-aminoethyl)-*N*-[2-(2-amino ethylamino) ethyl]ethane-1,2-diamine	TEPA	112-57-2	$C_8H_{23}N_5$	24.0		15.0	10.0	Liposoluble	Permeable	Cu^{2+} pentadentate ligand
N,*N*′-*bis*(2-aminoethyl)ethane-1,2- diamine	Trientine	112-24-3	$C_6H_{18}N_4$	20.4		12.1	18.0	Liposoluble	Permeable	Cu^{2+} tetradentate ligand
N, *N*,*N*′,*N*′-tetrakis(2-pyridyl-methyl) ethylenediamine	TPEN	16858-02-9	$C_{26}H_{28}N_6$	20.5		15.5	14.6 (Fe^{2+})	Liposoluble	Permeable	Cu^{2+} hexadentate ligand
2-[4,8,11-tris(carboxymethyl)-1,4,8,11-tetrazacyclotetradec-1-yl]acetic acid	TETA	60239-22-7	$C_{18}H_{32}N_4O_8$	27.0				Liposoluble	Permeable	Cu^{2+} tetradentate ligand
2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetrazacyclododec-1-yl]acetic acid	DOTA	60239-18-1	$C_{16}H_{28}N_4O_8$	27.0				Liposoluble	Permeable	Cu^{2+} tetradentate ligand
(2*S*)-2-amino-3-methyl-3-sulfanylbutanoic acid	D-pen	52-67-5	$C_5H_{11}NO_2S$	7.1				Hydrosoluble	Permeable	Cu^{1+} and Cu^{2+} ligand with reductibility
bis(Sulfanylidene)molybdenum sulfanide	TTM	16330-92-0	$H_2MoS_4^{-2}$	8.0				Hydrosoluble	Impermeable	Inorganic ligand
5-Chloro-7-iodo-8-hydroxy-quinoline	Cliquinol	130-26-7	C_9H_5ClINO	8.9		7.0		Liposoluble	Permeable	Cu^{2+} bidentate ligand
Diethylcarbamodithioic acid	DDC	147-84-2	$C_5H_{11}NS_2$	14.9				Liposoluble	Permeable	Cu^{2+} ligand with reductibility
2,9-Dimethyl-1,10-phenanthroline	Neocuproine	484-11-7	$C_{14}H_{12}N_2$		19.1	3.1		Liposoluble	Permeable	Cu^{1+} bidentate ligand
2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline	Bathocuproine	4733-39-5	$C_{26}H_{20}N_2$					Liposoluble	Permeable	Cu^{1+} bidentate ligand
4-[2,9-Dimethyl-7-(4-sulfophenyl)-1,10-phenanthrolin-4-yl]benzenesulfonic acid	BCS	52698-84-7	$C_{26}H_{20}N_2O_6S_2$	6.1	19.8			Hydrosoluble	Impermeable	Cu^{1+} bidentate ligand
N,*N*′-*bis*(Cyclohexylideneamino)oxamide	Cuprizone	370-81-0	$C_{14}H_{22}N_4O_2$					Liposoluble	Permeable	Cu^{2+} bidentate ligand

The affinity constant ($log\ Kt$) for the chelators was obtained from reference [20].
[a] Stability constant (K) is a consecutive or stepwise constant and calculated as follows: $ML_{n-1} + L \rightleftharpoons ML_n$ $K = \frac{[ML_n]}{[ML_{n-1}][L]}$.

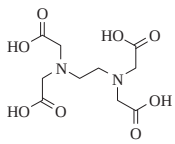
of ethanediamine, with six donor groups including two aliphatic amino nitrogens and four heterocyclic nitrogens (Fig. 2).

Demonstrating their high affinity for copper, TEPA, trientine, TPEN and 30 additional compounds were investigated systematically on their ability to inhibit purified Cu,Zn-SOD in erythrocytes [26]. The three acyclic amino chelators were considered the best chelators for the purpose of inactivating Cu,Zn-SOD rapidly and effectively, with no cytotoxicity induced by the chelators per se.

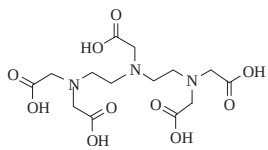
TEPA, trientine and TPEN have been used to create a functional copper deficiency in cell cultures [29]. These chelators were evaluated for not only their capability of decreasing copper levels, but also for their effect on the activity of copper-requiring enzymes such as Cu,Zn-SOD and CcO in HL-60 cells. In comparison with trientine and TPEN, TEPA distinctively illustrated the ability to reduce both copper levels and Cu,Zn-SOD activity in a time dependent manner. TEPA-induced copper-deficiency in the HL-60 cells did not result in changes in the cell viability or alterations in the stage of differentiation. The effects of TEPA on copper levels, and on the activities of Cu,Zn-SOD and CcO may readily be reversed by copper supplementation. Compared with TEPA alone, simultaneous incubation of cells with zinc and TEPA caused a similar reduction in cellular copper concentration.

All these experimental results indicate that TEPA is an ideal chelator for the aim of effectively removing copper from a cell without markedly affecting the viability or causing any fundamental changes in the cell phenotype. For this reason, in the studies of the role of copper in the regression of cardiomyocyte hypertrophy [30,31], neuronal differentiation [32], hepatocyte apoptosis [33], and hematopoietic progenitor cell proliferation and differentiation [34–36], TEPA was extensively used to manipulate cellular copper concentrations.

Previous observations acknowledge that trientine competes for copper bound to albumin in serum with high efficiency [37]. Although the molecular basis of trientine is unclear, the clinical application of

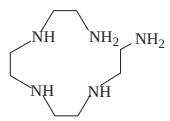


Ethylenediaminetetraacetic acid (EDTA)

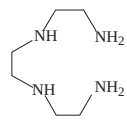


Diethylenetriaminepentaacetic acid (DTPA)

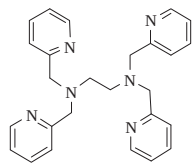
Fig. 1. Structure of polyaminocarboxylate chelators.



Tetraethylenepentamine (TEPA)



Trientine



N,N,N',N'-tetrakis(2-pyridyl-methyl)ethylenediamine (TPEN)

Fig. 2. Structure of acyclic amino chelators.

trientine for the treatment of Wilson's disease, a genetic disease with abnormal metabolism of copper within liver, has been exploited for several decades [15,37]. With the development of understanding the mechanism of copper in angiogenesis, many attempts have been made to apply trientine to the treatment of cancer [38–40]. Trientine is alternatively used for the treatment of diabetic heart disease [41], even though the mechanism of action of trientine in this particular application has not been understood.

TPEN is capable of crossing natural and artificial membranes and chelating divalent metals preferentially. This feature makes it useful in controlling the concentration of metal ions in biochemical experiments [29,42–45]. Regardless of the high affinity of TPEN for copper instead of for zinc, it is perplexing that TPEN failed to protect against the neuronal cytotoxicity of Cu^{2+} in rat hippocampus, whereas it protected against the toxicity induced by Zn^{2+} [46]. In fact, TPEN has been perpetually used as a "selective" chelator for zinc [42]. TPEN forms a distinctive and flexible complex with Cu^{1+} or Cu^{2+}, which exhibits dinuclear conformation with metal-metal bonds, bridging oxo and hydroxo ligands. The diversity of the composition of copper-TPEN complex depends on the stoichiometry, the condition of the reaction, and the presence of competing ligands [47,48]. This may partially explain the contradictory observation about the high stability of the copper-TPEN complex in vitro, but relative low affinity of TPEN for copper in vivo.

2.3. Macrocyclic amino chelators

The backbones of macrocyclic amino chelators are cyclam (1,4,8,11-tetra azacyclotetradecane) and cyclen (1,4,7,10-tetraazacyclododecane). Both of them demonstrated an extreme high affinity constant for copper, *log* K_l, found to be greater than 27. However, the powerful chelator for copper, cyclam failed to inhibit Cu,Zn-SOD activity [26]. In rational to being a large cyclic molecule exhibiting high affinity for copper in stereochemistry, cyclam yet prevents itself in the vicinity of protein bound copper. The clinically relevant chelators in this class are tetracarboxymethyl derivates of cyclam and cyclen, such as 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), respectively (Fig. 3). These two chelators have been used in conjunction with ^{64}Cu-labelled monoclonal antibodies as a radioimmunotherapeutic agent for medical diagnostic (γ-scintigraphy, single photon emission computed tomography, positron emission tomography) and therapeutic applications [49–53].

It has been shown that the stability of TETA or DOTA for ^{64}Cu was superior to acyclic chelating agents [49,50]. This extreme stability is most likely due to the greater geometrical constraint incorporated within the macrocyclic ligand that enhances the kinetic inertness and thermodynamic stability of their ^{64}Cu complexes. While DOTA has been used as a bifunctional chelator for ^{64}Cu, it is less ideal compared with TETA due to its affinity for other metal ions and relatively decreased stability. TETA, therefore, has been extensively used as a chelator for ^{64}Cu, and its successful derivatization has allowed it to conjugate to antibodies, proteins, and peptides [49,50,54]. Furthermore, the ethylene "cross-bridged" restructuring of TETA and DOTA has achieved encouraging results; their unique kinetic inertness and biological stability preferentially allow them to be superior bifunctional chelators for ^{64}Cu than previously observed [54,55].

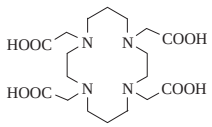
2.4. D-penicillamine chelator

D-penicilliamine (D-pen) contains amino, thiol and carboxylate groups within its molecule (Fig. 4). These functional groups are able to chelate both Cu^{1+} and Cu^{2+}. The complex of copper and D-pen is polymeric with various formations in aqueous solution depending on chloric ion, oxygen, time and copper/D-pen ratio [37,56,57]. D-pen reduces Cu^{2+} to Cu^{1+} in the process of chelation while simultaneously being oxidized to D-pen disulfide [57–59]. The reaction process of copper ions, D-pen and glutathione under anaerobic and aerobic conditions was monitored by means of ^{1}H nuclear magnetic resonance spectroscopy [57]. The cluster species of copper and D-pen was persistently the final product under aerobic conditions, regardless of the presence or absence of glutathione. It suggests that although the cluster species could be decomposed reductively by endogenous thiols, it is reproduced under oxidative conditions, observing that it is more thermodynamically stable than other copper-containing complexes such as D-pen disulfide and glutathione disulfide.

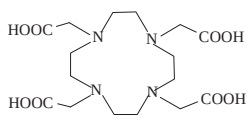
Although the metal-binding ability of D-pen is believed to underlie for an effective treatment for Wilson's disease, systematically experimental results implicated that D-pen performed relatively poorly in chelating copper bound to human albumin in serum in comparison with trientine [37]. A conspicuous feature of D-pen is the reductive activity that may exhibit from the thiolate group. Therefore, it has been hypothesized that the reduction in copper-D-pen complex would accompany a change in the conformation from preferred square planar geometry to tetrahedral geometry, as well as a change in charge. Both changes are less favorable for protein binding, so that D-pen, remarkably reducing Cu^{2+} to Cu^{1+} and chelating both Cu^{1+} and Cu^{2+} simultaneously, is presented with the ability to decrease excess copper levels in Wilson's disease [58]. In addition, hydrogen peroxide is generated during the process of copper-D-pen complex formation [59] and contributes to the cytotoxicity to cancer cells, suggesting a molecular mechanism of D-pen in cancer therapy [60,61].

2.5. Thiomolybdate chelators

Thiomolybdates, with $[MoO_nS_{4-n}]^{2-}$ units (n=0-2), are the only type of inorganic chelators discussed in this review (Fig. 5). Thiomolybdates continually attract biochemists because these compounds own specific features of suitability as models for various biological systems as well as exemplifying varieties in complex structures. Tetrathiomolybdate (TTM) reacts with inorganic copper, forming heterobimetallic complexes through the Mo-S-Cu cluster [62–64]. An important biological function of the Mo-S-Cu cluster is that the antagonism between copper and molybdenum can cause copper deficiency in ruminants [64]. In early biochemical and preclinical studies, TTM and dithiomolybdate (DTM) were intraperitoneally injected into rats for the purpose of comparing their mechanism of action [65]. Experimental results suggested that DTM removed copper from MT in a similar mechanism to that of TTM, but DTM was not appropriate as a therapeutic agent due to its liability in solution [65]. In addition, both TTM and DTM were susceptible to

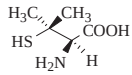


1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA)



1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)

Fig. 3. Structure of macrocyclic amino chelators.



D-penicillamine (D-pen)

Fig. 4. Structure of D-penicillamine chelator.

hydrolysis under acidic conditions and their weak hepatotoxicity may be attributed to the sulfide produced by the hydrolysis reaction [65].

TTM is the third significant medicine to treat Wilson's disease following D-pen and trientine. It acts by forming a tripartite complex with copper and proteins. A series of investigations have revealed the process of TTM selectively and directly removing copper bound in MT, forming MT-copper-TTM complex, solubilizing copper-TTM complex by liberating MT, and insolubilizing copper-TTM polymer gradually [66,67]. The unique mechanism of TTM can be used to generate two anti-copper effects. When given with meals, it forms complexes with copper and food proteins, thereby prevents the absorption of copper, which places patients into a negative copper balance. When given between meals, TTM is absorbed into blood, and binds copper and serum albumin to form a complex, from which copper is unavailable for cellular uptake. Lowering copper levels for therapeutic purposes with TTM in anti-angiogenesis, anti-fibrosis, and anti-inflammation has been proven in numerous in vitro and in vivo experiments [68–71]. Developing the basic understanding of pharmacological mechanism and clinical application of TTM has been reviewed recently [13,72].

2.6. Hydroxyquinoline chelators

Clioquinol, a derivate of 8-hydroxyquinoline, is a retired United States Pharmacopeia antibiotic medicine and orally bioavailable copper/zinc chelator (Fig. 6). It is a bidentant ligand and includes phenolate oxygen and pyridine nitrogen that situate in *ortho* position of benzene complementary to each other. Two clioquinol molecules likely bind one Cu^{2+} in vitro with a planar and pseudocentrosymmetric conformation [73,74]. In a biological buffer containing Ca^{2+} and Mg^{2+}, Cu^{2+} and clioquinol form a complex with a 1:2 stoichiometry with the conditional stability constant K_{C0} of 1.2×10^{10}, slightly greater than in neutral aqueous solution [74].

Clioquinol has been used in the treatment of Alzheimer's disease, characterized by elevated brain iron levels and accumulation of copper and zinc in the cerebral β-amyloid (Aβ) deposits. Patients suffering from Alzheimer's disease display higher levels of copper in the plasma but lower levels in the brain. The biological effects of clioquinol are most likely ascribed to the high lipophilicity of the free ligand to facilely access to the brain. Its selective metal chelation for Cu^{2+} and Zn^{2+} may account for its effect against neurodegeneration. In contrast, high-affinity copper/zinc chelators such as trientine failed to inhibit amyloid deposition in transgenic mouse model of Alzheimer's disease [75], suggesting that clioquinol has a different mechanism of action on Alzheimer's disease relative to the traditional systemic metal chelation [75,76].

It has been proposed by Bush and Tanzi [77] that clioquinol enters the brain and is attracted to the extracellular pool of metals such as copper and zinc that are in a dissociable equilibrium with Aβ. Clioquinol then binds copper and zinc in the Aβ deposits, possibly forming a ternary complex with Aβ. It has been shown that stripping metals away from Aβ leads to dissolution of Aβ aggregates back down to monomer, which can be readily cleared or degraded from the brain [77,78]. Considering the fact that abnormal brain copper distribution occurs in Alzheimer's disease with excessive accumulation of copper in amyloid plaques in addition to deficiency of copper in neighboring cells, copper mobilization by clioquinol from Aβ and transferring to other intracellular sites would contribute to a significant improvement for maintaining the balance of copper in the brain. Thus, clioquinol is not a simple copper chelator, but rather a copper chaperon for its ability to transfer copper between molecules.

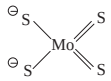
2.7. Dithiocarbamates chelators

Dithiocarbamates are sulfur-based chelators containing a functional group of dithiocarboxy conjugated with an aliphatic secondary amino (Fig. 7). Chelators in this class may be discriminated by the different alkyl group attached to their nitrogen atom. *N*,*N*-diethyldithiocarbamate (DDC) is a typical compound in this class, which has been reported to inhibit Cu,Zn-SOD in animal models as early as 1970s [28]. It has been confirmed that DDC is able to despoil copper from Cu,Zn-SOD, thus producing copper-depleted protein [27,28,79]. However, the mechanism by which DDC removes copper from Cu,Zn-SOD has been an ongoing debate, focusing on the existence of the ternary Cu^{2+}-DDC-protein complex during the process [28,79]. Further investigation showed a significant phenomenon of DDC in the process of inactivating Cu,Zn-SOD in erythrocytes, in which DDC reacted with oxyhemoglobin to produce reactive radicals, such as hydrogen peroxide and oxidized DDC that spontaneously reacted with thiols and resulted in intracellular glutathione depletion and other intracellular enzyme inactivation [80]. Severe cell damage occurs with the addition of DDC even at concentration below the requirement for Cu,Zn-SOD inhibition [26]. This makes DDC an inapt inhibitor for Cu,Zn-SOD in cultured cells.

There are various dithiocarbamates that have been exploited in the last decade for the studies of metal ion uptake and apoptosis in a variety of cells [81–87]. Strong evidence indicates that the pharmacological and toxicological effects of dithiocarbamates are derived from the formation of complexes between dithiocarbamates and copper [81,82]. The polarity of dithiocarbamate's nitrogen substitute influences the lipophilicity of the copper complexes. The lipophilicity potentially determines the ability for copper-dithiocarbamate complex to promote copper accumulation in target tissue and induce the toxicological effect [87,88].

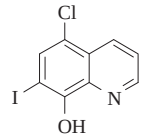
2.8. Diamine chelators

Diaminie chelators often contain 2,2′-bipyridine and 1,10-phenanthroline. The complexes of copper and 1,4-diamine ligands hold a



Tetrathiomolybdate (TTM)

Fig. 5. Structure of thiomolybdate chelator.



Clioquinol

Fig. 6. Structure of hydroxyquinoline chelators.

particular property of interdependence in their coordination geometry and their redox activity. The Cu^{1+} complex adopts a tetrahedral or pseudotetrahedral geometry and may be readily oxidized to a more stable square-planar Cu^{2+} species in the absence of restricting steric effects. For that reason, Cu^{1+} complexes of 2,2′-bipyridine and 1,10-phenanthroline exhibit an oxidative instability.

Unlike 1,10-phenanthroline, 2,9-dimethyl-substituted phenanthroline ligands disfavor octahedral tris-chelate or square-planar *bis*-chelate coordination because of the steric interference of the methyl substitute *ortho* with the imine nitrogen. In fact, when 2,9-dimethyl-substituted phenanthroline ligands bind to a metal ion in a *bis* complex, the metal ion is forced to exhibit a tetrahedral binding geometry with the two chelate ligands nearly perpendicular to each other. This tetrahedral geometry in combination with the size of the five-member chelate ring effectively traps Cu^{1+} over other metal ions, thus increasing the barrier for Cu^{1+} to Cu^{2+} inter-conversion and sufficiently allowing the former species to become stable.

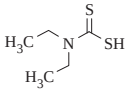
Neocuproine, bathocuproine and bathocuproine disulfonate (BCS) are three well-known chelators commonly used in cell biology experiments to selectively chelate Cu^{1+} (Fig. 8) [18,89–97]. These ligands are also known to bind to Cu^{2+}, forcing it into a tetrahedral geometry and inducing the reduction from Cu^{2+} to Cu^{1+}, indicating that these "Cu^{1+}-selective" chelators are not exclusive chelators for Cu^{1+}. It is important that although they are able to promote the reduction of Cu^{2+} to Cu^{1+}, once the reduced state of the copper complex is reached, it is stabilized and does not participate in redox cycling [18,19]. Both BCS and neocuproine have been shown to inhibit copper-dependent redox cycle [18,19].

Neocuproine is more hydrophobic among the three Cu^{1+} chelators. It is often used for intracellular and extracellular copper chelation since it can diffuse through the cell membrane [89,91,93,94]. It is important to note that neocuproine forms a lipophilic complex with Cu^{2+} and the Cu^{2+}-neocuproine complex has been found to be a more potent oxidant than Cu^{2+} alone [96], thus it potentiates copper-mediated toxicity [97,98]. On the contrary, BCS, a sulfonated derivative of bathocuproine, which has been developed to offer a water soluble characteristic, inhibits copper-mediated cytotoxicity due to the formation of a hydrophilic complex with Cu^{2+} [97]. But, the Cu^{2+}-BCS complex also increases the oxidative activity in comparison with Cu^{2+} [99,100]. Because BCS is charged and membrane impermeable, it is commonly used in the studies that need an extracellular copper-limiting agent [90,91,95].

2.9. Cuprizone chelator

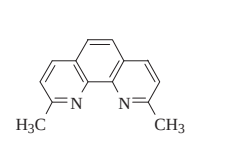
Cuprizone is a bidentate ligand with two hydrazides and is used to selectively bind Cu^{2+} in the cell [89,92,93] (Fig. 9). It has been demonstrated that Cu^{2+}-cuprizone complex forms in 1:2 stoichiometry in aqueous [101–103], but the actual structure of the complex has not been obtained until recently. Some current evidence shows that cuprizone stabilizes Cu^{3+} oxidation state in a square planar d^8 complex [102,103]. Therefore, the Cu^{2+}-Cu^{3+} redox cycling induced by cuprizone under biological conditions may be related to the cytotoxic and neurotoxic effects of this chelator [103].

Cuprizone was reported to possess unique neurotoxic properties and serve as a valuable pharmacological tool for central nervous system demyelination and spongiosis in experimental animals. The

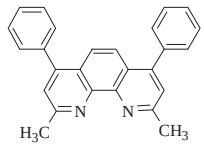


N, N-diethyldithiocarbamate (DDC)

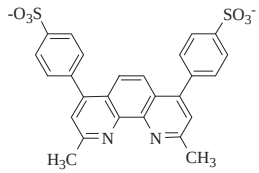
Fig. 7. Structure of dithiocarbamates chelators.



Neocuproine



bathocuproine



Bathocuproine disulfonate (BCS)

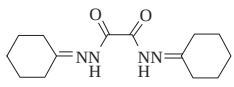
Fig. 8. Structure of diamine chelators.

cuprizone model for demyelination contributed to an important advance for multiple sclerosis research. Young mice fed cuprizone diet develop oligodendrocyte death and reversible demyelination [104,105]. Spontaneous remyelination can be seen as early as 4 days after withdrawal of cuprizone [104]. Although the molecular basis for the specific effect of cuprizone on the oligodendrocytes is uncertain, cuprizone might directly interfere with brain copper metabolism, resulting in inhibition of the copper-dependent mitochondrial enzymes, such as CcO and monoamine oxidase. Thus, it was hypothesized that the imbalance of copper metabolism and the disturbance in energy generation lead to apoptosis in the oligodendrocytes, causing demyelination [104–106].

3. Copper nutrition and metabolic disorders

The nutritional essentiality of copper for mammals was first reported in 1928 [107]; supplementing with 1.0 mg copper sulfate once a day for 6 days/week rapidly promoted anemic and stunted rats growth and blood hemoglobin elevation. Chronic dietary copper deficiency has been suggested as a contributing factor to three major pathological conditions; osteoporosis[9,10], neurodegenerative [5,6] and cardiovascular diseases [7,8,108]. Copper deficiency in infants causes iron-unresponsive anemia, neutropenia and bone abnormalities [1]. In elderly, the most frequent clinical manifestations of copper deficiency are compromised cardiovascular system, as well as hematologic and bone abnormalities [1,109,110]. Hematologic changes are characterized by the existence of hypochromic, normocytic or macrocytic anemia, accompanied by a reduced reticulocyte count, hypoferremia, neutropenia and thrombocytopenia [1,109]. These hematologic changes can be used as systemic indices for organ system copper deficiency. However, there are conditions that hematologic changes may not occur, but organ system copper deficiency may take place.

An important and interesting phenomenon is that under certain circumstances, the pathological changes of certain organ systems for example the brain and the heart are associated with a decrease in copper concentrations in the organs, accompanying with an increase in copper concentrations in the plasma [108,111–113]. An important



Cuprizone

Fig. 9. Structure of cuprizone chelator.

question that has not been widely raised is that why the high levels of copper in the plasma cannot be utilized by the diseased organs, such as the brain and the heart, where the deficiency of copper is predominantly observed under Alzheimer's disease[113] or ischemic heart disease condition[108,111,112]. It is widely accepted that copper is absorbed and transported into the blood stream through the intestinal epithelial cells and presented in the protein- or peptide-bound form in the peripheral circulation before it enters the liver for processing. Copper in the liver is either released in the form of ceruloplasmin into the peripheral circulation or secreted into the bile for excretion. Under physiological conditions, the use of copper by extra-hepatic organs relies on ceruloplasmin. But under the aceruloplasmin condition, there is no apparent copper deficiency in extra-hepatic organs, suggesting the existence of other copper delivery mechanisms for extra-hepatic organs. But under the brain or heart disease condition, it appears that high levels of copper in the plasma cannot be processed for reutilization by the brain or the heart.

Under these circumstances, i.e., copper deficiency in organ systems but increased copper concentrations in blood, hematologic indices for copper status may not be appropriate as a diagnostic parameter. The activities of copper-containing proteins including CcO, Cu,Zn-SOD, DBH, PrP, tyrosinase, XIAP, lysyl oxidase, ceruloplasmin and metallothionein are often used as a probe for systemic copper status. However, it has been shown that under organ system copper deficiency conditions, some copper-binding proteins may increase its concentrations or activities. For example, under dietary copper deficiency, liver metallothionein concentrations increase along with decreased activities of CcO [114]. It thus is important to understand copper metabolic changes and copper-binding protein speciation alterations under specific disease conditions in order to make an attempt to manipulate copper transport and distribution. In this context, copper-related diseases such as neurodegenerative symptoms [5,6], cardiovascular structural and functional defects [7,8], bone metabolism disorders [9,10], musculature diseases [11,12] and deregulation of inflammatory responses [12,13] are all uncertain targets for copper manipulation therapy.

4. The significance of copper chelation therapy

Copper chelation is a straightforward approach to improve the condition of copper overload or accumulation in organisms. There are multiple therapeutic applications of copper chelators in clinical trials and in the treatment of several diseases [13,41,72,77]. The clinical use of copper chelators focuses on removal of copper or limitation of the bioavailability of copper in the organ system. The typical and successful utilization of copper chelators has been in the treatment of Wilson's disease. Three chelators, including D-pen, trientine and TTM have been used with regard to this application and all three have achieved satisfactory therapeutic efficacies. However, the mechanisms of action of these three chelators are different, even though the biological effects, with the exception of copper removal, derived from these differences have not been understood. It would not be surprising if some critical differences can be identified among these chelators in the treatment of Wilson's disease, but the current application basically focuses on the removal of copper from the liver. Increased excretion of copper from the liver has been observed [13,72], but it would be expected that inter-organ transport of copper by these chelators could occur, which has not been explored.

Promoting copper inter-organ transport is an important issue, which has not been fully recognized in clinical practice. A common mistake in the assessment of copper status in the organ systems is the measurement of copper concentrations in the plasma, and the elevation of the plasma copper concentrations has often been considered copper overload. However, as discussed above, under certain circumstances, the disorder of certain organ systems for example the brain and the heart is associated with a decrease in copper concentrations in the organs, accompanying with an increase in copper concentrations in the plasma [107,108]. Therefore, under these conditions, the application of a copper chelation (copper removal) therapy may not be appropriate.

Clinical practice has provided important clues regarding the difference in the application of different copper chelators for the treatment of particular diseases. For instance, the use of copper chelators in the treatment of Alzheimer's disease has proven a simple removal of the plasma copper may not necessarily be effective. Patients with Alzheimer's disease have higher levels of copper in the plasma but lower levels in the brain. Moreover, copper is accumulated in the amyloid plaques, leading to an imbalanced distribution of copper in the brain. In the treatment of this disease, clioquinol was effective in contrast to high-affinity copper/zinc chelators such as trientine and EDTA [75]. Upon entering the brain, clioquinol is able to strip copper and zinc from the amyloid plaques, leading to dissolution of the Aβ aggregates. The Aβ monomers are readily cleaned or degraded [77,78]. Thus, clioquinol may mobilize copper from one site, and then transfer the copper to another, without causing significant changes in total copper concentrations. This mechanism of action is different from that of traditional copper chelators such as EDTA, which may simply remove copper from the target sites.

It is vital to know the change in copper speciation under the disease conditions such as neurodegenerative and cardiovascular diseases under which copper is often effluxed from the organs. It appears that copper released from the diseased organs is in a different protein- or peptide-bound form from that released from the intestinal epithelial cells, so that these unusual species of copper cannot be processed by the liver or other mechanisms for reutilization. It is interesting to observe that in the mouse model of pressure overload-induced cardiac hypertrophy, copper concentrations decrease in the heart, but increase in the plasma [30]. However, only when copper is supplemented in food can cardiac copper concentrations be normalized, suggesting that in the total copper pool in the plasma, only the portion that is derived from the intestinal epithelial cells can be processed for the utilization by the hypertrophic heart. However, is it possible that different species of copper in the plasma can be exchangeable so that some idle copper become available?

There are copper chelators that can change the oxidation state of copper from Cu^{2+} to Cu^{1+}, as discussed in the above section. The proteins that bind to Cu^{2+} are different from that which bind to Cu^{1+}. Therefore, one of the potential effects of the copper chelators is to remove Cu^{2+} from one copper-binding protein, convert Cu^{2+} to Cu^{1+}, then transfer Cu^{1+} to another copper-binding protein. For instance, neocuproine, bathocuproine and BCS are capable of binding to Cu^{2+}, forcing it into a tetrahedral geometry and inducing the reduction from Cu^{2+} to Cu^{1+}. Should the newly reduced Cu^{1+} become available from the chelators, different proteins other than those binding to Cu^{2+} would become the targets.

It is also significantly beneficial to the organ system when the reduction from Cu^{2+} to Cu^{1+} occurs in the copper-chelator complexes. An important aspect of this reduction is that once the reduced state of the copper complex is reached, it is stabilized and does not participate in redox cycling [18,19]. Therefore, BCS has been used in experimental setting to inhibit copper-dependent redox cycle for two important reasons: (1) BCS is charged and not membrane permeable so that it can limit the availability of copper for cells, and (2) since Cu^{2+} is the dominant form of copper in extracellular environment, the use of BCS can promote the reduction of Cu^{2+} to Cu^{1+} and the stabilized copper-chelator complex can suppress the copper-dependent redox cycle.

If copper speciation, such as its binding to different proteins or peptides, can be modified by copper chelators, the imbalance of copper distribution between the plasma and vital organs under certain disease conditions would be corrected. For instance, exploring

the use of copper chelators in pressure overload-induced cardiac hypertrophy would make the high levels of plasma copper available for the heart, in which a deficiency of copper has been demonstrated [7,8]. Exploring this use would deem copper chelators more valuable in the clinical practice.

5. Conclusions

The history of copper chelation therapy is nearly as extensive as the investigation of copper function in organisms. It is an important strategy to change the bioavailability of copper ions by using metal chelating agents for studying cellular processes related to copper transport, storage and usage. When taking advantage of live cells in probing for biomolecules related to copper homeostasis, a typical approach is to change the availability of copper and observe the subsequent changes in cell processes. Up-regulation or down-regulation of a specific set of genes can indicate the proteins either directly or indirectly related to copper handling. Copper chelators have been extensively used in conjunction with the copper-binding protein studies.

Since the ability of a chelator to limit the bioavailability of copper is crucial to the integrity of an experiment and to the interpretation of the results, the selectivity of a copper chelator for copper over other metals is the issue that has been addressed for a long time and continues to be the mainstay in copper chelation therapy and studies. However, the selectivity does not imply exclusivity. While an agent may have a thermodynamic preference for copper, it does not mean that it ignores to bind other metals. For instance, TEPA in culture media can limit the bioavailability of copper, but at the same time it also reduces the availability of other metals such as zinc. In this case, modest supplementation with zinc would provide cells with adequate levels of zinc when implementing a model of copper deficiency.

Many copper chelators, such as D-pen, trientine, TTM, and clioquinol have been used in clinical practice in the treatment of copper-related diseases. However, with the development of the understanding of the role of copper in pathogenesis, and the molecular pharmacological mechanism of the chelators, we may no longer consider the “chelation therapy” in the same light as metal depletion. Chelators can not only despoil copper from copper-binding proteins and increase its excretion from organ systems, but also offer a chaperon or delivery mechanism for copper redistribution in organisms. In addition, copper chelators can serve as an invaluable regulator of copper-dependent redox cycling. These last two aspects of copper chelators have been explored in experimental setting, but should be further expanded to clinical practice.

Novel application of copper in experimental and clinical settings requires more extensive usage of copper chelators. For instance, copper-based radionuclides have recently attracted more attention and are extensively evaluated in clinical application. Copper-based radionuclides offer a varying range of half-lives and positron energies. For example, the half-life (12.7 h) and decay properties (β^+, 0.653 MeV, 17.8%; β^-, 0.579 MeV, 38.4 %) of ^{64}Cu make it an ideal radioisotope for positron emission tomography imaging and radiotherapy. In addition, an important advantage of copper is the well-established coordination chemistry, allowing it to react with wide variety of chelator systems that can potentially be linked to antibodies, proteins, peptides and other biologically relevant molecules. Therefore, novel application of copper chelators in radioactive diagnostic and therapy is forthcoming.

In conclusion, copper chelators are an indispensable accompaniment for copper biology and medicine. There are many compounds that demonstrate the ability to chelate copper, but only those that are of clinical relevance have been presented in this discussion. It is obvious that these chelators have been under-utilized, although they have proven to be excellent tools in limiting the bioavailability of copper in organ systems. More valuable application of these invaluable tools in copper biology and medicine should be seen in the future.

References

[1] Uauy R, Olivares M, Gonzalez M. Essentiality of copper in humans. Am J Clin Nutr 1998;67:952S–9S.
[2] Rae TD, Schmidt PJ, Pufahl RA, Culotta VC, O'Halloran TV. Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. Science 1999;284:805–8.
[3] Kim B, Nevitt T, Thiele DJ. Mechanisms for copper acquisition, distribution and regulation. Nat Chem Biol 2008;4:176–85.
[4] Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984;219:1–14.
[5] Waggoner DJ, Bartnikas TB, Gitlin JD. The role of copper in neurodegenerative disease. Neurobiol Dis 1999;6:221–30.
[6] Cerpa W, Varela-Nallar L, Reyes AE, Minniti AN, Inestrosa NC. Is there a role for copper in neurodegenerative diseases? Mol Aspects Med 2005;26:405–20.
[7] Nath R. Copper deficiency and heart disease: molecular basis, recent advances and current concepts. Int J Biochem Cell Biol 1997;29:1245–54.
[8] Klevay LM. Cardiovascular disease from copper deficiency-a history. J Nutr 2000;130:489S–92S.
[9] Strause LG, Hegenauer J, Saltman P, Cone R, Resnick D. Effects of long-term dietary manganese and copper deficiency on rat skeleton. J Nutr 1986;116: 135–41.
[10] Eaton-Evans J, McIlrath EM, Jackson WE, McCartney H, Strain JJ. Copper supplementation and the maintenance of bone mineral density in middle-aged women. J Trace Elem Exp Med 1996;9:87–94.
[11] Vonk WIM, Klomp LWJ. Role of transition metals in the pathogenesis of amyotrophic lateral sclerosis. Biochem Soc Trans 2008;36:1322–8.
[12] Rayman MP, Pattison DJ. Dietary manipulation in musculoskeletal conditions. Best Prac Res Clin Rheum 2008;22:535–61.
[13] Brewer GJ. Anticopper therapy against cancer and diseases of inflammation and fibrosis. Drug Discovery Today 2005;10:1103–9.
[14] Pagendorf GK, Margerum DW. Mechanism for the proton-transfer reaction of a peptide hydrogen in copper(II) trglycine. J Am Chem Soc 1968;90:6963–7.
[15] No author list. Chelating agents in medicine. Br Med J 1971;2:270–2.
[16] Haas KL, Franz KJ. Application of metal coordination chemistry to explore and manipulate cell biology. Chem Rev 2009;109:4921–60.
[17] Yang P, Gao F. Principle of Bioinorganic Chemistry, 1st ed.Bei Jing: Science Publishing Company; 2002. p. 54–60.
[18] Patel RP, Svistunenko D, Wilson MT, Darley-Usmar VM. Reduction of Cu(II) by lipid hydroperoxides: implications for the copper dependent oxidation of low-density lipoprotein. Biochem J 1997;322:425–33.
[19] Seng HL, Tan KW, Maah MJ, Tan WT, Hamada H, Chikira M, et al. Copper (II) complexes of methylated glycine derivatives: effect of methyl substituent on their DNA binding and nucleolytic property. Polyhedron 2009;28:2219–27.
[20] Sillen LG, Martell AE. Stability Constants of Metal-ion Complexes, special publication No.17. London: The Chemical Society; 1964.
[21] Endo N, Nishiyama K, Okabe M, Matsumoto M, Kanouchia H, Oka T. Vitamin B6 suppresses apoptosis of NM-1 bovine endothelial cells induced by homocysteine and copper. Biochim Biophy Acta 2007;1770:571–7.
[22] Habtemariam S, Dagne E. Prooxidant action of knipholone anthrone: copper dependent reactive oxygen species generation and DNA damage. Food Chem Toxicol 2009;47:1490–4.
[23] Rivero-Müller A, Vizcaya-Ruiz AD, Plant N, Ruiz L, Dobrota M. Mixed chelate copper complex, Casiopeina IIgly®, binds and degrades nucleic acids: a mechanism of cytotoxicity. Chem Biol Interact 2007;165:189–99.
[24] Jiménez I, Speisky H. Effects of copper ions on the free radical-scavenging properties of reduced gluthathione implications of a complex formation. J Trace Elem Med Biol 2000;14:161–7.
[25] Letelier ME, Lepe AM, Faúndez M, Aracenaa P, Speisky H. Possible mechanisms underlying copper-induced damage in biological membranes leading to cellular toxicity. Chem Biol Interact 2005;151:71–82.
[26] Keiner MJ, Bagnell R, Hale B, Alexander NM. Inactivation of intracellular copper-zinc superoxide dismutase by copper chelating agents without glutathione depletion and methemoglobin formation. Free Radic Biol Med 1989;6:355–60.
[27] Ye MW, English AM. Binding of polyaminocarboxylate chelators to the active-site copper inhibits the GSNO-reductase activity but not the superoxide dismutase activity of Cu,Zn-superoxide dismutase. Biochemistry 2006;45:12723–32.
[28] Misra HP. Reaction of copper-zinc superoxide dismutase with diethyldithiocarbamate. J Biol Chem 1979;254:11623–8.
[29] Percival SS, Dem-Patrice ML. HL-60 cells can be made copper deficient by incubating with tetraethylenepentamine. J Nutr 1992;122:2424–9.
[30] Jiang Y, Reynolds C, Xiao C, Feng W, Zhou Z, Rodriguez W, et al. Dietary copper supplementation reverses hypertrophic cardiomyopathy induced by chronic pressure overload in mice. J Exp Med 2007;204:657–66.
[31] Feng W, Ye F, Xue W, Zhou Z, Kang YJ. Copper regulation of hypoxia-inducible factor-1 activity. Mol Pharmacol 2009;75:174–82.
[32] Birkaya B, Aletta JM. NGF promotes copper accumulation required for optimum neurite outgrowth and protein methylation. J Neurobiol 2005;63:49–61.

[33] Narayanan VS, Fitch CA, Levenson CW. Tumor suppressor protein p53 mRNA and subcellular localization are altered by changes in cellular copper in human Hep G2 cells. J Nutr 2001;131:1427–32.
[34] Peled T, Landau E, Prus E, Treves AJ, Fibach E. Cellular copper content modulates differentiation and self-renewal in cultures of cord blood-derived CD34+ cells. Br J Haematol 2002;116:655–61.
[35] Peled T, Landau E, Mandel J, Glukhman E, Goudsmid NR, Nagler A, et al. Linear polyamine copper chelator tetraethylenepentamine augments long-term ex vivo expansion of cord blood-derived CD34+ cells and increases their engraftment potential in NOD/SCID mice. Exp Hematol 2004;32:547–55.
[36] Peleda T, Glukhmana E, Hasson N, Fibach E. Chelatable cellular copper modulates differentiation and self-renewal of cord blood–derived hematopoietic progenitor cells. Exp Hematol 2005;33:1092–100.
[37] Sarkar B, Sass-Kortsak A, Clarke R, Laurie SH, Wei P. A comparative study of in vitro and in vivo interaction of D-penicillamine and triethylenetetramine with copper. Proc R Soc Med 1977;70:13–8.
[38] Yoshii J, Yoshiji H, Kuriyama S, Ikenaka Y, Noguchi R, Okuda H, et al. The copper-chelating agent, trientine, suppresses tumor development and angiogenesis in the murine hepatocellular carcinoma cells. Int J Cancer 2001;94:768–73.
[39] Yoshiji H, Yoshii J, Kuriyama S, Ikenaka Y, Noguchi R, Yanase K, et al. Combination of copper-chelating agent, trientine, and methotrexate attenuates colorectal carcinoma development and angiogenesis in mice. Oncol Rep 2005;14:213–8.
[40] Hayashi M, Nishiya H, Chiba T, Endoh D, Kon Y, Okui T. Trientine, a copper-chelating agent, induced apoptosis in murine fibrosarcoma cells in vivo and in vitro. J Vet Med Sci 2007;9:137–42.
[41] Cooper GJ, Phillips AR, Choong SY, Leonard BL, Crossman DJ, Brunton DH, et al. Regeneration of the heart in diabetes by selective copper chelation. Diabetes 2004;53:2501–8.
[42] Bruns CK, Kopito RR. Impaired post-translational folding of familial ALS-linked Cu, Zn superoxide dismutase mutants. The EMBO J 2007;26:855–66.
[43] Benders AA, Li J, Lock RAC, Bindels RJM, Wendelaar BSE, Veerkamp JH. Copper toxicity in cultured human skeletal muscle cells: the involvement of Na^+ /K^+ -ATPase and the Na^+/Ca^{2+} -exchanger. Eur J Physiol 1994;428:461–7.
[44] Strozyk D, Launer LJ, Adlard PA, Cherny RA, Tsatsanis A, Volitakis I, et al. Zinc and copper modulate Alzheimer Aβ levels in human cerebrospinal fluid. Neurobiol Aging 2009;30:1069–77.
[45] Burlando B, Evangelisti V, Dondero F, Pons G, Camakaris J, Viarengo A. Occurrence of Cu-ATPase in dictyostelium: possible role in resistance to copper. Biochem Biophys Res Commun 2002;291:476–83.
[46] Armstrong C, Leong W, Lees GJ. Comparative effects of metal chelating agents on the neuronal cytotoxicity induced by copper (Cu), iron (Fe) and zinc in the hippocampus. Brain Res 2001;892:51–62.
[47] Hirayamaa N, Iimuroa S, Kubonob K, Kokusenc H, Honjo T. Formation of dinuclear copper complex with *N,N,N′,N′*-tetrakis(2-pyridylmethyl)-1,2-ethanediamine in aqueous solution. Talanta 1996;43:621–6.
[48] Blindauer CA, Razi MT, Parsons S, Sadler PJ. Metal complexes of *N,N,N′,N′*-tetrakis (2-pyridylmethyl)ethylenediamine (TPEN): variable coordination numbers and geometries. Polyhedron 2006;25:513–20.
[49] Anderson CJ, Pajeau TS, Edwards WB, Sherman ELC, Rogers BE, Welch MJ. In vitro and in vivo evaluation of copper-64-octreotide conjugates. J Nucl Med 1995;36: 2315–25.
[50] Anderson CJ, Dehdashti F, Cutler PD, Schwarz SW, Laforest R, Bass LA, et al. McCarthy DW. ^{64}Cu-TETA-octreotide as a PET imaging agent for patients with neuroendocrine tumors. J Nucl Med 2001;42:213–21.
[51] Lewis MR, Wang M, Axworthy DB, Theodore LJ, Mallet RW, Fritzberg AR, et al. In vivo evaluation of pretargeted ^{64}Cu for tumor imaging and therapy. J Nucl Med 2003;44:1284–92.
[52] Wu Y, Zhang X, Xiong Z, Cheng Z, Fisher DR, Liu S, et al. Micro PET imaging of glioma integrin $\alpha_v\beta_3$ expression using ^{64}Cu-labeled tetrameric RGD peptide. J Nucl Med 2005;6:1707–18.
[53] McQuade P, Miao Y, Yoo J, Quinn TP, Welch MJ, Lewis JS. Imaging of melanoma using ^{64}Cu- and ^{86}Y-DOTA-ReCCMSH(Arg^{11}), a cyclized peptide analogue of α-MSH. J Med Chem 2005;48:2985–92.
[54] Sun X, Wuest M, Weisman GR, Wong EH, Reed DP, Boswell CA, et al. Radiolabeling and in vivo behavior of copper-64-labeled cross-bridged cyclam ligands. J Med Chem 2002;45:469–77.
[55] Boswell CA, Sun X, Niu W, Weisman GR, Wong EH, Rheingold AL, et al. Comparative in vivo stability of copper-64-labeled cross-bridged and conventional tetraazamacrocyclic complexes. J Med Chem 2004;47:1465–74.
[56] Birker PJ, Freeman HC. Structure, properties, and function of a copper(I)-copper (II) complex of D-penicillamine: pentathallium(I) μ8-Chloro- dodeca (D-penicillaminato)-octacuprate(I) hexacuprate(II) n-Hydrate. J Am Chem Soc 1977;99:6890–9.
[57] Kato N, Nakamura M, Uchiyama T. ^{1}H NMR studies of the reactions of copper(I) and copper(II) with D-penicillamine and glutathione. J Inorg Biochem 1999;75:117–21.
[58] Peisach J, Blumberg WE. A mechanism for the action of penicillamine in the treatment of Wilson's disease. Mol Pharmacol 1969;5:200–9.
[59] Gupte A, Mumper RJ. An investigation into copper catalyzed D-penicillamine oxidation and subsequent hydrogen peroxide generation. J Inorg Biochem 2007;101:594–602.
[60] Brem SS, Zagzag D, Tsanaclis AM, Gately S, Elkouby MP, Brien SE. Inhibition of angiogenesis and tumor growth in the brain. Suppression of endothelial cell turnover by penicillamine and the depletion of copper, an angiogenic cofactor. Am J Pathol 1990;137:1121–42.
[61] Gupte A, Mumper RJ. Copper chelation by D-penicillamine generates reactive oxygen species that are cytotoxic to human leukemia and breast cancer cells. Free Radic Biol Med 2007;43:1271–8.
[62] Zhu H, Huang X, Deng Y, Wu D, Chen C, Liu Q. Synthesis and structural characterization of a linear copper(I) tetrathiomolybdate complex containing the Me_2dtc^- ligand, $(Et_4N)_2[MoS_4(CuMe_2dtc)_2]$. Inorg Chim Acta 1997;256: 29–34.
[63] Beheshti A, Clegg W, Sadr MH. Synthesis and crystal structure of a copper(I) complex containing tetrathiomolybdate and dihydrobis(3,5-dimethylpyrazolyl) borate ligands: $[Et_4N]_2[(Bp')CuMoS_4Cu_2(\mu\text{-}Bp')_2Cu_2MoS_4Cu(Bp')](Bp'=H_2B(3,5\text{-}Me_2Pz)_2)$ and crystal structure of $[(Bp')_2Cu]$. Polyhedron 2001;20:179–83.
[64] Mills CF. Trace elements in animals. Phil Trans R Soc Lond B 1979;288:51–63.
[65] Ogra Y, Komada Y, Suzuki KT. Comparative mechanism and toxicity of tetra- and dithiomolybdates in the removal of copper. J Inorg Biochem 1999;75:199–204.
[66] Ogra Y, Ohmichib M, Suzuki KT. Mechanisms of selective copper removal by tetrathiomolybdate from metallothionein in LEC rats. Toxicology 1996;106: 75–83.
[67] Ogra Y, Chikusa H, Suzuki KT. Metabolic fate of the insoluble copper/tetrathiomolybdate complex formed in the liver of LEC rats with excess tetrathiomolybdate. J Inorg Biochem 2000;78:123–8.
[68] Khan MK, Miller MW, Taylor J, Gill NK, Dick RD, Golen KV, et al. Radiotherapy and antiangiogenic TM in lung cancer. Neoplasia 2002;4:164–70.
[69] Omoto A, Kawahito Y, Prudovsky I, Tubouchi Y, Kimura M, Ishino H, et al. Copper chelation with tetrathiomolybdate suppresses adjuvant-induced arthritis and inflammation-associated cachexia in rats. Arthritis Res Ther 2005;7: 1174–82.
[70] Pan Q, Kleer CG, Golen KL, Irani J, Bottema KM, Bias C, et al. Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis. Cancer Res 2002;62:4854–9.
[71] Pan Q, Bao LW, Merajver SD. Tetrathiomolybdate inhibits angiogenesis and metastasis through suppression of the NFKB signaling cascade. Mol Cancer Res 2003;1:701–6.
[72] Brewer GJ. Copper in medicine. Curr Opin Chem Biol 2003;7:207–12.
[73] Di Vaira M, Bazzicalupi C, Orioli P, Messori L, Bruni B, Zatta P. Clioquinol, a drug for Alzheimer's disease specifically interfering with brain metal metabolism: structural characterization of its zinc(II) and copper(II) complexes. Inorg Chem 2004;43:3795–7.
[74] Ferrada E, Arancibia V, Loeb B, Norambuena E, Olea-Azar C, Huidobro-Toro JP. Stoichiometry and conditional stability constants of Cu(II) or Zn(II) clioquinol complexes; implications for Alzheimer's and Huntington's disease therapy. Neurotoxicology 2007;28:445–9.
[75] Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jone WD, McLean CA, et al. Treatment with a copper-zinc chelator markedly and rapidly inhibits β-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 2001;30:665–76.
[76] Deraeve C, Pitie M, Meunier B. Influence of chelators and iron ions on the production and degradation of H_2O_2 by β-amyloid - copper complexes. J Inorg Biochem 2006;100:2117–26.
[77] Bush AI, Tanzi RE. Therapeutics for Alzheimer's disease based on the metal hypothesis. Neurotherapeutics 2008;5:421–32.
[78] Bush AI. Metal complexing agents as therapies for Alzheimer's disease. Neurobiol Aging 2002;23:1031–8.
[79] Cocco D, Calabrese L, Rigog A, Argeseg E, Rotilio G. Re-examination of the reaction of diethyldithiocarbamate with the copper of superoxide dismutas. J Biol Chem 1981;256:8983–6.
[80] Kelner MJ, Alexander NM. Inhibition of erythrocyte superoxide dismutase by diethyldithiocarbamate also results in oxyhemoglobin-catalyzed glutathione depletion and methemoglobin production. J Biol Chem 1986;261:1636–41.
[81] Cen D, Brayton D, Shahandeh B, Meyskens FL, Farmer PJ. Disulfiram facilitates intracellular Cu uptake and induces apoptosis in human melanoma cells. J Med Chem 2004;47:6914–20.
[82] Daniel KG, Chen D, Orlu S, Cui QC, Miller FR, Dou QP. Clioquinol and pyrrolidine dithiocarbamate complex with copper to form proteasome inhibitors and apoptosis inducers in human breast cancer cells. Breast Cancer Res 2005;7: 897–907.
[83] Valentine HL, Amarnath K, Amarnath V, Valentine WM. Dietary copper enhances the peripheral myelinopathy produced by oral pyrrolidine dithiocarbamate. Toxicol Sci 2006;89:485–94.
[84] Tonkin EG, Valentine HL, Milatovic DM, Valentine WM. *N,N*-diethyldithiocarbamate produces copper accumulation, lipid peroxidation, and myelin injury in rat peripheral nerve. Toxicol Sci 2004;81:160–71.
[85] Viquez OM, Valentine HL, Amarnath K, Milatovic D, Valentine WM. Copper accumulation and lipid oxidation precede inflammation and myelin lesions in *N, N*- diethyldithiocarbamate peripheral myelinopathy. Toxicol Appl Pharmacol 2008;229:77–85.
[86] Viquez OM, Lai B, Ahn JH, Does MD, Valentine HL, Valentine WM. *N,N*-diethyldithiocarbamate promotes oxidative stress prior to myelin structural changes and increases myelin copper content. Toxicol Appl Pharmacol 2009;239:71–9.
[87] Valentine HL, Viquez OM, Amarnath K, Amarnath V, Zyskowski J, Kassa EN, et al. Nitrogen substituent polarity influences dithiocarbamate-mediated lipid oxidation, nerve copper accumulation, and myelin injury. Chem Res Toxicol 2009;22:218–26.
[88] Warshawsky A, Rogachev I, Patil Y, Baszkin A, Weiner L, Gressel J. Copper-specific chelators as synergists to herbicides: 1. amphiphilic dithiocarbamates, synthesis, transport through lipid bilayers, and inhibition of Cu/Zn superoxide dismutase activity. Langmuir 2001;17:5621–35.

[89] Gordge MP, Meyer DJ, Hothersall J, Neild GH, Payne NN, Noronha-Dutra A. Copper chelation-induced reduction of the biological activity of S-nitrosothiols. Br J Pharmacol 1995;140:1083–9.
[90] Xiao Z, Loughlin F, George GN, Howlett GJ, Wedd AG. C-terminal domain of the membrane copper transporter Ctr1 from *Saccharomyces cerevisiae* binds four Cu (I) ions as a cuprous-thiolate polynuclear cluster: sub-femtomolar Cu(I) affinity of three proteins involved in copper trafficking. J Am Chem Soc 2004;126: 3081–90.
[91] Rasoloson D, Shi L, Chong CR, Kafsack BF, Sullivan DJ. Copper pathways in plasmodium falciparum infected erythrocytes indicate an efflux role for the copper P-ATPase. Biochem J 2004;381:803–11.
[92] Göçmen C, Giesselman B, De Groat WC. Effect of neocuproine, a copper(I) chelator, on rat bladder function. J Pharmacol Exp Ther 2005;312:1138–43.
[93] Kumcu EK, Büyüknacar HSG, Göçmen C, Evrüke İC, Önder S. Differential effect of neocuproine, a copper(I) chelator, on contractile activity in isolated ovariectomized non-pregnant rat, pregnant rat and pregnant human uterus. Eur J Pharmacol 2009;605:158–63.
[94] Bhat SH, Azmi AS, Hadi SM. Prooxidant DNA breakage induced by caffeic acid in human peripheral lymphocytes: involvement of endogenous copper and a putative mechanism for anticancer properties. Toxicol Appl Pharmacol 2007;218:249–55.
[95] Itoh S, Kim HW, Nakagawa O, Ozumi K, Lessner SM, Aoki H, et al. Novel role of antioxidant-1 (Atox1) as a copper-dependent transcription factor involved in cell proliferation. J Biolog Chem 2008;283:9157–67.
[96] Apak R, Guclu K, Ozyurek M, Karamedemir SE, Altun M. Total antioxidant capacity assay of human serum using copper (II)-neocuproine as chromogenic oxidant: the CUPRAC method. Free Radic Res 2005;39:949–61.
[97] Zhu BZ, Chevion M. Copper-mediated toxicity of 2,4,5-trichlorophenol: biphasic effect of the copper(I)-specific chelator neocuproine. Arch Biochem Biophys 2000;380:267–73.
[98] Peters ZJ, Nykamp JA, Passaperuma K, Carlson JC, DeWitte-Orr SJ, Greenberg BM, et al. Effect of copper on the cytotoxicity of phenanthrene and 9,10-phenanthrenequinone to the human placental cell line, JEG-3. Reprod Toxicol 2007;23:513–20.
[99] Sayre LM. Alzheimer's precursor protein and the use of bathocuproine for determining reduction of copper(II). Science 1996;274:1933–4.
[100] Laggner H, Hermann M, Gmeiner BMK, Kapiotis S. Cu^{2+} and Cu^{+} bathocuproine disulfonate complexes promote the oxidation of the ROS-detecting compound dichlorofluorescin (DCFH). Anal Bioanal Chem 2006;385:959–61.
[101] Fritsky IO, Kozłowski H, Sadler PJ, Yefetova OP, Świątek- Kozłowska J, Kalibabchuk VA, et al. Template synthesis of square-planar nickel(II) and copper(III) complexes based on hydrazide ligands. Dalton Trans 1998;19: 3269–74.
[102] Messori L, Casini A, Gabbiani C, Sorace L, Muniz-Miranda M, Zatta P. Unravelling the chemical nature of copper cuprizone. Dalton Trans 2007;21:2112–4.
[103] Fritsky IO, Kozlowski H, Kanderal OM, Haukka M, Swiatek-Kozlowska J, Gumienna-Kontecka E, et al. Efficient stabilization of copper(III) in tetraaza pseudo -macrocyclic oxime-and-hydrazide ligands with adjustable cavity size. Chem Commun 2006;39:4125–7.
[104] Torkildsen Ø, Brunborg LA, Myhr KM, Bø L. The cuprizone model for demyelination. Acta Neurol Scand 2008;117:72–6.
[105] Matsushima GK, Morell P. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Pathol 2001;11:107–16.
[106] Zatta P, Raso M, Zambenedetti P, Wittkowski W, Messori L, Piccioli F, et al. Copper and zinc dismetabolism in the mouse brain upon chronic cuprizone treatment. Cell Mol Life Sci 2005;62:1502–13.
[107] Hart EB, Steenbock H, Waddell J, Elvehjem CA. Iron nutrition. VII. Copper is a supplement to iron for hemoglobin building in the rat. J Biol Chem 1928;77: 797–812.
[108] Kim B-E, Turski ML, Nose Y, Casad M, Rockman HA, Thiele DJ. Cardiac copper deficiency activates a systemic signaling mechanism that communicates with the copper acquisition and storage organs. Cell Metab 2010;11:353–63.
[109] Williams DM. Copper deficiency in humans. Semin Hematol 1983;20:118–28.
[110] Heller RM, Kirchner SG, O'Neill Jr JA, Hough Jr AJ, Howard L, Kramer SS, et al. Skeletal changes of copper deficiency in infants receiving prolonged total parenteral nutrition. J Pediatr 1978;92:947–9.
[111] Khan SN, Rahman MA, Samad A. Trace elements in serum from Pakistani patients with acute and chronic ischemic heart disease and hypertension. Clin Chem 1984;30:644–8.
[112] Ford ES. Serum copper concentration and coronary heart disease among US adults. Am J Epidemiol 2000;151:1182–8.
[113] Bayer TA, Multhaup G. Involvement of amyloid β precursor protein (AβPP) modulated copper homeostasis in Alzheimer's disease. J Alzheimers Dis 2005;8: 201–6.
[114] Suzuki KT, Someya A, Komada Y, Ogra Y. Role of metallothionein in copper homeostasis: responses to Cu-deficient diets in mice. J Inorg Biochem 2002;88: 173–82.