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BIOCHEMISTRY

Metabolism of copper and possibilities for its regulation

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Abstract. Copper is an indispensable biometal participating as a redox catalyst in many important biochemical processes. However, if uncontrolled, copper ions induce the formation of reactive oxygen species and become toxic. For this reason, cellular copper metabolism is tightly regulated and specific proteins – copper chaperones – participate in the metalation of cellular copper transporters and enzymes. The thermodynamic background for cellular copper distribution is known, and copper is driven to cellular destinations according to shallow affinity gradients. Copper metabolism is disturbed in the case of Wilson's, Menkes, and Alzheimer's disease (AD), characterized by copper overload, deficiency, and misdistribution, respectively. Wilson's and Menkes disease could be treated by copper chelators and supplements, respectively; however, with AD, a search for effective molecular tools for the correction of copper metabolism is ongoing. One natural copper-binding ligand – α-lipoic acid – has shown positive results in cellular and fruit fly models of AD and serves as a promising candidate for the regulation of copper metabolism in the case of AD.

Keywords: copper metabolism, Wilson's disease, Menkes disease, Alzheimer's disease, copper chelation, copper ionophores, lipoid acid.

INTRODUCTION

Copper is an essential biometal whose chemical and redox properties are exploited for promoting a variety of biological functions. In the majority of cases, copper is functioning as a crucial cofactor of the key enzymes involved in cellular energy production (cytochrome c oxidase (CCO)), antioxidative defense (Cu,Zn-superoxide dismutase (Cu,Zn-SOD)), cross-linking of elastin, collagen (lysyl oxidase) and keratin (sulfhydryl oxidase), melanin production (tyrosinase), and metabolism of iron (ceruloplasmin (CE)). In the brain, some copper proteins play specific roles in the synthesis of noradrenaline and neuropeptides (dopamine β-hydroxylase and peptidylglycine α-amidating monooxygenase) [1]. Copper is also involved in myelination, in the regulation of circadian rhythms, blood coagulation and angiogenesis [2]. In some cases, the "free" copper ions have a biological function. It has been established that in a certain type of brain neurons, copper ions are packed into secretory vesicles, which are upon stimulation released into the synaptic cleft, where copper ions play a neuromodulatory role [3].

Besides being an essential trace element, copper is also a potentially toxic element. Namely, "free" or weakly complexed copper ions can generate reactive oxygen species (ROS), including highly reactive hydroxyl radicals by interacting with oxygen metabolites such as hydrogen peroxide [4]. Hydroxyl radicals are able to react indiscriminately with proteins, nucleic acids, or lipids by causing irreversible damage. This bifacial nature of copper ions dictates the requirement for their tight control in biological systems, which is granted by the combined action of copper transporters, intracellular copper chaperones, metallothioneins, and extracellular copper-buffering proteins like serum albumin (HSA) [5,6]. Defects in the functioning of these proteins cause copper dysregulation, such as deficiency, misdistribution, or excessive accumulation leading to various diseases [7]. Traditional examples of excessive accumulation and deficiency of copper are Wilson's disease (WD) and Menkes disease

(MD), respectively. Copper misdistribution is characteristic for Alzheimer's disease (AD).

CELLULAR COPPER METABOLISM

Copper enters the eukaryotic cell in the form of Cu(I) ions mainly through high-affinity plasma membrane copper transporters Ctr1/2 [8]. Although there are only two principal copper enzymes CCO and Cu,Zn-SOD inside the cells, cellular copper metabolism is very complex (Fig. 1). Copper influx and efflux systems are highly regulated and, moreover, within the cell, copper ions are delivered to the sites of utilization by special proteins called copper chaperones [9]. Copper chaperones bind and transport Cu(I) ions to either intracellular copper enzymes or to membrane copper transporters that transfer Cu(I) ions into the trans-Golgi network (TGN) for incorporation into secretory copper enzymes such as Cu,Zn-SOD3, CP and others. The cytoplasmatic copper chaperones HAH1 and CCS deliver copper to their partners, namely the soluble cytosolic domains of a Cu(I)-ATPases – ATP7A (Menkes protein) or ATP7B (Wilson's protein) located in the TGN membrane and the Cu,Zn-SOD1, respectively [10]. Cox17 shuttles between the cytoplasm and mitochondrial lumen, delivering Cu(I) ions to Sco1, Sco2, and Cox11, located in the inner mitochondrial membrane. Sco1/2 pass Cu(I) ions to the copper A (CuA) site and Cox11 acts as a copper donor to the copper B (CuB) site of CCO [10,11]. Besides copper chaperones, cellular cytosol is rich in ubiquitous cysteine-rich proteins – metallothioneins [12] – and contains millimolar concentrations of glutathione (GSH) [13], which both bind Cu(I) ions and are also important regulators of copper metabolism.

The distribution of copper ions within cells is determined by both thermodynamic and kinetic factors. By using a unified ESI-MS-based approach, the Cu(I)-binding constants for a representative set of key cellular copper proteins and small Cu(I)-ligands have been determined [14,15]. According to the results of these studies, Cu(I)-binding affinities (log K_D values) of cellular copper proteins lie in the range of 17–20, and copper ions are directed toward target proteins by affinity gradients, which are quite shallow. The difference between the affinities of copper chaperones and their partner proteins is approximately 10-fold [14]. Metallothioneins have high Cu(I)-binding affinity, and they participate in the binding of excessive copper ions into redox-silenced complexes [16]. Through binding into high-affinity protein complexes, the

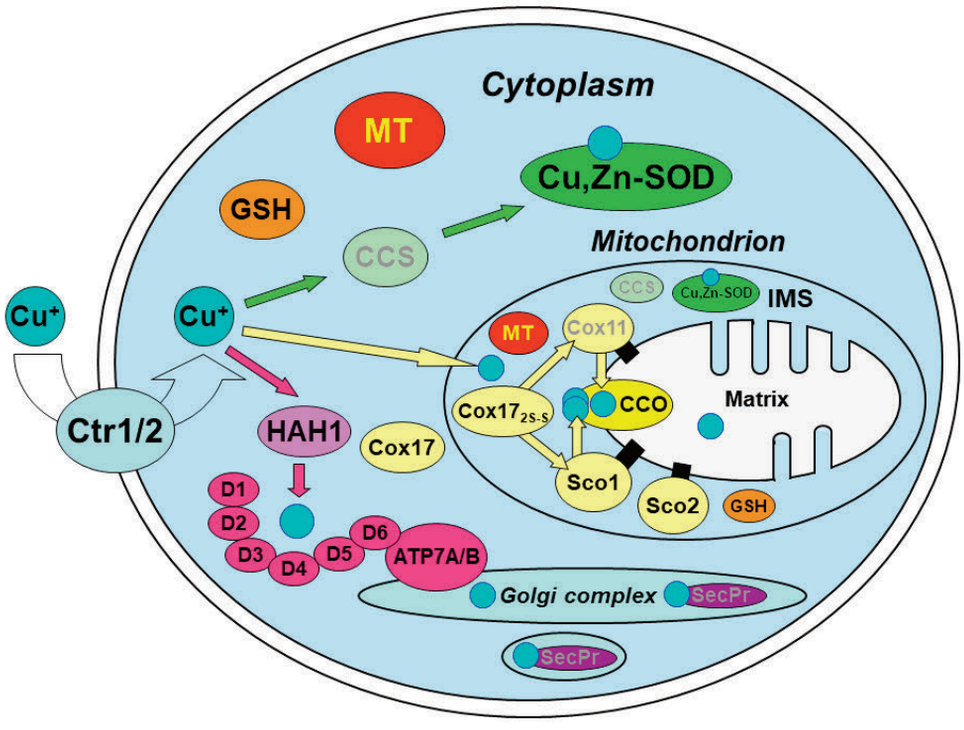

Fig. 1. Cellular copper metabolism. Abbreviations: Ctr1/2 – copper transporter 1 and 2; MT – metallothionein; CCS – copper chaperone for Cu,Zn-superoxide dismutase (Cu,Zn-SOD); HAH1 – copper chaperone for ATP7A (Menkes protein) or ATP7B (Wilson's protein); D1–D6 – copper-binding domains of ATP7A/B; Cox11, Cox17, Sco1 and Sco2 – copper chaperones for cytochrome c oxidase (CCO); GSH – glutathione; SecPr – secretory copper proteins.

intracellular concentration of "free" or non-protein-bound Cu(I) ions is extremely low under normal conditions and lower than one copper atom per cell [17]. Apparently, this situation protects cells from the uncontrolled copper-catalyzed generation of reactive oxygen radicals; however, it necessitates the delivery of copper ions to the target proteins through specific protein–protein interactions, which is the primary role of copper chaperones.

COPPER IN THE EXTRACELLULAR SPACE

The extracellular copper pool exists mainly in the blood, which is the central medium for copper transport and distribution in the body. In the blood, copper ions are assumingly distributed mainly between three proteins: CP (appr. 70%), HSA (appr. 15%), and alpha-2-macroglobulin (α2M) (appr. 5–10%) [18,19]. The remaining 5% of copper is found in Cu,Zn-SOD3, clotting factors V and VIII, amine and diamine oxidases, ferroxidase II, and some other enzymes [19].

In a recent study, it was attempted to determine the Cu(II)-binding affinities of CP, HSA, and α2M by using a unified approach, which is prerequisite for understanding the distribution of copper in the blood [20]. Determination of Cu(II)-binding affinities for HSA, CP, and α2M were attempted through their competition with a set of low-molecular-weight Cu(II)-binding compounds (DTPA, EDTA, NTA, His) by using a LC-ICP MS-based approach [20]. By using NTA, which forms 1:1 complex with Cu(II) ions and shows slow demetalation of Cu•HSA, a K_D value equal to 0.90 pM was determined. However, titration with His, which forms 2:1 complex and shows fast demetalation of Cu•HSA, yielded K_D equal to 34.7 fM [20]. The difference in K_D value, determined from the competition with NTA and His, might be explained by the fact that the formation of a putative ternary complex between HSA, Cu(II) ion, and His was omitted in the binding scheme. Thus, the K_D value determined with NTA should be considered a more accurate estimate.

It was found that CP binds Cu(II) ions at pH 7.4 extremely tightly and can be demetalated by high millimolar EDTA only at non-physiologically high pH values (pH 11) [20]. The inertness of the Cu•CP complex at pH 7.4 is granted most probably due to the entrapment of metal ions in the protein interior, which hinders their dissociation. This conclusion is supported by structural data indicating that in Cu•CP, the copper ions are located in three mononuclear and one trinuclear binding sites, which are not exposed to the solvent [21]. Kinetical inertness of the Cu•CP complex does not allow the determination of Cu(II)-binding affinity of CP, which confirms earlier conclusions that CP-bound copper ions are practically nonexchangeable at physiological pH values [19].

Experiments with α2M demonstrated that *in vitro* α2M does not bind Cu(II) ions [20]. The obtained thermodynamic and kinetic data are important for understanding copper distribution in human blood as well as in other extracellular media, e.g., cerebrospinal fluid (CSF).

COPPER METABOLISM IN WILSON'S DISEASE

The prevalence of WD is around 1/30 000 live births [22]. WD is characterized by loss-of-function mutations in a P-type copper ATPase, ATP7B, which is expressed mostly in the liver [23,24]. The WD protein has a dual role – at normal copper levels, it is located in TGN membranes and transports copper into the TGN for incorporation into the plasma copper proteins, including CP [25,26]. At excessive copper levels, ATP7B firstly translocates to lysosomal membranes and transports copper into their lumen and, secondly, activates lysosomal exocytosis and stimulates the release of copper into bile [27]. Defective ATP7B functioning causes reduced incorporation of copper into CP and copper accumulation primarily in the liver and then in the brain, where copper concentrations are typically increased by a factor of 5–20 and 10–15, respectively [22]. Copper accumulation leads to liver disorders (liver cirrhosis and cancer) and/or neuropsychiatric symptoms like movement disorders (tremors, involuntary movements), seizures, etc. [28,29]. The symptoms usually begin between the ages of 5 and 35 years and, if untreated, tend to become progressively worse and are eventually fatal due to the liver, kidney, or hematological complications. Cytotoxicity arises from oxidative damage induced by the excess of copper, but may additionally result in the disruption of Zn-related metabolic systems, which is ultimately cytotoxic [30].

CP in blood serum is decreased in the case of WD, and the activity of CP is decreased even more as a considerable fraction of CP is not metalated [22,31]. Under normal conditions, serum CP levels are very low in neonates and elevate to a maximum 300–500 mg/L concentration between 2–3 years of age and then gradually decrease to the adult range (>200 mg/L) until the teenage period [32]. The concentration of serum non-CP bound copper is elevated above 250 μg/L in most untreated WD patients (normal level <150 μg/L), and it has also been proposed as a diagnostic marker for WD [22,28,33].

COPPER METABOLISM IN MENKES DISEASE

MD is a relatively rare disease with incidence ranging from 1/50 000 to 1/360 000 live births, depending on the population studied [2]. MD is caused by loss-of-function mutations in ATP7A [24]. Under normal physiological

copper concentrations, ATP7A is localized to TGN membranes, transporting copper into the lumen for inclusion into the copper-dependent enzymes [34]. Under increased copper concentrations, ATP7A is translocated to the vesicles in a mouse model [35] or to the plasma membrane in CHO cells [36]. In the case of MD, transport of copper from intestine enterocytes to the blood is defective and leads to organismal copper deficiency affecting mainly the liver and the brain [2]. Copper deficiency leads to reduced activities of copper-dependent enzymes, such as CCO, Cu,Zn-SOD, lysyl oxidase, tyrosinase and dopamine β-hydroxylase, which are associated with most of the clinical features of MD [2]. MD is a progressive disorder leading usually to death before the third year of life; only some patients survive above 5 years of age [2].

Serum copper and CP levels are typically low in MD, in the range of 0–55 μg/dL (normal level 70–150 μg/dL) and 10–160 mg/dL (normal level 200–450 mg/dL), respectively. These levels are typically low in less than six months old babies [2,37].

COPPER METABOLISM IN ALZHEIMER'S DISEASE

AD is the most common neurodegenerative disease, comprising around 75% of dementia cases in the elderly [38]. In the USA, there are currently 6.7 million people living with AD, which is 2% of the population [39]. The disease is characterized by the extracellular deposition of β-amyloid (Aβ) peptides in the form of senile plaques and the intracellular deposition of hyperphosphorylated tau protein, oxidative damage, and neuronal death in the brain [40]. The progressive loss of neurons throughout the brain during the disease slowly destroys memory and cognitive skills and leads eventually to death. According to the amyloid cascade hypothesis, the Aβ peptide aggregation and formation of amyloid plaques is the key event, which in turn causes neurofibrillary tangles and cell death [41,42].

Copper metabolism in AD is characterized by copper misdistribution. Quantitative meta-analyses of numerous independent studies show that in the case of AD, copper levels in blood serum are substantially elevated [43,44] and, simultaneously, copper levels in brain tissue are decreased [45]. A later study confirmed that in AD, brain copper levels are substantially (53–70%) decreased in the seven studied brain regions, resembling MD, which is also characterized by neurodegeneration [46]. The results of a recent meta-analysis also show that Cu decreases in AD brain samples, whereas Cu and non-CP Cu are increased in serum/plasma samples, and that CP does not change [47].

COPPER LEVELS IN BLOOD AFFECTED BY AGING AND ITS RELATION WITH ALZHEIMER'S DISEASE

It has been known for a long time that in the normal population, copper levels increase with age in serum [48] and decrease in brain tissue [49]. Moreover, in the same age groups, there are significant interindividual differences in copper levels both in serum [48] as well as in the brain [49]. The age-related tendencies in copper levels in serum and in the brain are similar to the changes observed in AD, whereas in the latter case, the changes are more pronounced. Therefore, it is reasonable to hypothesize that an individual increase of copper level in serum and a concomitant decrease in the brain is a normal age-dependent process, but after the crossing of a certain level, copper dysregulation might lead to AD pathology. According to such a scenario, dysregulation of copper metabolism is an early event in AD pathology and its normalization might be an effective strategy for the prevention of AD.

There have been attempts to find the genetic background behind elevated non-CP bound copper in the serum of AD patients, which have been, however, limited to the analysis of *ATP7B* [50]. Some positive correlation between certain variants of *ATP7B* and elevated copper levels has been found [50]; however, the situation could be much more difficult as systemic copper levels are regulated by multiple copper transporters, including ATP7A, Ctr1/2, and others, as well as by cellular and extracellular copper proteins and ligands.

Analysis of *ATP7A* and *ATP7B* copper transporter genes supports the complexity of organismal copper regulation and its connections with the genetic background. It is impressive that in WD, which is an autosomal recessive disease, there are currently 685 mutations found in *ATP7B* (The Human Gene Mutation Database), which lead to an enormous variability of the disease phenotype from mild to severe. At the same time, there are also heterozygotic carriers of these mutations, where systemic copper metabolism should be affected. MD is an X-linked recessive disease where 70% of cases are inherited and 30% arise due to new mutations in copper transporter ATP7A. There are currently 174 mutations found in *ATP7A* (The Human Gene Mutation Database). MD affects mainly men, but there are both male and female carriers where systemic copper metabolism should be affected. There is also substantial polymorphism in these major copper regulating genes, which brings us to the conclusion that copper metabolism is highly individual. This conclusion is also supported by the facts that the levels of serum copper determined by different authors are very heterogeneous [51] and variable at the individual level [48].

TREATMENT OF WILSON'S AND MENKES DISEASE

Unlike many other genetic disorders, WD and MD can be treated by correcting the abnormal copper metabolism [52,53].

WD is treatable, primarily by copper-chelation therapy, which promotes copper excretion. Several de-coppering drugs have been used for the treatment of Wilson's disease, two of which have also been approved by the FDA. D-penicillamine, the first orally administered copper-chelating agent available, was approved for therapeutic use in 1956 [54]. D-penicillamine induces copper excretion into urine [55]; however, its usage causes many adverse side effects [28]. The second oral copper-chelating drug, trientine, was approved in 1982. Trientine also acts through enhancing the urinary excretion of copper, whereas it is better tolerated than D-penicillamine [56]. The third de-coppering drug is tetrathiomolybdate, which was introduced in 1984 and was used in a limited number of WD patients [57]. Initial studies with ammonium tetrathiomolybdate [57] and a completed phase II clinical study with bis-choline tetrathiomolybdate [58] demonstrated that the drug acts rapidly and improves copper control by stabilizing liver function. It also improved neurologic symptoms and showed a favorable safety profile [59]. In addition to these three major approved drugs, two other copper-chelating compounds have been used for the treatment of WD in the past: an injectable drug, British anti-Lewisite or dimercaptopropanol was used in the UK in 1951 [60], and dimercaptosuccinate has been administred to hundreds of patients in China [61,62]. In Western medicine, British anti-Lewisite and dimercaptosuccinate are used primarily for the treatment of arsenic, mercury, and lead poisoning [63,64].

The treatment of MD in major cases is mainly symptomatic; however, clinical reports suggest that careful medical care and early copper supplementation may substantially modify disease progression and extend the life span up to 13 years or even more [2]. The purpose of a specific treatment for MD is to provide copper to copper-dependent enzymes. Copper should be supplemented parenterally or subcutaneously because orally administrated copper is trapped in the intestines and is ineffective. Among the available copper compounds, copper–His complex has been proven to be the most effective [65,66]. The positive outcome of copper–His supplementation is dependent on early initiation and the presence of at least partially functional ATP7A [2].

TREATMENT OF ALZHEIMER'S DISEASE BY MODIFYING COPPER METABOLISM

The role of altered metal homeostasis as a pathogenic factor in AD has been intensively studied; moreover, the assumption about the causative role of metal ions in AD has laid the basis for the elaboration of metal chelation therapeutic approach in AD [67]. The final aim of this approach is finding therapeutic agents, which modulate metal distribution in the brain and have the potential to ameliorate the dysfunctional copper metabolism characteristic of AD.

Several attempts have been made to treat AD by modifying copper metabolism, and three different strategies – copper supplementation, chelation, and redistribution – have all been tested in laboratory experiments as well as in clinical trials. Copper supplementation was tested with copper orotate [68,69], chelation with D-penicillamine [70], and copper redistribution with copper ionophores clioquinol (CQ, 5-Chloro-8-hydroxy-7-iodoquinoline) and its derivative PBT2 (5,7-dichloro-8-hydroxy-2-[(dimethylamino) methyl]quinoline) [71,72]. In clinical trials, copper supplementation showed no effect on the progression of AD phenotype after a 12-month treatment period [69]. D-penicillamine promoted decoppering of the organism and reduced oxidative stress but did not affect the clinical progression of the disease in a 6-month trial [70]. CQ was tested in phase II clinical trial with 36 patients [73], which did not show any statistically significant difference in cognition between the treatment and placebo groups after 36 weeks of treatment [73,74]. CQ, however, has been withdrawn from development due to safety concerns as it did not succeed in reducing a mutagenic ingredient "diiodo" CQ to an acceptable level [74]. PBT2, an improved version of CQ, was tested in two clinical trials [75]. In one of them, PBT2 showed a favorable safety profile [73] and did reduce Aβ42 concentration in CSF, compared with the patients treated with a placebo [76]. Initially, it was concluded that PBT2 does not improve the cognitive function of AD patients [76]; however, a later analysis of the results detected some improvement of cognition [75,77]. The second trial of PBT2 was more thoroughly conducted and showed that, after 12 weeks of treatment, this compound was safe and well tolerated in people with mild AD; however, it was concluded that larger and longer trials are required for a reliable demonstration of cognitive efficacy [73]. In addition to clinically tested compounds, numerous other synthetic Cu(II)-binding ligands [78–84], including Trientine [85], an FDA-approved WD drug, as well as zinc treatment [86], have been proposed for the treatment of AD. Trientine and all other drugs of this type as well as zinc treatment result in the decoppering of the organism [87,88], which may undesirably further decrease copper levels in the brain. These and earlier attempts are largely trial and error attempts, which are not based on a comprehensive understanding of the organismal copper metabolism, in general, and copper-binding properties of the ligands in comparison with organismal copper-binding proteins, in particular.

In a recent study, we proposed molecular tools to normalize copper metabolism in AD, based on systematic knowledge about the metal-binding properties of AD drug candidates, which have the potential to normalize dysregulated copper metabolism in AD. Thus far, copper chelators with preferential binding of Cu(II) ions have been proposed for AD treatment [78,79]. These chelators are active in extracellular space such as blood, interstitial fluid, and CSF. In the case of AD, such drugs might decrease the elevated copper levels in the extracellular space; however, an undesirable decrease in the intracellular copper levels in the brain cells might also occur. Therefore, such ligands are not suitable for the translocation of extracellular excessive copper to intracellular space necessary for the normalization of copper levels in the case of AD. Alternatively, we proposed to normalize copper metabolism in AD by using Cu(I) ligands, binding copper ions only in intracellular space and having the potential to shift the equilibrium of copper distribution from extracellular to intracellular location. Cu(I)-binding properties of Cu(I)-binding ligands should, however, be moderate to avoid the demetalation of cellular copper proteins. Cu(I)-binding properties of the cellular copper proteome were known from our earlier study [14], and Cu(I)-binding affinities of a set of copper chelators, including WD drugs and the reduced form of a natural compound α-lipoic acid (LA), were also determined [15]. It turned out that dihydro-LA (DLA) has substantial Cu(I)-binding affinity, which is higher than that of GSH but lower than those of intracellular copper chaperones and enzymes [14,15]. Thiol groups of LA are reduced inside the cell and oxidized to a disulfide bond in the extracellular environment. These properties make LA a suitable candidate for translocating copper to the intracellular space without disturbing the function of intracellular copper proteins.

We have performed two types of experiments, testing the involvement of LA in the regulation of cellular copper metabolism and suitability for the treatment of AD. We first performed experiments with SHSY-5Y cell lines and demonstrated that supplementation with LA significantly increases the intracellular copper level in a dose-dependent manner. Next, by using transgenic AD fruit fly models, we showed that LA can alleviate the phenotype of these mutant flies in a negative geotaxis experiment. The obtained results support the new copper-regulating mechanism of LA cellular action and its applicability for the prevention or treatment of AD [89].

LA is a natural ligand, synthesized enzymatically in the mitochondrion from octanoic acid [90]. LA is primarily functioning as a covalently linked cofactor of mitochondrial α-ketoacid dehydrogenases [91]. In addition, LA is absorbed from food and has a number of biochemical activities in the unbound form [90]. Currently, the biological effects of LA are explained with its antioxidant action; however, its potential in detoxification of heavy metals like Hg has also been recognized [92]. In connection with AD and aging, it is worth mentioning that LA improves the memory of aged nontransgenic (NMRI) mice [93] as well as transgenic AD (Tg2576) mice [94]. Importantly, LA has also been tested in clinical trials and has therapeutic value in the treatment of diabetic polyneuropathy [95]. In AD clinical trials, a daily dose of 600 mg showed a positive effect by slowing the progression of cognitive impairment in patients with mild AD (43 patients, trial duration 48 months) [96,97] and in patients with mild to moderate AD with and without insulin resistance (126 patients, duration of trial 16 months) [98]. Despite these promising results, clinical trials with LA have not been taken forward. The therapeutic effect of LA in these trials has mainly been attributed to its antioxidative effect, but its metalloregulatory properties have not been considered and studied.

There are many benefits of LA over other synthetic compounds in drug development as well as in further therapeutic use. LA has been approved for the treatment of diabetic polyneuropathy [95] and could be repurposed for therapeutic application in the case of AD [99]. Known toxicology and pharmacodynamic profiles of repurposed drugs significantly accelerate the drug development process, decrease the related costs, and increase the probability of success. LA is extremely well-tolerated and causes no adverse effects at doses up to 2400 mg/day in human clinical trials [100]. LA is water-soluble, moderately lipophilic and has a molecular mass less than 500 Da, which match the characteristics of a successful oral drug [101]. Moreover, LA is a natural compound, approved as a food supplement in many countries and is freely and cheaply available in pharmacies.

PARTICIPATION OF LIPOYLATED PROTEINS IN COPPER-INDUCED CELL DEATH – CUPROPTOSIS

Recently, a new copper-dependent mechanism of regulated cell death – cuproptosis – has been discovered, and was demonstrated to be distinct from all known mechanisms of regulated cell death, including apoptosis, ferroptosis, pyroptosis, and necroptosis [102]. Cuproptosis is dependent on mitochondrial respiration and is triggered by direct binding of excessive Cu(I) ions to lipoylated proteins functioning in the tricarboxylic acid (TCA) cycle. The affected proteins include four enzymes: dihydrolipoamide branched chain transacylase E2, glycine cleavage system protein H, dihydrolipoamide S-succinyltransferase, and dihydrolipoamide S-acetyltransferase, an essential component of the pyruvate dehydrogenase com-

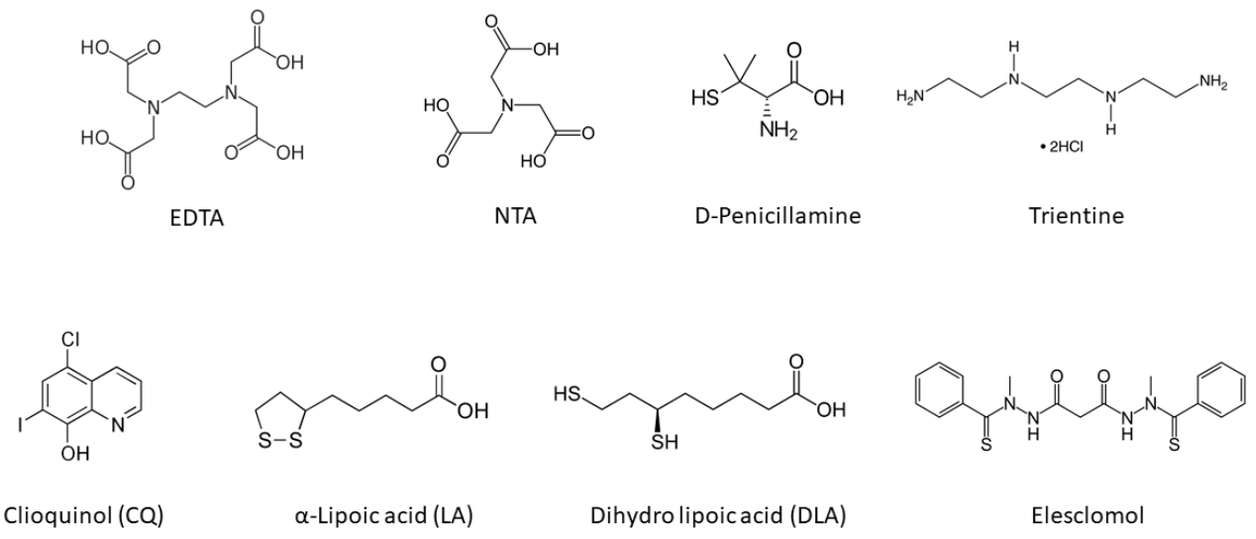

Fig. 2. Ligands used for studies and regulation of copper metabolism.

plex [91]. Lipoylation of these proteins is required for enzymatic function; however, Cu(I) binding results in the oligomerization and aggregation of the lipoylated proteins. This leads to subsequent iron-sulfur cluster protein loss, induction of HSP70, indicative for acute proteotoxic stress, and ultimately cell death [102].

Cuproptosis was induced by ionophores such as elesclomol, disulfiram, 8-hydroxy quinoline and others, inducing a 5- to 10-fold increase in the levels of intracellular copper [102]. This result shows that synthetic copper ionophores may cause a substantial increase of cellular copper content, reaching to toxic levels, which explains why their application for regulation of copper metabolism in AD and other diseases should be taken with caution. Depletion of the endogenous intracellular copper chelator GSH by buthionine sulfoximine sensitized cells to cuproptosis [102], which shows that cuproptosis is dependent on cellular oxidative stress.

The term "cuproptosis" was widely accepted among copper scientists, and, already in 2022, there were 269 research papers mentioning this term in PubMed.

CONCLUSION

Organismal copper metabolism is highly regulated, and its misregulation occurs in the case of various diseases such as Wilson's disease, Menkes disease, and Alzheimer's disease. Wilson's and Menkes diseases can be treated by using copper chelators or copper complexes, respectively. Alzheimer's disease is in search of a therapeutic ligand able to reduce extracellular copper levels and simultaneously increase intracellular levels. Many synthetic ionophores including clioquinol and PBT2 have been used for such a purpose. However, the usage of synthetic copper ionophores should be taken with care as they can substantially increase intracellular copper content and induce regulated cell death called "cuproptosis". One promising ligand for the normalization of copper metabolism in the case of Alzheimer's disease is the natural compound α-lipoic acid, which has been proven to be effective in cellular and fruit fly experiments.

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Photo: private collection/ Priit Palumaa

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Vase metabolism ja selle reguleerimise võimalused

Peep Palumaa

Vask on asendamatu biometall, mis osaleb redoks-katalüsaatorina paljudes olulistes biokeemilistes protsessides. Samaaegselt kutsuvad kontrollimatud ehk nn vabad vaskioonid esile reaktiivsete hapnikuühendite moodustumist ja muutuvad toksiliseks. Sel põhjusel on raku vase metabolism rangelt reguleeritud ja spetsiifilised valgud – vaskšaperoonid – osalevad raku vase transporterite ja ensüümide metaleerimisel. Rakulise vase jaotumise termodünaamiline taust on teada ja vaskioonid juhitakse raku sihtkohtadesse vastavalt lamedatele afiinsusgradientidele. Vase ainevahetus on häiritud Wilsoni, Menkesi ja Alzheimeri tõve (AD) puhul, mida iseloomustab vastavalt vase ülekoormus, defitsiit ja vale jaotumine. Wilsoni ja Menkesi tõbe saab ravida vastavalt vase kelaatorite ja toidulisanditega, kuid AD puhul otsitakse tõhusaid molekulaarseid vahendeid vase metabolismi korrigeerimiseks. Üks looduslik vaske siduv ligand – α-lipoehape – on näidanud positiivseid tulemusi AD puuviljakärbse- ja rakumudelites ning on paljutõotav kandidaat vase metabolismi reguleerimiseks AD korral.