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REVIEW ARTICLE

Does Ceruloplasmin Defend Against Neurodegenerative Diseases?

Bo Wang[1,2] and Xiao-Ping Wang[1,3,*]

[1]*Shanghai General Hospital of Nanjing Medical University, Shanghai, 200080, China;* [2]*Department of Neurology Baoshan Branch, Shanghai General Hospital, Shanghai, 200940, China;* [3]*Department of Neurology, Shanghai Tong-Ren Hospital, Shanghai Jiao-Tong University School of Medicine, Shanghai, 200080, China*

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Abstract: Ceruloplasmin (CP) is the major copper transport protein in plasma, mainly produced by the liver. Glycosylphosphatidylinositol-linked CP (GPI-CP) is the predominant form expressed in astrocytes of the brain. A growing body of evidence has demonstrated that CP is an essential protein in the body with multiple functions such as regulating the homeostasis of copper and iron ions, ferroxidase activity, oxidizing organic amines, and preventing the formation of free radicals. In addition, as an acute-phase protein, CP is induced during inflammation and infection. The fact that patients with genetic disorder aceruloplasminemia do not suffer from tissue copper deficiency, but rather from disruptions in iron metabolism shows essential roles of CP in iron metabolism rather than copper. Furthermore, abnormal metabolism of metal ions and oxidative stress are found in other neurodegenerative diseases, such as Wilson's disease, Alzheimer's disease and Parkinson's disease. Brain iron accumulation and decreased activity of CP have been shown to be associated with neurodegeneration. We hypothesize that CP may play a protective role in neurodegenerative diseases. However, whether iron accumulation is a cause or a result of neurodegeneration remains unclear. Further research on molecular mechanisms is required before a consensus can be reached regarding a neuroprotective role for CP in neurodegeneration. This review article summarizes the main physiological functions of CP and the current knowledge of its role in neurodegenerative diseases.

Keywords: Ceruloplasmin, iron, copper, oxidative stress, free radicals, neurodegeneration, neurodegenerative diseases.

1. INTRODUCTION

Ceruloplasmin (CP) is a glycoprotein of the serum and was first isolated from plasma by Holmberg and Laurell in 1948 [1]. It is characterized as a copper (Cu)-containing protein binding 40-70% of Cu in the plasma and is mainly produced by the liver [2, 3]. Notably, its membrane-anchored form glycosylphosphatidylinositol (GPI)-linked CP has been found in glial cells (central nervous system and retina) and Sertoli cells (testis) [4-6]. To date, many physiological functions of CP have been demonstrated (Table **1**), including transportation of Cu, regulation of iron homeostasis, ferroxidase activity, oxidation of organic amines and ascorbate oxidase activities, as well as antioxidant activity through the prevention of free radicals formation [7, 8]. In turn, CP has also been shown to have prooxidant properties [9]. Furthermore, CP is reported to be an acute phase reactant in inflammation, infection, trauma, diabetes and pregnancy, which is mostly attributed to its antioxidant properties [10-12].

*Address correspondence to this author at the Shanghai General Hospital of Nanjing Medical University, Shanghai, 200080, China; Tel: +86-21-63240090; Fax: +86-21-63243755; E-mail: x_p_wang@sjtu.edu.cn

In addition, an increasing evidence suggests that abnormal metabolism of Cu and iron is observed in many neurodegenerative diseases, such as Wilson's disease (WD), aceruloplasminemia, Alzheimer's disease (AD) and Parkinson's disease (PD) [13-16]. Therefore, it is reasonable to hypothesize that CP might play a neuroprotective role in neurodegenerative diseases by regulating homeostasis of cellular Cu and iron and protecting tissues from oxidative damage. Herein, we reviewed the most recent findings on the physiological functions of CP, and discussed its roles in neurodegenerative diseases.

2. CP STRUCTURE AND EXPRESSION

CP is widely distributed in various types of eukaryotes, including mammals, fish, plants and fungi [17, 18]. The human CP gene is located on chromosome 3q23-q24 ,encodes in 20 exons and spans about 65 kb of DNA [19]. CP is constituted by a single chain of 1046 amino acids and has a total weight of about 132 kDa [20]. X-ray crystallography of copper-containing (holo) CP shows overall three sequential homologous regions each with 2 domains and each domain tightly bound to one Cu atom [9]. Among these domains, there is a trinuclear cluster between the first and last do-

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Table 1. Physiological functions of CP.

Items	Functions	Target of Reaction
Enzyme activities	ferroxidase	oxidize Fe^{2+} to Fe^{3+}
	amine oxidase	O_2 (be reduced to H_2O/H_2O_2), phenylenediamine
	catechol oxidase	catechol, adrenaline, noradrenaline, dopamine, serotonin, tryptophan
Ion metabolism	Cu metabolism	combine, transport and release Cu
	Fe metabolism	oxidize Fe^2 to Fe^{3+} which is charged to transferrin
Antioxidant activities	ferroxidase	inhibit Fenton reaction to produce ROS
	Inhibit xanthine oxidase	scavenge anion radicals
	Inhibit lipid peroxidation antagonize hydrolysis of red blood cells	combine with Cu^{2+} to reduce copper oxidative toxicity
	Induce the expression of nitric oxide synthase	promote the synthesis of NO combating free radicals
	acute-phase protein	Inflammation, trauma, pregnancy and diabetes
Prooxidant properties	lipid oxidation negate NO bioactivity	Increased formation of ROS disrupt Cu binding to CP, while liberating Cu promote oxidative pathology

Abbreviations: CP: ceruloplasmin; ROS: reactive oxygen species; NO: nitric oxide.

mains, an important three-Cu catalytic center, and plays an essential role in the oxidation reaction [21].

Plasma CP is primarily synthesized in the endoplasmic reticulum (ER) of hepatocytes as apo-CP (Cu-free) and incorporates with Cu within the trans-Golgi network resulting in the holo-CP, Cu binding form. Thereafter, CP is secreted to general circulation to reach other organs [15]. Other organs also produce CP, including the brain, kidney, trachea, prostate, mammary gland, and placenta [22-25]; CP is also produced in macrophages and mononuclear cells in the blood during inflammation [18, 26, 27]. GPI-linked CP is another form of CP by the alternative splicing of exons 19 and 20, which was first discovered in the astrocytes and neuroglia of the brain [4, 28, 29]. Although GPI-CP is significantly expressed within the brain, several other tissues are known to exhibit relatively small amounts of expression, *i.e*. spleen, kidney, heart, liver, and testis [6, 30].

The normal circulating level of plasma CP in human adults is approximately 300 mg/L and about 10% of the total is discovered as apo-CP, which is unstable, has no enzymatic activity, and is rapidly catabolized with a half-life of about five hours [8]. The activities and levels of CP depend on several main factors, including copper deficiency, inflammatory cytokines, and estrogens/progestogens [31]. Although studies with radioactive Cu have demonstrated that Cu has no influence on the rate of synthesis or secretion of CP, CP is highly sensitive to Cu deficiency. It has been shown that low copper diet results in a marked decline in the activity and amount of CP, whereas an increase of CP concentration is not observed with an increase of Cu level [32]. During the acute phase, the levels of CP increase about 2-3 times as a response to infections and inflammation. In addition, inflammatory cytokines such as IL-1, IL-6 and TNF were recorded in rabbits and cultured human hepatoma cells for the regulation of CP levels and iron transport in the acute phase [33, 34]. The roles of CP in immunity remain to be investigated, and a complex process might be involved along with the scavenging of radicals [3]. As already noted, estrogen and progesterone have well been established on enhancing the expression of CP in plasma. Studies in mice have illustrated that estrogen increases the synthesis of CP 3-4 fold during pregnancy [35]. On the other hand, Guller *et al*. have suggested that the high expression of CP in pre-eclampsia is associated with its ferroxidase activity by mitigating the effects of reperfusion injury [36]. In fact, the details of the roles CP play in gestation remain to be worked out, however, these observations indicate that CP is indispensable for the development of newborns.

3. PHYSIOLOGICAL FUNCTIONS OF CP

3.1. Enzyme Activities

To date, CP is regarded as a promiscuous enzyme, acting on multiple substrates by binding to different Cu sites. However, it is best-known to function to oxidize Fe^{2+} to Fe^{3+}, and CP is the principal ferroxidase in the blood plasma [37]. As a multicopper oxidase, CP can accept electrons of substrates in the center of type I Cu ions located in domains 2, 4 and 6, and then delivers electrons to the trinuclear Cu cluster, where O_2 is bound and reduced to two H_2O molecules [38]. In this process, metal ions, such as Cu and iron ions can be used as the substrate. CP can oxidize Fe^{2+}/Cu^{1+} to Fe^{3+}/Cu^{2+} which are regarded as the less toxic ion form, and enable them to undertake transport and metabolism in the body [39, 40]. Nevertheless, in physiological pH, Fe^{2+} undergoes spontaneous oxidation even without the presence of protein catalyst, triggering the formation of oxygen radicals *via* Fenton chemistry. Thus CP would defend against iron-induced oxidative stress by ferroxidation. Furthermore, CP has the activity of

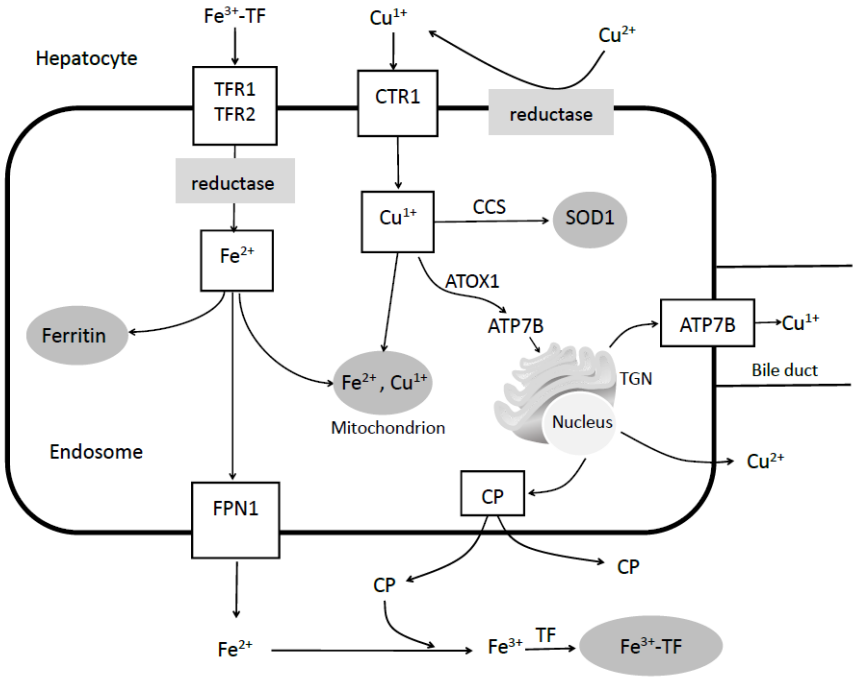

Fig. (1). CP and iron-copper homeostasis in hepatocytes. Iron is assimilated into hepatocytes *via* endocytosis of transferrin (TF) *via* transferrin receptor (TFR)1/2. In endosomes, iron is released from TF by the action of a hydrogen ATPase. Iron is used in cells, stored in ferritin, or exported by ferroportin 1 (Fpn1) which may be influenced by copper levels. After reduction by reductase, Cu^{+} is taken up *via* copper transporter 1 (CTR1) and distributed within cells by chaperones (*e.g.* CCS, copper chaperone for SOD1). Antioxidant 1 copper chaperone (ATOX1) delivers copper to ATP7B, which pumps copper into the *trans*-Golgi network (TGN) for incorporation into the CP, which oxidize iron released from hepatocytes to permit binding to TF. ATP7B also mediates copper excretion across the canalicular membrane into bile.

amine oxidase, promoting the reduction of O_2 to H_2O_2. Present evidence shows that the reaction of amine oxidation is complex; optimum pH (about 5.2) is required for effective response, and chloride ions take part in the process as potent activators [41]. So far, an extensive group of substrates has been attested in both organic amines and biogenic amines, including catechol, adrenaline, noradrenaline, dopamine, serotonin and tryptophan which are necessary for maintaining normal neurotransmission in the brain [42].

3.2. Copper and Iron Homeostasis

It has been recognized that Cu and iron are essential metal elements that exist in two oxidation states and are highly redox active, as enzyme cofactors. Furthermore, deficiencies or excesses of both minerals can cause impaired cellular functions and eventually cell death. Studies have shown the important roles of CP in the homeostasis of Cu and iron [43-45] (Fig. **1**). Dietary Cu is primarily absorbed in the small intestine by ATP7A (a P-type ATPase). Following absorption by enterocytes, Cu is bound to albumin or α2-macroglobulin, transported to the liver *via* the portal circulation, and then is delivered to the hepatocytes by Cu transport protein 1 (CTR1) [45-50]. Antioxidant protein 1 (Atox1), a Cu chaperone, directs Cu to ATP7B (the Wilson disease protein) in the trans-Golgi network (TGN), where Cu is incorporated into CP. In addition, ATP7B transports excess Cu to the canalicular membrane and mediates the excretion of Cu into bile [51, 52]. Recent research suggests that the transfer of Cu from holo-CP occurred at the cell surface by interacting with reductase and transporters, producing apo-CP, with no change in the total amount of CP [53]. In the brain, astrocytes, where CP is synthesized, are considered as important regulators of Cu homeostasis. Previous studies have shown that impaired metabolism of Cu in the brain is associated with neurodegeneration in humans such as Aceruloplasminemia, Wilson's disease, Alzheimer's disease and Parkinson's disease [54-56]. Although several Cu-dependent enzymes and Cu-binding proteins are known to exist, the molecular mechanism of Cu metabolism in the brain remains unclear. Nevertheless, it has been demonstrated that CP does play important roles in Cu transport and iron homeostasis [3, 57, 58].

Iron is essential for multiple functions in CNS, including DNA synthesis, gene expression, myelinization, neurotransmission, and mitochondrial functions. Iron accumulation or deficiency in the brain may impair the normal function of a cell and promote its death [59]. Dietary iron mainly as Fe^{3+} must first be reduced to Fe^{2+} to be transported into enterocytes. This process is mediated by duodenal cytochrome B (DcytB), a ferrireductase on the top membranes of enterocytes [60]. Then Fe^{2+} is transported across the enterocytes by divalent metal transporter-1(DMT1), leaves the enterocyte through ferroportin (Fpn), expressed on the basolateral membrane, and is delivered by transferrin (TF) to the liver *via* the portal circulation [61, 62]. Once in the liver, iron is utilized to synthesize iron-containing proteins or exported *via* Fpn following oxidation by CP and distribution in the blood as Fe^{3+}-TF [63]. Molecular mechanisms of iron transport in CNS appear to be similar to those described in the peripheral tissues [64]. Despite needing Cu for its functions,

CP plays a more important role in iron metabolism, identified as early as 1966 [65]. The patients of aceruloplasminemia, which is caused by genetic defects in the CP gene, suffer from excessive iron accumulation but not copper deficiency. An increasing body of evidence demonstrates an essential role for CP in iron efflux from cells *via* its ferroxidase activity [66]. It has been reported that CP can stimulate iron release from macrophages in the presence of apotransferrin and hypoxia [67]. Targeted CP gene disruption in mice revealed an obvious impairment in iron efflux from reticuloendothelial cells and hepatocytes [68]. Moreover, increased deposition of iron was found in several regions of the CNS in CP-/-mice [69], and the presence of GPI-CP for iron efflux from astrocytes was noted [70]. In line with this, some studies suggest that holo-CP, but not apo-CP, has the function to stabilize ferroportin by defending hepcidin, which is secreted by hepatocytes as a suppressor of iron release [71, 72].

3.3. Antioxidant Activities and Prooxidant Effect

CP is an important antioxidant, which can inhibit reactive oxygen species (ROS) produced by the mediation of ferrous ion. Meanwhile, CP can further reduce metal toxicity in the body by antioxidation, to avoid tissue damage and functional disorder [73, 74]. CP was shown to inhibit xanthine oxidase, similar to superoxide dismutase (SOD), that performs scavenging of anion radicals. When oxidative stress poison trinitrotoluene (TNT) enters in mice, SOD can scavenge O^{2-} generated from reduction to H_2O_2, which then undergoes Fenton reaction with Fe^{2+} ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^+$). Taking advantage of the ferroxidase activity, CP competitively binds to Fe^{2+}, oxidize Fe^{2+} to Fe^{3+}, and finally eliminates the products of this reaction [75]. In addition, CP can also induce the expression of nitric oxide synthase in the body, promote the synthesis of NO combating free radicals and indirectly play the role of an antioxidant [76]. By generating CP-/- mice model treated with rotenone, A. Hineno *et al*. found that CP protects against oxidative stress and neurotoxicity induced by rotenone, possibly utilizing its antioxidant properties [77]. At the same time, there is evidence that CP is an endogenous protectant against kainate neurotoxicity in epileptic samples from both animals and humans, through its inhibition of oxidative damage, Fe^{2+} accumulation, and change in ferritin immunoreactivity [78]. In turn, CP has also been shown to have prooxidant properties [79, 80]. In addition, CP may significantly inhibit lipid peroxidation and effectively antagonize hydrolysis of red blood cells induced by Cu^{2+} [81]. Studies of CP-mediated vasculopathy showed that oxidative stress may change CP from a protective to a vasculopathic factor [9]. Data indicate that CP depending on the integrity of its structure and its bound Cu can exert a potent prooxidant rather than antioxidant action on low-density lipoprotein (LDL). It also demonstrates that disruption of Cu bound to CP may alter its antioxidant function and that again a certain quotient of Cu is labile.

4. CP IN NEURODEGENERATIVE DISEASES

4.1. Wilson's Disease

Wilson's disease (WD) is an inherited autosomal recessive disease of Cu metabolism disorder, and was first defined in 1912. The main clinical features in WD include hepatic dysfunction and neurological deficits presenting as dystonia and parkinsonism [82]. It is now believed that mutation detection for ATP7B gene contributes to the diagnosis of WD, and to date, over 500 mutations have been identified with no clear correlation between genotype and phenotype [83, 84]. There is growing evidence that WD might be much more common than previously estimated [85-88]. Different levels of ATP7B expression were found in the liver, kidney, placenta and the brain [89]. In the brain, ATP7B is mainly expressed in the choroid plexus, basal ganglia, cerebellum and cortex [90]. ATP7B plays a role in Cu transportation to TGN where Cu is incorporated into CP, and it is involved in the biliary excretion of Cu. Binding of Cu to Atox1 in cytosol and shifting to TGN where Cu is incorporated into CP with the help of ATP7B is among the important processes of Cu transport [84]. Mutation of the ATP7B gene results in the impairment of Cu excretion and excess Cu in tissue, leading to a series of harmful reactions, especially oxidative stress, which can damage cells [91-93]. Accumulation of free Cu (non-CP bound) is the central pathological feature of Wilson's disease. The low levels of CP cause higher values of free copper (>1.6 micromolar) in general circulation which causes damage and oxidative stress also in the brain (*e.g.* Keiser Flescher ring). In the brain, the accumulation of Cu in astrocytes leads to impairment of the blood-brain barrier and consequent damage to neurons and oligodendrocytes [94]. In addition, dysfunction of ATP7B in astrocytes may cause impaired production of holo-CP with the consequence of decreased ferroxidase activity and impaired Fe efflux [95]. The accumulation of Cu, Fe and Mn elements was also indicated in the brain of WD by MRI studies [96, 97]. A PET study demonstrated an increased uptake of radioactively labeled Fe in WD patients as compared to healthy volunteers [98]. Of note, iron accumulation may result from low CP levels in WD, which was demonstrated in WD patients with less than 5% of the normal CP levels [99]. CP also interacts with Mn and its decreased activity can alter the neurotoxicity of Mn. Unlike iron, a study in an aceruloplasminemia mouse model has reported that CP does not play a role in the loading and partitioning of Mn. CP did, however, affect the retention of Mn in blood and its distribution to tissues, most notably kidney and to a lesser extent brain and lung. Results also indicate that CP interacted with chronic elevated Mn exposures to produce greater levels of brain oxidative stress [100]. Additional research with ATP7B (−/−) mice showed that there was not Cu accumulation in the brain, and Cu intoxication did not result in Fe accumulation in the brain or liver of mice [101-103]. All of these studies indirectly suggest that impaired Fe metabolism in WD is affected by decreased CP, rather than Cu accumulation. Overall, besides Cu, the accumulation of other metals in WD remains unclear, but there is evidence that both Fe and Mn may cause neurodegeneration. Further research is needed to explore the precise roles of CP in WD.

4.2. Neurodegeneration with Brain Iron Accumulation

Along with the advance in MRI techniques, a group of neurodegenerative diseases characterized by iron accumulation in the brain were gradually recognized, which are named neurodegeneration with brain iron accumulation (NBIA). The location of iron accumulation is mainly in the basal gan-

glia, specifically, in the globus pallidus and substantia nigra (SN), and the characteristic clinical symptoms include movement disorder, spasticity, and cognitive impairment [104]. So far, ten gene mutations associated with NBIA have been identified, including CP, ferritin light chain (FTL), Pantothenate Kinase 2 (PANK2), Phospholipase A2 group 6 (PLA2G6), *etc*. The molecular mechanisms of these genetic diseases are seemingly unrelated, but both CP and FTL are directly involved in iron homeostasis [105].

Aceruloplasminaemia belonging to the group of NBIA is an autosomal recessive inherited disease caused by mutation in the CP gene. It is a typical disease showing the relationship between iron deposition and neurodegeneration, which helps us to understand the role of CP in CNS. Neurological symptoms of this disease include blepharospasm, orolingual mandibular dystonia, chorea, dysarthria, ataxia, parkinsonism, and cognitive decline [106]. The pathological findings in the brain of the patients showed obvious iron accumulation in both the astrocytes and neurons, and neurodegenerative changes were found in the cerebral cortex, basal ganglia, as well as dentate nuclei, and cerebellar cortices [107]. Redox-active iron accumulation was found to be more prominent in the astrocytes than in the neurons. The most characteristic findings were deformed astrocytes and globular structures of astrocytes. The inclusions vary in size from 10 to 60 um in diameter. Many structures show a grumose internal structure and iron deposition [105]. Studies of molecular pathogenesis in aceruloplasminemia were investigated by analyzing the mammalian cells and murine models characterized by CP mutants [69, 108, 109]. To date, the proposed molecular mechanisms of nerve damage by iron accumulation are attributed to the result of ROS and free radical stress [110, 111]. In the absence of CP activity, Fe^{2+} cannot be oxidized and accumulates remarkably within astrocytes, as observed in the pathology [112]. At the same time, in brain tissues and cerebral fluid, a marked increase was recorded in oxidative stress including lipid peroxidation and protein carbonylation in response to excess iron-toxicity [113]. A ceruloplasmin-deficient model showed that neuronal cell loss may result from iron starvation in regions where iron in astrocytes is not effectively mobilized for uptake into neurons, and the excess iron accumulation in astrocytes could also result in oxidative damage to these cells, with subsequent loss of the glial-derived growth factors critical for neurons. Neuronal cell injury may result from iron deficiency in the early stage in addition to iron-mediated oxidation in the late stage [15]. In addition to CP, hephaestin, the second MCO and CP homolog, is also abundantly expressed in the mouse CNS. It reflects not only the ability of these MCOs as ferroxidases, but also their role in superoxide and H_2O_2 inactivation, as cuprous oxidases and an NO oxidase/nitrite synthase [114, 115]. The mechanism by which a lack of MCOs causes neurodegeneration and whether iron accumulation is a primary or secondary event remain unknown. However, existing data show that CP plays a protective effect in neurodegeneration.

4.3. Alzheimer's Disease

AD is a multifactorial disease caused by several genetic and environmental factors and is the most common form of dementia. It is characterized by progressive neurodegeneration associated with senile plaques (SPs) deposition and neurofibrillary tangles (NFTs) in the brain [116, 117]. Although the mechanisms of AD development are still being debated, evidence supports the idea that metals, such as Cu, Fe, Zn, and Mg are involved in the processes of amyloid-β (Aβ) deposition in SP and the inclusion of phosphorylated tau in NFTs [118, 119]. Metal ions not only could result in oxidative stress, neurotoxicity and apoptosis, but also induce neurogenesis and synaptic plasticity. If the balancing mechanism of metal ions is disrupted, it will finally lead to AD development [120]. As already noted, Cu and CP have been implicated in the pathogenesis of both AD and PD [121]. Several studies argued that deregulation of Cu metabolism contributes to these pathogenetic pathways and can be a risk factor accelerating the disease cascade [122, 123]. Noda, Y. *et al*. demonstrated that Cu enhanced dimerization of amyloid precursor protein (APP) and increased extracellular release of Aβ. On the other hand, D-penicillamine, a Cu chelator, suppressed the above process [124]. In addition, some meta-analyses showed an increase of free Cu (non-CP bound) levels in the serum and cerebrospinal fluid of AD patients [125, 126]. In line with this, a longitudinal study in AD patients showed that higher free Cu levels in serum are associated with unfavorable evolution of cognitive function [125]. Besides Cu, iron is also widely researched in the pathogenesis of AD. Increased iron concentrations are reported in the cortex and cerebellum of cases with preclinical AD and mild cognitive impairment [127]. It was speculated that iron may have a direct impact on plaque formation through its effects on APP. Iron can bind to Aβ and tau, so as to induce the aggregation of Aβ and the hyperphosphorylation of tau, enhancing the toxicity to neurons [128, 129]. Increasing evidence suggests that the disruption of Zn homeostasis is involved in AD [130, 131]. Zn is abundantly present in the synaptic vesicles of the hippocampus, amygdala, cerebral cortex, thalamus, and olfactory cortex in the brain. Synaptic Zn is released with neuronal excitation, and plays essential roles in learning and memory [132]. So far, both increased and decreased concentrations of Zn have been measured in AD brains. Therefore, the role of Zn in the pathogenesis of AD is still controversial. Studies demonstrated that Zn may act as a contributor to AD pathogenesis, as well as a protector to attenuate Aβ-induced neurotoxicity. Elena Atrián-Blasco, *et al* found that APP, like CP, possesses ferroxidase activity mediated by a conserved H-ferritin-like active site, which is inhibited specifically by Zn [133]. The role of CP in Zn metabolism remain unknown. Further research about the molecular mechanism of metals in AD is required. Indeed, we now know that CP plays an essential role in Cu and iron homeostasis, whether CP acts as a protective factor in AD development remains to be determined. Recently, a case-control study in AD patients showed that eCP/iCP (enzymatic activity CP/concentration of immunological CP), a variable that explains the specific anzymatic activity of CP (enzymatic activity per mg of CP concentration in $IU/mg*10^{-1}$) which is actually active, is associated with a decreased risk of developing AD. Conversely, in addition to age and APOE-ε4, non-CP Cu increases the risk of developing AD [125]. Thus, eCP/iCP is a potential protective factor that contrasts the effects of an altered non-CP Cu [16]. Inter-

estingly, there is emerging evidence of a genetic risk for AD associated with alleles of ATP7B, and there are increasing levels of apo-CP in general circulation [134, 135]. Taken together, these findings suggest that CP may play a similarly protective role in neurodegenerative diseases.

4.4. Parkinson's Disease

PD is another common neurodegenerative disease, characterized by tremor at rest, muscle rigidity, slowness of movement, and changes in posture. The main pathological features in PD include the loss of dopaminergic neurons in the SN, the presence of alpha-synuclein and Lewy bodies [136]. Similar to AD, PD is also a disease caused by multiple factors such as genetic defects, age and environment. In this regard, many theories have been proposed to elucidate the pathological mechanisms of PD, including the excess of free radicals, changes of the ubiquitin-proteasome system, and inflammation [137-139]. The possibility that alterations of homeostasis in the metal ions play a role in PD has gradually gained attention. Recent studies suggest that free Cu is related to increased oxidative stress, alpha-synuclein oligomerization, and Lewy body formation in PD. The decrease of CP-Cu may promote iron accumulation and the related oxidative stress [140-142]. Some studies reported that there is low CP activity in the cerebrospinal fluid and iron accumulation in the SN of PD, and particularly in patients bearing the AT genotype of the rs707753 CP gene variant (corresponding to a D544E protein change) [143-145]. The dysfunctions of CP in PD have also been documented in other studies. It has been found that decreased ferroxidase activity was associated with earlier disease onset and markers of iron deposition in the SN of PD patients [146]. In line with this, approximately 80% loss of CP ferroxidase activity was demonstrated in the SN of PD patients [147]. Notably, a study using a mice model of PD found that peripheral infusion of CP could attenuate neurodegeneration and nigral iron accumulation [148]. Johannesson, T. *et al*. suggested that CP concentration and oxidative activity are significantly lowered with iron accumulation in the SN and basal ganglia. By inducing neuromelanin synthesis, CP may protect neurons in the SN [149]. Thus, CP upregulation may be a potential disease-modifying therapy for PD [150]. Grolez G, *et al*. showed that most of the deferiprone (the iron chelator)-treated patients displayed clinical and radiological improvements, those with the lower CP activity appeared to respond better to iron chelation [143]. Nevertheless, larger RCTs are now needed to establish whether pharmacological modulation of CP activity could be an innovative neuroprotective strategy in PD, and whether the D544E CP polymorphism is a suitable biomarker. In addition, the observed CP prooxidant properties in PD CSF suggest that the CP-based therapeutic approach in humans might not be efficacious [151]. On account of the complex genotype and phenotype in PD patients, as well as the different mechanism of ferroxidase activity impairment, it is presently unknown whether the potential therapeutic strategy is valid. Similar to PD, ALS (amyotrophic lateral sclerosis), another fatal neurodegenerative disease shares a conspicuous feature: selective neuronal loss. The mechanism(s) of this neuronal loss remain unknown and the studies about the role of CP in ALS are poor. Conti A, *et al*. suggested that there is CP functional impairment in the CSF of ALS patients compared with that of the controls by evaluating ferroxidase activity [152]. In conclusion, previous studies have shown that CP may play a protective role in neurodegenerative diseases by its multiple functions, but the pathophysiological mechanisms remain unclear, and further research is needed.

CONCLUSION

The association between neurodegeneration and the absence of CP was first identified in patients with aceruloplasminemia three decades ago. Numerous investigations have revealed an essential role of CP in iron homeostasis by its ferroxidase activities. Moreover, they have also shown that CP has antioxidant activities, which can protect tissues from oxidative damage. GPI-CP is the predominant form in astrocytes in the brain. Currently, the neurotoxicity of iron and Cu accumulation resulting from the insufficiency or decreased activity of CP is regarded as one of the mechanisms in the development of neurodegenerative diseases, such as WD, AD and PD. However, whether the deposition of those ions is a cause or a result of neurodegeneration remains unknown. There is converging evidence that CP may play a neuroprotective role in neurodegenerative diseases. Some researchers have shown the positive results of the treatment by upregulating the CP activities in neurodegenerative diseases. Future studies should aim to address the molecular mechanisms of neurodegeneration, and investigate the other roles of CP. This will hopefully lead to the development of therapeutically effective drugs, which will successfully retain the balance of essential metals within the brain.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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