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Aceruloplasminemia- A Neurogenerative Disorder

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Abstract: Aceruloplasminemia is an iron-overloading disorder caused by mutations in the ceruloplasmin (CP) gene, and is clinically characterized by diabetes mellitus, retinal degeneration, mild iron deficiency anemia, and neurological symptoms. It facilitates oxidation of ferrous ion to ferric ion and encourages binding ferric ion to transferrin and ferritin. Clinical and pathologic studies in patients with aceruloplasminemia revealed a marked accumulation of iron in affected parenchymal tissues, a finding consistent with early work identifying ceruloplasmin as a ferroxidase and with recent findings showing an essential role for a homologous copper oxidase in iron metabolism. To decrease the iron accumulation, systemic iron chelation treatment has been introduced in some patients. Deferoxamine is a high-affinity iron chelator, the administration of deferoxamine was effective for reducing the hepatic iron overload and leading to a partial progress of the neurological symptoms and brain iron accumulation. This review paper was mainly focused to understand the mechanism, neurological consequences, pathogenesis and treatment of the neurodegenerative disorder.
Keywords: Ceruloplasmin, iron, neurodegenerative disorder, iron metabolism, pathogenesis, chelation therapy.

1. Introduction

Iron is an indispensable bioactive metal that involves in a variety of brain functions, comprising the biosynthesis of neurotransmitters, myelin formation and energy metabolism. In additional, extreme iron in the brain causes neuronal injury and cell death, as redox-active ferrous iron (Fe2+) enriches oxidative stress via the generation of the highly cytotoxic hydroxyl radical.[1] Aceruloplasminemia is an autosomal recessive disease resulting in the absence of ceruloplasmin in blood. Ceruloplasmin is a plasma ferroxidase, which contains copper and is related to the oxidation of Fe2+ to Fe3+, allowing it to be transported by transferrin[3]. Its deficiency leads to the accumulation of iron in the CNS, particularly in basal ganglia, in retina, liver and pancreas. Clinically, patients exhibit diabetes mellitus, retinal degeneration and a progressive neurological syndrome combining extrapyramidal signs, cerebellar ataxia and dementia, usually between the age of 25 and 60 years[2–5]. Another manifestation is sideropenicanemia[3]. Diagnosis is suspected by the presence of elevated levels of ferritin, anemia, decreased serum copper and absence of ceruloplasmin in serum [6];this disease may exhibit some similarities to Wilson's disease[7]. From a biochemical standpoint, ceruloplasmin knockout mice are characterized by enhanced lipid peroxidation caused by iron-mediated cellular radical injury[2]. Blood studies in aceruloplasminemia show low serum concentrations of copper and iron, a microcytic anemia, which contrasts with a high serum ferritin concentration. Hepatic concentrations of iron are increased. Brain MRI shows low intensities due to iron accumulation, mainly in striatum, thalamus and cerebellum. MRI of the liver is also suggestive. Clinical manifestations are related to iron deposits in tissues, treatment is mainly based on controlling the overload of this ion through iron chelation therapy. Possible drugs, deferasirox isa oral chelator

taken daily that has already demonstrated good results in the treatment of transfusional overload of iron[8]. Iron chelators, such as desferrioxamine, are recommended[4]. Fresh-frozen human plasma (FFP) decreases iron contents in the liver and may improve neurologic defects. Antioxidants, such as vitamin E, and the oral administration of zinc might prevent tissue damage[5].

2. Genetic expression of ceruloplasmin

The gene encoding ceruloplasmin is located on the long arm of chromosome 3 (3q23-q24) and it is expressed primarily in the hepatocytes and in the brain, but also in the lungs, the heart, the spleen, the kidneys, the testis and in the placenta[9,10]. Membrane proteinATP7B, located in the endoplasmic reticulum of the hepatocytes, binds 6–8 atoms of copper to the molecule of apoceruloplasmin and holoceruloplasminis formed (commonly referredas ceruloplasmin),which shows full enzyme activity. Then, holoceruloplasminis excreted into the plasma[11]. Changes in the structure of ATP7B results in the ineffective binding of copper into the molecule of apoceruloplasmin. Then, mainly apoceruloplasminis produced, which shows a lowered activity and shorter half-life[10]. Lower activity and shorter half-life of apoceruloplasmin are the result from decrease number of atoms of copper. Ceruloplasmin is mainly produced in the liver. It bounds and transports about 90–95% of copper contained in the blood[12]. Ceruloplasminin the brain is produced in astrocytes and it is bound with glycosylphosphatidylinositol (GPI)[13,14,15].

3. Functional role of Ceruloplasmin

Ceruloplasmin is a multicopper-containing protein generally synthesized by the liver. Although the amount of synthesis is not influenced by copper consumption, ceruloplasmin lacking bound copper has a shorter half-life[11]. Ceruloplasmin is involved insignificant functions, acting as an iron oxidase, an amine oxidase, an antioxidant and a glutathione peroxidase[16,17]. As a multicopper oxidase, ceruloplasmin reduces dioxygen, O_2, to two water molecules. Ceruloplasmin is a scavenger of reactive oxygen species (ROS). The genuine link between copper metabolism and iron metabolism is facilitated by ceruloplasmin. Its soluble form controls the oxidation of iron to be included into transferrin. A deficit in copper reduces the ferroxidase activity of ceruloplasmin (Fe2+ to Fe3+). Dietary and recycled iron are in the Fe2+ oxidation state, but iron is transported in serum by transferrin only as Fe3+ after its export by ferroportin[18]. Iron is involved in the formation of ROS. A deficit of dietary copper leads not only to an accumulation of iron in the liver, but also to an impaired distribution within the spinal cord (see zinc-induced myeloneuropathy in the next section). In the central nervous system (CNS), a glycosylphosphatidylinositol-linked ceruloplasmin bound to the cell membranes is the major isoform of this protein [19]. Astrocytes can synthesize their own ceruloplasmin. This glial ceruloplasmin controls the process of iron oxidation, which allows the clearance of iron from the CNS[20]. Therefore, functional ceruloplasmin promotes the synthesis of proteins involved in iron efflux. The maintenance of the iron balance in the brain is thus closely linked to the metabolism of copper.

Aceruloplasminemia revealed the vital role for ceruloplasmin in brain iron homeostasis. Serum ceruloplasmin does not invade the blood-brain barrier in the normal brain. Instead, GPI-linked ceruloplasmin is bound to thecell membranes of astrocytes, where it plays anchief role in iron efflux from astrocytes due to the activity of ferroxidase, which oxidizes ferrous iron following its transfer to the cell surface via ferroportin, and transports ferric iron to extracellular transferrin **(Fig. 1).** Ferroportin is post translationally regulated through internalization triggered by hepcidin binding[21].Hepcidin is synthesized by astrocytes and microglia, as well as the hepatocytes. The prevention of hepcidin-mediated ferroportin internalization was observed in glioma cells lines expressing endogenous ceruloplasmin, as well as in the cells transfected with GPI linked ceruloplasmin under low levels of hepcidin[22].Reduction in the extracellular level of ferrous iron by an iron chelator and by incubation with purified ceruloplasminin the culture medium prevented hepcidin-mediated ferroportin internalization, while the reconstitution of apoceruloplasmin was not able to prevent ferroportin internalization. Mutant ceruloplasmin cannot stabilize ferroportin because of the loss-of-function in the ferroxidase activity, which has been stated to perform an essential role in the stability of ferroportin[23].Ceruloplasmin regulates the (i) efficiency of iron efflux, (ii) functions as a ferroxidase and regulates the oxidation of ferrous iron (Fe2+) to ferric iron (Fe3+), (iii) does not bind to transferrin directly, (iv) ceruloplasmin stabilizes the cell surface iron transporter, ferroportin and (v) GPI-linked ceruloplasmin is the predominant form expressed in the brain[24]. The ceruloplasmin-ferroportin system represents the central pathway for cellular iron egress, and it is accountable for the physiological regulation of the cellular iron levels. In patients with aceruloplasminemia, the serum hepcidin levels and hepatic hepcidin mRNA levels are lower than those in control subjects[24,25]. Ceruloplasmin

knockout mice also exhibited reduced hepatic hepcidin mRNA levels in comparison to wild type and heterozygous mice[26]. The low serum hepcidin levels may induce amplified iron absorption in the intestine, where the ceruloplasmin homolog protein, hephaestin, retains ferroxidase activity is involved in basolateral intestinal iron transport. Therefore, the low hepcidin level in the serum and the loss of cell surface ferroportin due to mutant ceruloplasmin may enhance the cellular iron accumulation.

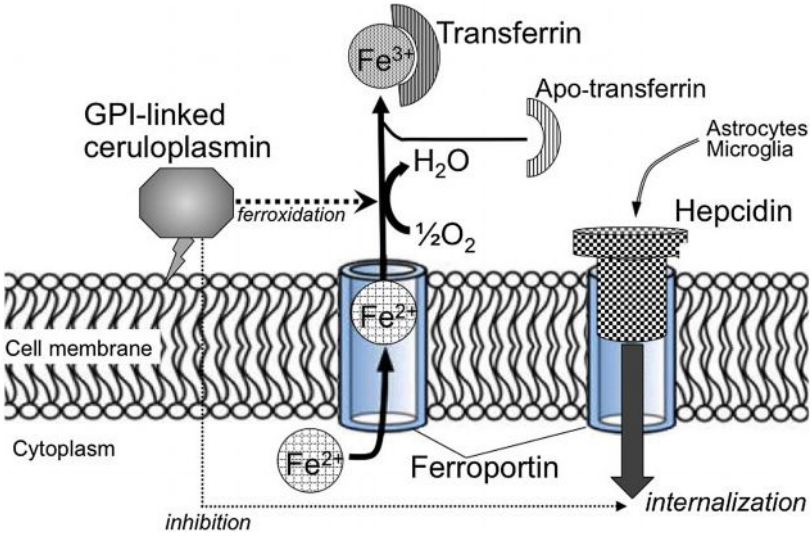

Figure:1 Iron transport at the cell membrane of the astrocytes.

4. Methods to determine the concentration of ceruloplasmin

In the diagnostics of syndromes connected with distressed production of ceruloplasmin, two predictable methods of this protein are available –enzymatic and nefelometric method. Enzymatic method is the oldest one; it was developedin the 1960s by Ravin. This method employs the enzymatic ability to oxidase p-phenylenediamine by ceruloplasmin. At the final stage ofreaction, absorbance of the mixture is measured at the wave length of 530 nm. The multiplication ofthe values obtained from the measurement of absorbance by Holmberg-Laurell factor (87.5), enzymatic activity measured were measuredin mg/dl,[27]. This evidence is significant because enzymatic activity of ceruloplasminin neurodegenerative disorders is lowered under *in vivo* conditions. The determination of enzymatic activity is essential in diagnostics of neurodegenerative disorders, and, in the case of Wilson's disease, it may be supportive in establishing a diagnosis.

In nefelometric methods of determination of ceruloplasmin concentration, a monoclonal antibody against apoceruloplasmin and holoceruloplasmin is added into the evaluated serum. As a result of the antigen–antibody reaction, soluble complexes are produced, and then the intensity of light dispersed by produced molecules of immune complexes is measured. By determining the derivative of the light dispersion, protein concentration is established and the values are provided in mg/dl. Nefelometric method is significant in determining the value of protein concentration and it reveals higher repeatability than enzymatic method (one), which is performed manually. However, in the case of nefelometric methods of determination of ceruloplasmin concentration, it was unable to precisely determine the enzyme activity, because monoclonal antibody is directed not only against the precursory form of ceruloplasmin with a lower activity (apoceruloplasmin), but also against a full active form of enzyme (holoceruloplasmin).

5. Clinical features of aceruloplasminemia

Despite indication of systemic iron overload, features of aceruloplasminemia are neurologic, resulting from progressive neurode generation of the retina and basal ganglia in association with iron accumulation in these tissues. This distinctive involvement of the central nervous system distinguishes aceruloplasminemia from other hereditary and acquired syndromes of iron metabolism and suggests that ceruloplasmin plays an essential role in normal brain iron homeostasis. Under usual circumstances, if any ceruloplasmin crosses the blood-brain barrierany proposed direct role for this protein in ironhomeostasis in the central nervous system implies

extrahepatic expression at this site. Consistent with this theory, in situhybridization of murine and human central nervous system tissues reveal abundant cell-specific ceruloplasmin gene expression[28]. Ceruloplasmin gene expression in the murine brain is observed abundantly in astrocytes surrounding the cerebral microvasculature. Additional studies reveal ceruloplasmin gene expression in glia surrounding specific neurons within the substantianigra and other basal ganglia tissues.The expression of the ceruloplasmin gene in astrocytes observed by *in situ* hybridization is confirmed by *in vitro* studiesthat have identified ceruloplasmin gene expression in cultured astrocytes. Ceruloplasmin is synthesized and secreted from these astrocytes with size characteristics and kinetics identical to those observed in hepatocytes. The patients with aceruloplasminemia suggests a direct role for the absence of this protein in the accumulation of iron and tissue injury. **Figure: 2.** Under normal circumstances, iron exits the cerebral microvasculature after receptor-mediated endocytosis of transferrin and release of iron across the blood-brain barrier. Ferrous iron is oxidized by astrocyte-secreted ceruloplasmin and incorporated into transferrin synthesized by oligodendrocytes. In the absence of ceruloplasmin, ferrous iron is take nup rapidly by the brain parenchymal cells, analogous to the occurance in the periphery. The accumulation of ferrous iron with inglia leads to cell-specific injury with resultant loss of glial-derived neurotrophic factors essential for neuronal survival[29].

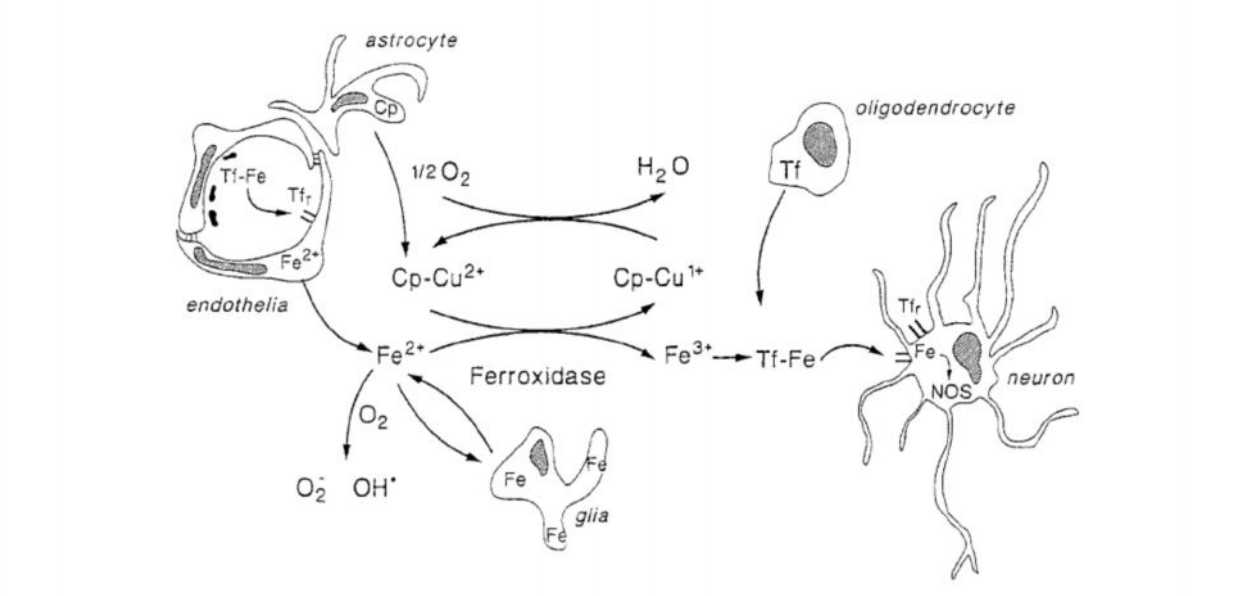

Figure: 2 Illustration of synthesis and function of ceruloplasmin(Cp) and transferrin(Tf)

6. Molecular Pathogenesis

Aceruloplasminemia was examined by analysis of Cp mutants expressed in mammalian cell culture [30,31,32,33] and characterizing murine models[34, 35, 36].

The *in vitro* biological examination of Cpmutants revealed three different types of pathologica lmechanisms, resulting in loss of the protein ferroxidase activity. The protein structural variations induced by mutations can lead to:(i) retention of Cpin the endoplasmic reticulum (ER), (ii) miss-incorporation of copper into apoceruloplasmin, and (iii) reduced ferroxidaseactivity[30, 31, 32, 33].

These events deteriro export from the cell, leading to cellular iron overload.

The *invivo* experiments were accomplish edon different mice models obtaining variable results. The first knockout mice generatedonaC57BL/6J genetic background were defined by Harris *et al.*[34]. The mice showed increased iron content with lipid peroxidation in the brain, but didn't suffer from neurological symptoms; however, recent work discovered that these CP-deficient young adult mice showed an anxiety phenotype, without discernable effects on memory or motor performance. The authors determined that in contrast to peripheral tissues, iron levels in the hippocampus were significantly reduced in *CP*-KO mice and,

paradoxically, that the anxiety phenotype resulted from reductions in the levels of iron, serotonin, and brain-derived neurotrophic factor expression in the hippocampus[37].

A second knockout mice model was developed with a different strategy by[35]. Also in this case *CP*-null mice showed increased iron deposition and lipid peroxidation in several regions of the CNS, but ,in addition, they showed deficits in motor coordination that were associated with a loss of brain stem dopaminergic neurons. Astrocytes isolated from the CNS of these *CP*-nullmice were used to investigate the functional role of Cp in CNS[38]. The authors assessed the 59Fe influx and efflux from astrocytes and concluded that GPI-Cp is essential for iron efflux, while is not involved in regulating iron influx. Furthermore, they identified the co-localization of GPI-Cp on the astrocyte cell surface with the divalent metal transporter IREG1 (now renamed ferroportin) and defined that the harmonized actions of GPI-Cp and IREG1 is required for iron efflux from neural cells. If disruption of this equilibrium occurs, it could lead to iron accumulation in the CNS and neurodegeneration, highlighting the importance of Cp in maintaining iron homeostasis in brain.

In 2002, Yamamoto using genetic background described a third type of knock- out obtained on C57BL/10 and BALB/c[36]. The mice had hepatic iron overload but no brain iron accumulation was detected. This murine model was then also investigated for the age dependent expression of hephestin. The authors detected regional difference in age-dependent hephestin expression and concluded that hephestin may compensate for the loss of Cp in aregion-specificmanner[39]. These interesting results might explain the evidence that adult *CP*-null mice have increased iron deposition in the cerebellum and brain- stem, while other regions(such as the caudate and putamen), appeared to have normal iron level[35]. Unfortunately, the latter brain regions show iron accumulation in aceruloplasminemia patients, indicating the limit of this murine model in recapitulating the aceruloplasminemia human phenotype.

7. Treatment of Aceruloplasminemia

Aceruloplasminemia is a lethal disease, and its initial diagnosis and primary treatment of patients are issues of paramount importance. Iron-mediated lipid peroxidation and oxidative stress are considered to be the leading cause of the neuronal degeneration in aceruloplasminemia patients. To decrease the iron accumulation, systemic iron chelation treatment has been introduced in some patients. Deferoxamine is a high-affinity iron chelator that combines with ferric iron in a 1:1 molarratio. It has been shown to cross the blood-brain barrier and to promote the excretion of excess iron in patients with inherited and acquired forms of iron overload [40]. The administration of deferoxamine was effective for reducing the hepatic iron overload and leading to a partial progress of the neurological symptoms and brain iron accumulation, as reported in a single case report [41]. However, consequent studies showed little effect of deferoxamine on the central nervous symptoms, despite normalization of the serum ferritin and hepatic iron concentrations and improvement in the insulin requirement and the regional brain iron levels in T2*- weighted MRI[42,43,44]. The treatments with deferoxamine were discontinued because of a concomitant reduction in hemoglobin and the serum iron level was detected after several months of the therapy, signifying that deferoxamine sequestered the iron available for erythropoiesis. Deferiprone which has a lower molecular weight and more lipophilic properties had no favourable effects in a patientin a preceding report[45]. Iron chelation therapy with deferasirox, an oral iron chelating agent, shows no progress in the neurological symptoms or brain iron accumulation, and it was discontinued because of anemia[46], while deferasirox therapy has been conveyed to show mild improvementin clinical symptoms including cognitive performance, gait and balance in an aceruloplasminemia patient who had no response to both deferoxamine and fresh–frozen plasma therapy[47]. In contrast, dramatic neurological improvement was found after 15 months of treatment withoral zinc sulfate therapy (200 mg/day) in a patient with extrapyramidal and cerebellar-mediated movement disorder caused by a heterozygous mutation in the ceruloplasmin gene [44]. Although the patient was bedridden before the zinc treatment, she was able to withstand for a short time after undergoing this treatment. The antioxidant properties of zinc, as well as its effects on iron absorption, are well-established[48,49]. While the mechanisms of antioxidation are not fully understood, the induction of metallothionein synthesis is considered to be one relevant aspect. Combination therapy with fresh frozen plasma for 6 weeks to replenish the blood ceruloplasmin levels, and thereafter, administration of deferoxamine for an additional 6weeks to deplete ferric iron stores showed unprecedented improvement in neurological symptoms [50]. Short-term iron chelation therapy is therefore effective for reducing the hepatic iron overload and improving the diabetic mellitus, but is ineffective for the treatment of neurological symptomsdue to brain iron accumulation. In most aceruloplasminemia patients, side effects prohibited the long-term therapy that may be

required to mobilize iron from the brain. Although a high dose of iron chelator has a high chelation efficiency for visceral organs, a low dose of the iron chelator may have amore favourable side effect profile, which in turn may allow for both longer-term administration and combination therapy, which may progress the patient outcome. Significantly, zinc therapy could be used as an alternative treatment when iron chelation therapy is discontinued due to side effects or progression of the symptoms, because the zinc therapy shows no side effects and may ameliorate the neurological symptoms in aceruloplasminemia patients.

Conclusion

The presence of mutations in the ceruloplasmin gene in combination with clinical and pathologic findings suggest an essential role for ceruloplasmin in human biology and identify aceruloplasminemia as an autosomal recessive syndrome of iron Metabolism. Studies on the biochemical mechanisms of copper incorporation into this protein as well as data representing a loss of ceruloplasmin oxidase activity with aging suggest that further analysis of the function of ceruloplasmin within the central nervous system will be of worth in elucidating the mechanisms of neuronal loss in several neurodegenerative disorders in which abnormalities in iron metabolism have been indicated.

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Conflict of interest statement

The authordeclare that there is no conflict of interest.

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