CERULOPLASMIN ITS ROLE AND SIGNIFICANCE: A REVIEW

Vinayak Gaware*1, Kiran Kotade2, Kiran Dhamak1, Sachin Somawanshi3

1Department of Pharmaceutical Chemistry, College of Pharmacy Chincholi, Sinnar, Nashik, M.S, 422101.
2Department of Pharmacology, College of Pharmacy Chincholi, Sinnar, Nashik, M.S, 422101.
3Department of Pharmaceutics, College of Pharmacy Chincholi, Sinnar, Nashik, M.S, 422101.

Corresponding author*: vins_gaware1@rediffmail.com

ABSTRACT

Ceruloplasmin is a ferroxidase enzyme that in humans is encoded by the CP gene. Ceruloplasmin is the major copper-carrying protein in the blood and in addition plays a role in iron metabolism. It is an enzyme synthesized in the liver containing 6 atoms of copper in its structure. Mutations in the ceruloplasmin gene can lead to the rare genetic human disease aceruloplasminemia, characterized by iron overload in the brain, liver, pancreas and retina. The most important clinical application of the ceruloplasmin test is in the diagnosis of various dreadful diseases like Wilson's disease, copper deficiency syndrome, Menkes kinky hair syndrome, nephrotic syndromes, malabsorption and with some cases of advanced liver disease in which decreased level of serum proteins have occurred. Ceruloplasmin is high in a variety of neoplastic and inflammatory states. The antioxidant effects of ceruloplasmin could have important implications for various neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease in which iron deposition is known to occur.

KEY WORDS: Aceruloplasminemia, Ceruloplasmin, Wilson's disease.

INTRODUCTION

Ceruloplasmin (or caeruloplasmin) is a ferroxidase enzyme that in humans is encoded by the CP gene. Ceruloplasmin is the major copper-carrying protein in the blood and in addition plays a role in iron metabolism. It was first described in 1948. Another protein, hephaestin, is noted for its homology to ceruloplasmin and also participates in iron and probably copper metabolism.

FUNCTIONS OF CERULOPLASMIN

It is an enzyme synthesized in the liver containing 6 atoms of copper in its structure. Ceruloplasmin carries about 70% of the total copper in human plasma while albumin carries about 15%. The rest is accounted for by macroglobulins. Albumin may be confused at times to have a greater importance as a copper carrier because it binds copper less tightly than ceruloplasmin. Ceruloplasmin exhibits a copper-dependent oxidase activity, which is associated with possible oxidation of Fe²⁺ (ferrous iron) into Fe³⁺ (ferric iron), therefore assisting in its transport in the plasma in association with...
transferrin, which can only carry iron in the ferric state. The molecular weight of human ceruloplasmin is reported to be 151kDa.

PATHOLOGY

Like any other plasma protein, levels drop in patients with hepatic disease due to reduced synthesizing capabilities.

- Mechanisms of low ceruloplasmin levels:
  - Gene expression genetically low: aceruloplasminemia.
  - Copper levels are low in general.
  - Malnutrition/trace metal deficiency in the food source
  - Copper does not cross the intestinal barrier due to ATP7A deficiency in Menkes disease.
  - Delivery of copper into the lumen of the ER-Golgi network is absent in hepatocyte due to absent ATP7B in Wilson's disease.

Copper availability doesn't affect the translation of the nascent protein. However, the apoenzyme without copper is unstable. Apoceruloplasmin is largely degraded intracellularly in the hepatocyte and the small amount that is released has a short circulation half life of 5 hours as compared to the 5.5 days for the holo-ceruloplasmin. Mutations in the ceruloplasmin gene can lead to the rare genetic human disease aceruloplasminemia, characterized by iron overload in the brain, liver, pancreas and retina.

CERULOPLASMIN NEED

It is needed when someone has signs and symptoms that the doctor suspects may be due to diseases such as:

- anemia
- nausea, abdominal pain
- jaundice
- fatigue
- behavioral changes
- tremors
- difficulty walking and/or swallowing
- dystonia

Rarely, ceruloplasmin may also be ordered along with copper tests when your doctor suspects that you have a copper deficiency and periodically if monitoring is recommended.

CERULOPLASMIN TEST

- Ceruloplasmin Plasma Test
  Ceruloplasmin plasma test is a blood test that is ordered to diagnose Wilson’s disease. This is an inherited disease that is associated with an excess of copper in the liver and other vital organs like the brain. With this excess of copper, the ceruloplasmin levels fall down drastically. Only in rare cases can this test be ordered to diagnose copper deficiencies. A clinician would generally order this test when a patient has symptoms of the Wilson’s disease. Some of these symptoms are nausea, jaundice, abdominal pain, dystonia, anemia, fatigue. Difficulty in walking, behavioral changes, mood swings, difficulty in swallowing and tremors are some of the symptoms of Wilson’s disease. In a rare case, the doctor will order for a ceruloplasmin test along with other tests when your doctor feels that you are suffering from a copper deficiency. When a person is diagnosed with low levels of ceruloplasmin, it does not necessarily correlate with any particular ailment. However, when the serum ceruloplasmin level is evaluated along with copper tests, the results may be associated with Wilson’s disease.

- Ceruloplasmin Serum Test
In a serum ceruloplasmin test, only those who have low serum ceruloplasmin and low copper in their blood and high copper levels in their urine, are said to experience Wilson’s disease. In some cases however, people who have been diagnosed with Wilson’s disease, exhibit normal ceruloplasmin levels. About 40% of those who exhibit hepatic symptoms also show normal ceruloplasmin levels. When the urine and blood concentrations of ceruloplasmin are low and the concentrations of copper are also low, the patient is simply suffering from a copper deficiency. Substances which may interfere with the body’s ability to metabolize copper may also have an effect on the serum ceruloplasmin levels.

- Ceruloplasmin Levels
  An increased level of ceruloplasmin may be due to inflammation or tissue damage. Severe infections or damaging diseases like cancers may also cause the serum ceruloplasmin levels to rise. During pregnancy, the hormone levels are high and could cause a rise in the serum levels of ceruloplasmin. If you are using medications that contain estrogen, oral contraceptives and some other medications that affect your hormones, it can cause the ceruloplasmin levels to increase.

Ceruloplasmin levels are not routinely tested. Therefore the serum ceruloplasmin test is not a routine test and is not performed unless you are exhibiting signs and symptoms of Wilson’s disease. The test may also be recommended if you have some visible problems of metabolizing copper.

Ceruloplasmin may be increased in a variety of circumstances where the test is not used as a clinical tool. These may include:

- Ceruloplasmin is an acute phase reactant. It is frequently elevated when someone has inflammation, severe infection, tissue damage and may be increased with some cancers.
- It may be increased during pregnancy and with the use of estrogen, oral contraceptives and medications such as carbamazepine, phenobarbital and valproic acid.

**CHANGES IN THE Cp GENE RELATED TO HEALTH CONDITIONS**

Aceruloplasminemia - caused by mutations in the Cp gene. Approximately 40 mutations in the CP gene that cause aceruloplasminemia have been identified. Some of these mutations substitute one protein building block (amino acid) for another amino acid in the ceruloplasmin protein, resulting in an unstable protein that quickly breaks down (degrades). Other mutations result in the production of an abnormally short, nonfunctional version of the protein or prevent the protein from being secreted by the cells in which it is made. Absence of functional ceruloplasmin results in iron transport problems that lead to the iron accumulation, neurological dysfunction and other health problems seen in aceruloplasminemia.

**CLINICAL APPLICATIONS**

- The most important clinical application of the ceruloplasmin test is in the diagnosis of Wilson's disease, where typically, concentrations of ceruloplasmin are reduced and concentration of dialyzable copper are increased. Unless treated with copper chelators, the disease is always progressive and fatal. Prompt diagnosis is important since the treatment takes 3-6 months to have the
desired effect. Ceruloplasmin assay should be considered in cases of central nervous system disease of obscure etiology. Neurological symptoms include problem of coordination.\textsuperscript{21}

- Excessive therapeutic zinc may lead to block of intestinal absorption of copper and a copper deficiency syndrome characterized by hypochromic microcytic anemia with leukopenia/neutropenia and zero level of ceruloplasmin. A prolonged period of time may be required to eliminate the excess zinc, overcome the block of intestinal copper absorption and obtain increase in serum copper and ceruloplasmin levels.\textsuperscript{22}

- Ceruloplasmin is low in Menkes kinky hair syndrome (in Menkes syndrome the defect is secondary to poor absorption and utilization of dietary copper) and with protein loss such as the nephrotic syndromes, malabsorption and with some cases of advanced liver disease in which decreases of serum proteins have occurred.\textsuperscript{23}

- Ceruloplasmin is high in a variety of neoplastic and inflammatory states since it behaves as an acute phase reactant, although levels rise more slowly than do those of other acute phase reactants.\textsuperscript{24} Increases are described in carcinomas, leukemia's, Hodgkin disease, primary biliary cirrhosis, systemic lupus erythematosus and rheumatoid arthritis. High levels occur in pregnancy, with estrogens and with oral contraceptive use when the agent contains estrogen as well as progesterone. It is also increased in copper intoxication.\textsuperscript{25}

- Another reported role of Cp is in the oxidation of LDL. Oxidized LDL (Ox-LDL) is a well-known atherogenic factor. Therefore, an increase in serum Cp levels is expected to act as an atherogenic factor.\textsuperscript{26} Increases in serum Cp levels have been reported under many conditions, including diabetes. Therefore, in diabetes, observable increased serum Cp levels should cause LDL oxidization. An increased level of Ox-LDL is known to inhibit nitric oxide (NO) production and a decreased level of NO impairs the endothelium-dependent relaxation of arteries, the impairment of which is a factor causing atherosclerosis. Thus, increased serum Cp levels in diabetes might account for the early progression of atherosclerosis.\textsuperscript{27}

CERULOPLASMIN AS MULTICOPPER OXIDASE

Copper is incorporated into ceruloplasmin during synthesis and is essential for oxidase activity. Within the plasma, ceruloplasmin catalyzes the oxidation of the signaling molecule NO concomitantly with cupric (Cu$^{2+}$) to cuprous (Cu$^{1+}$) reduction. Nitrite (NO$_2^-$) ions can therefore be used as a sink for NO production through reduction by deoxyhemoglobin, which allows for the mobilization of NO as a signaling molecule involved in hypoxic vasodilation and ischemia-reperfusion cytoprotection. In addition, nitrite acts independently as a signaling molecule necessary for cytoprotection and post-translational modifications such as iron nitrosylation and N-and S-nitrosation (figure 3).

DISEASE RELEVANCE OF CERULOPLASMIN

- During the acute-phase reaction, genes encoding CP, SAA, AGP and HP demonstrate unique extrahepatic tissue specific patterns of expression in kidney,
spleen, thymus, heart, brain, lung, testis and epididymis.

- Aceruloplasminemia is an autosomal recessive disorder caused by mutations in the ceruloplasmin (Cp) gene and is characterized by a unique combination of neurovisceral iron overload and iron deficiency anemia.
- Thus, Cp (-/-) have mild microcytic, hypochromic anemia consistent with normal red cell formation but defective iron availability \(^{29}\).

PSYCHIATRY RELATED INFORMATION ON CERULOPLASMIN

The antioxidant effects of ceruloplasmin could have important implications for various neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease in which iron deposition is known to occur \(^{30}\).

ASSOCIATIONS OF CERULOPLASMIN WITH CHEMICAL COMPOUNDS

- Ceruloplasmin (Cp) is a ferroxidase that converts highly toxic ferrous iron to its non-toxic ferric form.
- Ceruloplasmin is an abundant serum glycoprotein containing greater than 95% of the copper found in the plasma of vertebrate species.
- Immunocytofluorescence analysis demonstrated that digoxigenin-labeled Cp bound to P19 neurons and the proportion of responding neurons decreased with aging.
- It also plays an important role in detoxifying potentially harmful free ferrous iron to the less soluble ferric iron by virtue of the ferroxidase activity of the H subunit.
- Ferritin stimulation of a monokine inhibitor of lipopolysaccharide-augmented myelopoiesis is ferroxidase dependent \(^{31}\).

OTHER NAMES FOR THE Cp GENE OR GENE PRODUCTS \(^{32}\)

- CERU HUMAN
- Ceruloplasmin
- CP-2
- Ferroxidase

CONCLUSION

We are just beginning to understand the normal and pathological functions of Cp. The present review states the role of ceruloplasmin enzyme in variety of pathophysiological conditions. There is an abundance of epidemiological data that suggests that serum Cp may be an important risk factor predicting cardiovascular disease. The diagnosis of various dreadful diseases like Wilson's disease, copper deficiency syndrome, Menkes kinky hair syndrome, nephrotic syndromes, malabsorption and with some cases of advanced liver disease in which decreased level of serum proteins have occurred shows evidence of Cp. It also plays a vital role in various neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease in which iron deposition is known to occur. The discovery of aceruloplasminemia patients with iron overload confirms the long-held idea that Cp is important in iron homeostasis.

REFERENCES

1. Takahashi N, Ortel TL, Putnam FW “Single-chain structure of human ceruloplasmin: the complete amino acid sequence of the whole molecule".
1. Prog. Natl. Acad. Sci. U.S.A. 1984, 81 (2): 390–4.
2. Koschinsky ML, Funk WD. "Complete cDNA sequence of human preceruloplasmin". Proc. Natl. Acad. Sci. U.S.A. 1986, 83 (14): 5086–90.
3. Royle NJ, Irwin DM, Koschinsky ML. "Human genes encoding prothrombin and ceruloplasmin map to 11p11-q12 and 3q21-24, respectively". Somat. Cell Mol. Genet. 1987, 13 (3): 285–92.
4. Holmberg CG, Laurell C-B. "Investigations in serum copper. II. Isolation of the Copper containing protein and a description of its properties". Acta Chem Scand 1948, 2: 550–56.
5. Scheinberg IH, Gitlin D. "Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson's disease)". Science 1952, 116 (3018): 484–5.
6. Gitlin JD. "Aceruloplasminemia". Pediatr. Res. 1998, 44 (3): 271–6.
7. Lutsenko S, Gupta A, Burkhead JL, Zuzel V. "Cellular multitasking: the dual role of human Cu-ATPases in cofactor delivery and intracellular copper balance". Arch. Biochem. Biophys. 2008, 476 (1): 22–32.
8. Wolf TL, Kotun J, Meador-Woodruff JH. "Plasma copper, iron, ceruloplasmin and ferrooxidase activity in schizophrenia". Schizophr. Res. 2006, 86 (1-3): 167–71.
9. Virit O, Selek S, Bulut M, Savas HA, Celik H, Erel O, Herken H. 2008,23:123-128.
10. Sampath, P; Mazumder B, Seshadri V, Fox PL. "Transcript-selective translational silencing by gamma interferon is directed by a novel structural element in the ceruloplasmin mRNA 3' untranslated region". Mol Cell Biol 2003, 23 (5): 1509–1519.
11. Mazumder B, Sampath P, Fox PL. "Regulation of macrophage ceruloplasmin gene expression: one paradigm of 3'-UTR-mediated translational control." Mol Cells, 2005, 20 (2): 167–72.
12. Hellman NE, Gitlin JD. Ceruloplasmin metabolism and function. Annu Rev Nutr. 2002; 22:439-58.
13. Kono S, Miyajima H. Molecular and pathological basis of aceruloplasminemia. Biol Res. 2006; 39 (1):15-23
14. Kono S, Suzuki H, Oda T, Miyajima H, Takahashi Y, Shirakawa K, Ishikawa K, Kitagawa M. Biochemical features of ceruloplasmin gene mutations linked to aceruloplasminemia. Neuromolecular Med. 2006; 8(3):361-74.
15. Kono S, Suzuki H, Oda T, Shirakawa K, Takahashi Y, Kitagawa M, Miyajima H. Cys-881 is essential for the trafficking and secretion of truncated mutant ceruloplasmin in aceruloplasminemia. J Hepatol. 2007 ; 47(6):844-50.
16. Nittis T, Gitlin JD. The copper-iron connection: hereditary aceruloplasminemia. Semin Hematol. 2002; 39(4):282-9.
17. Vassiliev V, Harris ZL, Zatta P. Ceruloplasmin in neurodegenerative diseases. Brain Res Rev. 2005; 49(3):633-40.
18. Kingston IB, Kingston BL, Putnam FW. "Primary structure of a histidine-rich proteolytic fragment of human ceruloplasmin. I. Amino acid sequence of the cyanogen bromide peptides". J. Biol. Chem. 1980, 255 (7): 2878–85.
19. Zaitseva et al, Structrur sohed, J Biol Inorg Chem, 1995,1:15.
20. Schaefer M and Gitlin JD. Genetic disorders of membrane transport. IV.
Wilson's disease and Menkes disease. Am J Physiol 1999; 276: (2): G311-314.

21. Vanhoutte PM. Endothelial dysfunction and atherosclerosis. Eur Heart J 18 (Suppl. E): E19-E39, 1997.

22. Ehrenwald E. Chisolm GM, Fox PL. Intact human ceruloplasmin oxidatively modifies low density lipoprotein. J Clin Invest, 1994, 93: 1493-1501.

23. Deiss A. Wilson's disease. In: Cecil Textbook of Medicine, Vol 1, 19th Edition. W.B. Saunders Co, Philadelphia, PA; pp: 1378-1527, 1992.

24. Gahl WA. Wilson's disease. In: Cecil Textbook of Medicine, 21st ed. W.B. SaundersCo, Philadelphia, PA. 2000, 1130-2.

25. Hoffman HN II. Zinc-induced Copper Deficiency. Gastroenterology, 1988; 94(2): 508-12.

26. Cauza E. Maier-Dobersberger T. Screening for Wilson's disease on Patients with Liver Diseases by Serum Ceruloplasmin. J Hepatol, 1997; 27(2): 358-62.

27. H.V, Linehan, L.A, Bowman, B.H, Yang, F. Extrahepatic expression of plasma protein genes during inflammation. Kalmovarin, Inflammation, 1991, 44:378-383.

28. Yamamoto, K, Yoshida, K, Miyagoe, Y, Ishikawa, A, Hanaoka, K, Nomoto, S, Kaneko, K, Ikeda, S, Takeda, S. Quantitative evaluation of expression of iron-metabolism genes in ceruloplasmin-deficient mice. Biochim. Biophys. Acta 2002, 22:456-460

29. Cherukuri, S, Tripoulas, N.A, Nurko, S, Fox, P.L. Anemia and impaired stress-induced erythropoiesis in aceruloplasminemic mice. Blood Cells Mol. Dis. 2004, 33:567-569.

30. Letendre, E.D, Holbein, B.E. Ceruloplasmin and regulation of transferrin iron during Neisseria meningitidis infection in mice. Infect. Immun. 1984, 86:222-227.

31. Patel, B.N, Dunn, R.J, Jeong, S.Y, Zhu, Q, Julien, J.P, David, Ceruloplasmin regulates iron levels in the CNS and prevents free radical injury. Neurosci, 2002, 76:229-234.

32. Tamika K Samuel & Jonathan D Gitlin, Nature Chemical Biology, 2006, 2: 452 – 453.
### Table 1- Interpretation Of Ceruloplasmin Levels

| Decreased levels | • Menkes disease (Menke's kinky hair syndrome) (very rare)  
|                  | • Wilson's disease (a rare copper storage disease)\(^5\)  
| Lower-than-normal ceruloplasmin levels may indicate | • Overdose of Vitamin C  
|                  | • Copper deficiency  
|                  | • Aceruloplasminemia\(^6\)  
| Elevated levels  | • Pregnancy  
| Greater-than-normal ceruloplasmin levels may indicate or be noticed in | • Oral Contraceptive Pill Use \(^7\)  
|                  | • Lymphoma  
|                  | • Acute and chronic inflammation (it is an acute-phase reactant)  
|                  | • Rheumatoid arthritis  
|                  | • Angina \(^8\)  
|                  | • Alzheimer's disease  
|                  | • Schizophrenia  
|                  | • Obsessive-Compulsive Disorder \(^9\)  

Fig 1: Crystal Structure of Ceruloplasmin (Ferroxidase)
Fig 2- Human Structure of Ceruloplasmin

Fig 3- Ceruloplasmin As Multicopper Oxidase