



CERULOPLASMIN – AN UPDATE

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Accepted on: 23-04-2011; Finalized on: 25-07-2011.

ABSTRACT

Ceruloplasmin is a glycoprotein encoded by CP gene on chr 3q24 involved in transport of copper and also involved in iron metabolism by virtue of ferroxidase activity. It is synthesised primarily in liver and contains 6 to 7 atoms of copper. The most important and established clinical application is in diagnosis of Wilson's Disease, Menkes Kinky Hair syndrome, copper deficiency syndrome and sometimes in chronic liver disease, malabsorption and nephritic syndrome. Its role is coming up as an acute phase reactant as it is found to be elevated in inflammatory and neoplastic conditions. The antioxidant effect of ceruloplasmin has been observed in neuronal injury and neurodegenerative conditions and hence can be used for pharmaceutical research for developing therapeutic drugs.

Keywords: Ceruloplasmin, Wilson's disease, Acute Phase Reactant, Ferroxidase.

INTRODUCTION

Ceruloplasmin (CP) is a blue plasma glycoprotein that is synthesized primarily in hepatocytes which is involved in transport of copper throughout the body. It was first described in 1948. It is the major copper-carrying protein in the blood which carries about 70% of the total copper in human plasma while albumin carries about 15%¹. Ceruloplasmin in humans is encoded by the CP gene which has been mapped to chromosome 3q24². It is the product of an intragenic triplication and is composed of three homologous domains with an estimated molecular weight of 151kDa and has six or seven cupric ions per molecule. Two splice variants, CP-1 and CP-2, have differential expression in specific tissues. Ceruloplasmin mRNAs are expressed in human liver, macrophages and lymphocytes³. Another protein, hephaestin, is noted for its homology to ceruloplasmin and also participates in iron and probably copper metabolism.

FUNCTIONS

Apart from transport function, copper atom of Ceruloplasmin is essential for copper utilisation in the biosynthesis of cytochrome C oxidase and also Ceruloplasmin can transfer copper to metal-free superoxide dismutase. In addition, it has been shown to act as an enzyme, a serum ferroxidase playing a major role in oxidizing iron (II) to iron (III) in serum and at the cell surface, thereby assisting in its transport in the plasma in association with transferrin, which can only carry iron in the ferric state. It thereby converts the toxic ferrous form to its non-toxic ferric form⁴.

Ceruloplasmin also induces low density lipoprotein oxidation *in vitro*, an action that depends on the presence of a single, chelatable Cu atom⁵.

It is a late acute phase reactant synthesized by the liver. Acute phase reactant refers to proteins whose serum concentrations rise significantly during acute

inflammation due to causes including surgery, myocardial infarction, infections inflammations and tumours⁶.

Catabolism

Ceruloplasmin is proteolytically degraded to a short form, which still possesses ferroxidase activity. However, only the intact long form is able to catalyze iron loading into ferritin, indicating that the structural integrity of ceruloplasmin is essential for the enzyme to effectively catalyze iron loading into ferritin⁵.

ESTABLISHED CLINICAL ROLE OF CERULOPLASMIN ESTIMATION

Ceruloplasmin levels are not routinely tested. Therefore the serum ceruloplasmin test is not a routine test and is not performed unless the patient is exhibiting signs and symptoms of Wilson's disease like nausea, jaundice, abdominal pain, dystonia, anaemia, fatigue⁷. Difficulty in walking, behavioural changes, mood swings, difficulty in swallowing and tremors are some of these symptoms. The test is also recommended if patient is suspected to have some visible problems of metabolizing copper.

1. Role in Wilson's Disease

Wilson's disease is an autosomal recessive defect in the regulation of copper metabolism. Delivery of copper into the lumen of the ER-Golgi network is absent in hepatocyte due to absence of ATP7B in Wilson's disease.

The most important clinical application of the ceruloplasmin test is in the diagnosis of Wilson's disease, where typically, concentrations of ceruloplasmin are reduced. 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. 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.

2. Menke's Disease

Ceruloplasmin is low in Menkes kiny hair syndrome (in Menkes syndrome the defect is secondary to poor absorption and utilization of dietary copper). In this disease Copper does not cross the intestinal barrier due to ATP7A deficiency in Menkes disease

3. Copper deficiency

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. 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-ceruloplasmin⁸.

Conditions associated with Reduced Ceruloplasmin levels:

1. Aceruloplasminemia- Approximately 40 mutations in the CP gene that cause aceruloplasminemia have been identified. Some of these mutations cause substitution of one amino acid by another resulting in an unstable protein that quickly degrades. Other mutations result in the production of an abnormally short, non functional version of the protein or prevent the protein from being secreted by the cells in which it is made⁹. Absence of functional ceruloplasmin results in iron transport problems that lead to the iron accumulation, neurological dysfunction and other health problems.
2. Wilson's Disease
3. Malnutrition /Trace metal Deficiency
4. Hepatic Disease – Due to reduced synthesising capabilities of liver particularly billiary cirrhosis
5. Nephrotic syndrome
6. Malabsorption

Conditions Associated with Increased Ceruloplasmin levels:

1. An increased level of ceruloplasmin may be due to inflammation or tissue damage. Ceruloplasmin is an acute phase reactant. It is frequently elevated when someone has inflammation, severe infection, tissue damage
2. Increased ceruloplasmin levels are particularly notable in diseases of the reticuloendothelial system such as Hodgkin's disease
3. Pregnancy - During pregnancy, the hormone levels are high and could cause a rise in the serum levels of ceruloplasmin

4. Use of contraceptive pills and medications that contain estrogens, increase ceruloplasmin levels.
5. Cancers (Neoplastic Conditions) like leukemia's, Hodgkin disease,
6. Primary biliary cirrhosis, systemic lupus erythematosus and rheumatoid arthritis
7. Medications such as carbamazepine, phenobarbital and valproic acid
8. Copper intoxication
9. Chronic infections like TB and degenerative diseases

Role in ox LDL formation

Another reported role of Ceruloplasmin is in the oxidation of LDL. Oxidized LDL (Ox-LDL) is a well-known atherogenic factor. Therefore, an increase in serum Ceruloplasmin levels is expected to act as an atherogenic factor¹⁰. Increases in serum Ceruloplasmin 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 atherosclerosis¹¹.

Role in Neurodegenerative conditions

High levels of copper have pro-oxidant activity. Its free or unbound form may produce radicals such as the hydroxyl radicals, which could change the structure and solubility of proteins and results in tissue damage. Copper and iron levels both increase dramatically with aging and may lead to neuro-toxic H₂O₂ production which is associated to the pathogenesis of Alzheimer's Disease¹². The antioxidant effects of ceruloplasmin by binding free copper is found to have important implications for various neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease in which iron deposition is known to occur¹³.

Role in cancer

A balance between oxidant carcinogens and endogenous antioxidant defence is of particular relevance to the carcinogenesis. Oxidative stress is an imbalance between free radical damage and antioxidant protection in the body. Although zinc and iron levels remain unaltered, serum copper has been observed to be significantly increased in many cancers. Ceruloplasmin, the copper binding protein was also increased¹⁴. Cupric ions are reported to inhibit the production of singlet oxygen; this is of particular significance because of the latter's ability to cross the cell membrane and its high reactivity towards various bio molecules¹⁵. Ceruloplasmin is a copper binding protein, which increases in several carcinomas.



Lipid peroxidation is a well established mechanism of cellular injury which leads to production of lipid peroxides and their by products. Malonaldehyde peroxidation, elevation of serum Copper, Ceruloplasmin and their ratios have been reported to be useful in diagnosis and prognosis of other malignancies. The results of studies indicate that serum copper and ceruloplasmin may be used as a valuable predictor of the presence of malignant gynaecological tumour or specifically indicates the presence of advanced ovarian cancer along with the CA 125¹⁶.

An oxidative stress presented in non-treated patients with malignant haematological diseases is demonstrated by the increased levels of MDA as a consequence of abnormality in anti oxidative metabolism due to the cancer process. The oxidative stress might lead to compensatory increased level of the ceruloplasmin in these patients. The positive correlation observed between MDA level and ceruloplasmin activity in patients with malignant diseases confirmed that the oxidative stress appears to be compromised by augmented activity of ceruloplasmin in these patients.

Neuronal Injury

Ceruloplasmin expression is upregulated by stresses of various kinds, e.g., optic nerve crush and retinal photic injury. Acute optic nerve crush increases Ceruloplasmin dramatically within the inner retina in the rat¹⁷, and recent evidence has demonstrated the same is true in the most common human optic neuropathy, glaucoma¹⁸. The mechanisms by which axonal injury or other neuronal stresses could induce Ceruloplasmin expression are not known, but might parallel the rise in CP expression after cerebral ischemia. Given that optic nerve injury induces CP in retinal ganglion cells. The induction of a neuroprotective protein like CP after nervous system injury implies that the damage not only induces signals within the cell that transduce cell death, but also induces signals that transduce cell survival. This is expected, because if the homeostatic mechanisms for maintaining neuronal survival are not robust, then normal fluctuations or perturbations in the cell milieu could lead to progressive cell death over the life of the organism. Given that death and survival both result from an neuronal injury, it is probable that the damage transduces both positive and negative signals within the cell. This putative mechanism for CP regulation could explain its induction after cerebral ischemia, optic nerve crush, or photic stimulation. CP, a ferroxidase, oxidizes Fe⁺⁺ to Fe⁺⁺⁺ and thus prevents generation of OH via the Fenton reaction. CP therefore behaves like an endogenous neuroprotectant induced by injury, and as would be predicted, neurons from CP knockout mice are more susceptible to reactive oxygen species-mediated death in vitro.

A rise in reactive oxygen species is a likely signaling event for neuronal injury^{19,20}, and compensatory induction of CP could serve to dampen its deleterious effects.

IL-6 can induce CP expression via IL-6 response elements in the 5V upstream region, with the second site being most important for induction⁶. This provides a potential link between central nervous system injury and CP expression.

CONCLUSION

The present update is an effort to highlight the upcoming role of ceruloplasmin apart from the conventional role as a copper binding transport protein. The discovery of aceruloplasminemia resulting in iron overload established its role in iron transport. Its role a risk factor in cardiovascular disease by virtue of increasing ox LDL formation and NO level reduction has been studied widely. The established uses of estimation of ceruloplasmin are in Wilson's, Menke's syndrome and copper deficiency. Various studies have accumulated data stating its role in neuronal injury, Neurodegenerative conditions, neoplastic conditions and inflammatory conditions which is by virtue of primarily being an acute phase reactant.

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