Ceruloplasmin and iron in Alzheimer’s disease and Parkinson’s disease: a synopsis of recent studies

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Abstract: Ceruloplasmin (Cp) concentration and oxidative activity in serum are lowered in Parkinson’s disease (PD). In most PD patients, iron increases in the substantia nigra in the midbrain. In PD, the low Cp concentration and activity in serum and the high iron amounts in the substantia nigra appears to be correlated. An hereditary background is common in PD and variations in the Cp gene that have been found in PD are associated with high iron levels in the substantia nigra. Variations in Cp synthesis and in the incorporation of copper into the Cp molecule are essential features of PD. In Alzheimer’s disease (AD), the Cp activity in serum is lowered but not the concentration, except in the advanced stages of the disease. Generally, iron is not increased in the AD brain. In the AD brain, iron accumulates in neuritic plaques and in neurofibrillary tangles. There is also increased risk of iron-mediated tissue damage, which may possibly be counteracted by Cp. At the same time, the AD brain is short in copper, which presumably results in the deficient activity of many copper enzymes in the brain, in addition to Cp. Lowered Cp activity in serum most likely stems from lessened incorporation of copper in the Cp molecule and similar incorporation defects might also apply to other copper enzymes in AD.

Keywords: ceruloplasmin, iron, copper, Alzheimer’s disease, Parkinson’s disease

Introduction

The role of the oxidative (ferroxidase) activity of the multi-copper enzyme ceruloplasmin (Cp) in iron homeostasis was first described around 1970. Later experiments in mice with disruption of the Cp gene (located on chromosome 3) showed that Cp is essential in moving iron out of the reticuloendothelial cells and hepatocytes. It was subsequently found that Cp exists not only in a free, secreted form but also in a bound, glycosylphosphatidylinositol (GPI)-anchored form in astrocytes in the central nervous system (CNS). These experiments concluded that GPI-Cp is required for the cellular efflux of iron in the CNS and that defects in this activity might lead to the accumulation of iron in the brain and result in neurodegenerative lesions. Aceruloplasminemia is a rare autosomal, recessive disorder in which ceruloplasmin is, as the name indicates, missing in serum and in other tissues. In line with this, aceruloplasminemia is characterized by impaired iron homeostasis with iron deposits in the brain and other organs. There is considerable evidence that connects Alzheimer’s disease (AD) with disturbed iron homeostasis in the brain. Thus, alterations have been found in the normal cellular distribution of iron and iron proteins in the AD brain and it is hypothesized that the aberrant distribution of iron in the AD brain could lead to oxidative damage. Also, many hereditary causes of disrupted iron homeostasis, including aceruloplasminemia,
result in iron depositions in the brain and movement disorders that are reminiscent, at least partially, of Parkinson’s disease (PD) (reviewed by Ponka et al). In PD patients specifically, a negative correlation has been found between the Cp concentration and the oxidative activity in serum and iron deposits in the substantia nigra in the brain.\(^5\)\(^6\)

The gene for apoceruloplasmin is, as mentioned above, located on chromosome 3 near the gene areas for transferrin and transferrin receptors.\(^1^1\) The gene for the ATPase (\(\text{ATP7B}\)) that is responsible for the incorporation of copper into fully developed holoceruloplasmin is located on chromosome 13.\(^1^2\) Thus, disorders of these two synthetic processes obviously have different causes. In the authors’ studies, care was taken to determine both Cp parameters in order to elucidate what role Cp activity might play, specifically in these diseases.\(^1^3\)\(^1^4\)

The data from the two AD studies and the two PD studies of the authors are shown in Table 1.

In this short presentation, based on recent studies, the authors elaborate the contention that defective Cp mechanisms, which are evident in the serum of AD and PD patients, may relate to the etiology of these diseases, although they may manifest themselves in iron disorders of the CNS. However, a note of caution should be added in this context; in the living patient, there is no way of knowing whether alterations in Cp parameters in serum correspond to similar changes in Cp in the brain.

### Table 1: Cp concentration, oxidative activity, and Cp-specific oxidative activity in two studies of PD patients and two studies of AD patients compared to healthy and age- and sex-matched controls

<table>
<thead>
<tr>
<th>Determinations</th>
<th>Patients, mean (range)</th>
<th>Controls, mean (range)</th>
<th>Number of pairs</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PD patients (study 1)</strong>(^1^3)</td>
<td>Ceruloplasmin concentration, mg/mL (serum)</td>
<td>342 (160–560)</td>
<td>388 (180–600)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Ceruloplasmin oxidative activity, units/mL (serum)</td>
<td>110 (60–193)</td>
<td>139 (65–222)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Ceruloplasmin specific oxidative activity, units/mg (serum)</td>
<td>322 (195–515)</td>
<td>362 (202–544)</td>
<td>40</td>
</tr>
<tr>
<td><strong>PD patients (study 2; follow-up study)</strong>(^1^4)</td>
<td>Ceruloplasmin concentration, mg/mL (serum)</td>
<td>284 (210–390)</td>
<td>316 (240–460)</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Ceruloplasmin oxidative activity, units/mL (serum)</td>
<td>94 (42–126)</td>
<td>125 (52–236)</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Ceruloplasmin specific oxidative activity, units/mg (serum)</td>
<td>325 (173–518)</td>
<td>377 (217–544)</td>
<td>24</td>
</tr>
<tr>
<td><strong>AD patients (study 1)</strong>(^1^5)</td>
<td>Ceruloplasmin concentration, mg/mL (serum)</td>
<td>382 (247–562)</td>
<td>383 (222–655)</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Cp oxidative activity, units/mL (serum)</td>
<td>89 (47–135)</td>
<td>136 (79–227)</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Cp specific oxidative activity, units/mg (serum)</td>
<td>219 (144–322)</td>
<td>338 (255–442)</td>
<td>26</td>
</tr>
<tr>
<td><strong>AD patients (study 2)</strong>(^1^6)</td>
<td>Ceruloplasmin concentration, mg/mL (serum)</td>
<td>225 (100–400)</td>
<td>220 (150–340)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Cp oxidative activity, units/mL (serum)</td>
<td>119 (68–201)</td>
<td>136 (69–179)</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Cp specific oxidative activity, units/mg (serum)</td>
<td>568 (283–1093)</td>
<td>611 (345–814)</td>
<td>40</td>
</tr>
</tbody>
</table>

Notes: The mean values and the range of the individual results and the P-values relevant to comparison of the patient and the control groups are shown. PD study 2 was a follow-up study of the 28 patients living (out of 40) 5 years after participating in PD study 1 and their healthy controls. AD study 1 and AD study 2 were independent studies 12 years apart. In the AD studies, the values for Cp-specific oxidative activity are not comparable between the two rounds. This is due to methodological changes in the determination of Cp concentration in serum. \(^*\)Wilcoxon’s matched pairs test; \(^*\)Student’s t-test; \(^*\)Mann–Whitney test.

Abbreviations: AD, Alzheimer’s disease; Cp, ceruloplasmin; PD, Parkinson’s disease.

### Ceruloplasmin in serum and iron in Parkinson’s disease

PD is primarily a disjunction of dopaminergic neurons located in the substantia nigra in the midbrain and neurons in the basal ganglia, collectively named the extrapyramidal brain region. More iron is found in this region than in any other region in the brain. In PD, the dopaminergic neurons of substantia nigra degenerate and lose their blackish color, and the iron content usually increases further. Inside many of the degenerated neurons are amyloid formations, so-called Lewy bodies, which bind iron. Lewy bodies consist of many substances. In the core of the Lewy body is a protein, \(\alpha\)-synuclein, of uncertain biological function, and iron seems to be a requisite for its deposition and accumulation. In PD, there is also a loss of ferritin in the neurons of the midbrain.\(^1^7\)\(^1^8\) Thus, in PD, iron might be deposited in a disorderly manner in extrapyramidal structures, resulting in tissue damage, as is the case with neuromelanin, as discussed later.

In the authors’ study on 40 PD patients, the results showed that both the Cp concentration and oxidative activity in serum were significantly lower than in the age- and sex-matched healthy controls.\(^1^3\) Five years later, 28 of the patients were still alive and consented to further study. At that point, they were the same age as the original group but had progressed further in the disease. Results from the latter study were identical to the first study.\(^1^4\) Cp-specific activity (oxidative
activity related to mass) was calculated. In both studies, the Cp-specific activity was significantly lower in the patients than in the controls. Thus, the quality of the enzyme must have been significantly deteriorated in the patients and this can best be explained by lowered incorporation of copper into the Cp molecule.\textsuperscript{13,14} (see also Table 1).

Cp is a so-called acute phase reactant. Accordingly, serum concentration increases during various conditions associated with oxidative stress. In these circumstances, the acute increase of Cp concentration is generally ascribed to increased transcription of the Cp gene by cytokines, especially interleukin-6.\textsuperscript{11} In healthy persons, increased Cp concentration may be matched by increased special activity in serum, as is the case in advanced age.\textsuperscript{20} In disease, increased Cp concentration may, on the other hand, represent a compensatory mechanism for pathologically low Cp oxidative activity.\textsuperscript{21,22} In the opinion of the authors, this may have been the case in Arnal et al.\textsuperscript{23} These authors found that the Cp concentration increases with the deterioration of disease in PD patients and, at the same time, observed an increase of the free copper (not bound to Cp) in serum. The increase in free copper is indicative of a lower incorporation of copper into the Cp molecule and, consequently, the lower Cp activity was compensated by increased Cp concentration.

Two recent studies have dealt with the relation between Cp parameters in serum and iron in the substantia nigra. In the first study, by Jin et al.,\textsuperscript{9} iron was determined by magnetic resonance in seven brain regions in PD patients. Iron was significantly increased in the substantia nigra of these patients only if they also had low Cp concentration in serum. In PD patients with normal Cp, concentration in serum iron was not increased in the substantia nigra, nor was increased iron found in other brain regions. Thus, it was concluded from this study, that at least two variants of PD might exist: (1) PD with high iron in the substantia nigra and low Cp concentration in serum, and (2) PD with normal iron in the substantia nigra and normal Cp concentration in serum. In the second study, by Martinez-Hernandez et al.,\textsuperscript{10} Cp oxidative activity in serum was negatively correlated with the iron content of the substantia nigra. In this study, increased iron was found in the substantia nigra of all patients and the Hoehn and Yahr scores were, on average, about the same (2.45 and 2.40, respectively) as in the first PD study of the authors.\textsuperscript{13}

In another study, iron was found in pathologically high amounts in the substantia nigra of more than 90% of all the PD patients studied. Large amounts of iron were also found in the substantia nigra of close relatives of the PD patients, although only a few of them had manifest symptoms of the disease. Moreover, iron was found in large amounts in the substantia nigra of a relatively small number of healthy persons unrelated to the PD patients.\textsuperscript{24} Thus, the accumulation of iron in the substantia nigra, although common in PD, is seemingly not enough in itself to produce the clinical symptoms of PD. Extensive research should be instituted in this field and especially with a view to detecting possible defects in Cp synthesis. In this context, it is of interest that Cp gene variations have been associated with iron accumulation in the substantia nigra in PD patients.\textsuperscript{25}

The oxidative activity of ceruloplasmin is seemingly involved in the oxidation and polymerization of redundant catecholamines (dopamine, norepinephrine, epinephrine) in neurons where these amines function as transmitter substances.\textsuperscript{26} A common name for these polymerization products is neuromelanin. It binds iron and impacts the blackish-blue color on catecholaminergic neurons in the substantia nigra and locus coerulus in the midbrain and pons. By binding iron, neuromelanin obviously has a regulatory function in keeping the levels of noxious ferrous iron (Fe\textsuperscript{2+}) low in these iron-rich structures. In PD, degenerative or dying neurons release neuromelanin (and deplete their color), which is digested by microglia. The iron released from neuromelanin could subsequently lead to iron-mediated tissue damage.\textsuperscript{27} Thus, it is reasonable to assume that high Cp oxidative activity might counteract this process and obviate the occurrence of PD.

Rotenone is an organic substance found in plants (Derris root and some other tropical and subtropical plants). It is one of several substances that, in experiments with rats and mice, was found to induce a condition that could be used as a model of PD. Among these substances, rotenone has been found to have the most specific effect on dopaminergic neurons.\textsuperscript{28} Rotenone is an inhibitor of NADH dehydrogenase in the mitochondria (complex I), which oxidizes NADH to NAD. Blocking this enzyme results in deranged electronic transport with lowered energy production and increased risks of developing noxious oxygen radicals. Dopaminergic neurons are especially sensitive to blocking of mitochondrial dehydrogenases.\textsuperscript{28,29}

Two things are of special interest in animal studies with rotenone: first, rats and mice differ to a high degree in their sensitivity to the effect of rotenone; second, in mice at least, the difference in sensitivity to rotenone might refer to the levels of Cp in the animals.\textsuperscript{28–30}

In summary, in PD, lowered Cp concentration and oxidative activity in serum and increased iron in the substantia nigra are often concomitant occurrences. Deranged electronic transport and energy production in the mitochondria of nigral
dopaminergic neurons may be an important cause of PD, with Cp possibly counteracting the process.

**Ceruloplasmin in serum and iron in Alzheimer’s disease**

AD is the most common of the so-called dementias that result from the degeneration and death of cortical neurons in the brain. Prominent among the neurons that are affected early in AD are cholinergic neurons projecting to the hippocampus and hippocampal regions located in the temporal lobe. The pathological status in the AD brain is characterized by intracellular neurofibrillary tangles and intercellular neuritic plaques primarily located near synapses between neurons in the affected regions. Neurofibrillary tangles are considered to have more prospective value than the neuritic plaques in assessing the progress of the disease.\(^\text{18,31}\)

Tau is a neuronal, microtubule-associated protein, the physiological function of which is to regulate microtubule dynamics.\(^\text{32}\) In AD, the tau protein is phosphorylated to a pathological degree, leading to distortion of the microtubuli and splitting of the axonal structures. This results in the formation of neurofibrillary tangles.\(^\text{18,31}\) Thus, deficient axonal transport may be the real hallmark of the disease. In this context, it is interesting that oligodendrocytes, which are responsible for the synthesis of the myelin sheath, contain more iron than any other cells in the CNS, and they are also considered to put forward most of the transferrin in the brain.\(^\text{17,19,33,34}\)

Amyloid precursor protein (APP) is a glycosylated protein of unknown biological function that is uniformly found in the membranes of cells in the body, but most abundantly in the brain. The protein is cleaved into several peptides by the following three intracellular enzymes: \(\alpha\), \(\beta\), and \(\gamma\)-secretases. In the CNS, these cleavage products might possibly have some regenerating or protecting effect on the integrity of synaptic structures. Most of the cleavage products are soluble and never accumulate or are found in tissue deposits. In AD, however, a less soluble variant, a \(\beta\)-peptide with 42 amino acids, is more prevalent. This \(\beta\)-peptide constitutes the amyloid core in the neuritic plaques. It is usually called \(A\beta\) and comprises the essential part of the plaques.\(^\text{18,31,35}\)

Iron, along with copper and zinc (released during neural transmission), accumulates in the plaques and is, with or without the other metals, a requisite for the disposition of \(A\beta\) in the plaques.\(^\text{18}\) Iron binding to the hyperphosphorylated tau protein is also a precursory pattern to the formation of the neurofibrillary tangles.\(^\text{18}\) Thus, the two most prominent pathological features of the AD brain, plaques and tangles, are dependent on the presence of iron.\(^\text{17,19}\)

Occurrence of the deleterious Fenton reaction, which is dependent on free Fe\(^{2+}\), is often observed in the early stages of AD or at the prodromal stage of the disease (mild cognitive impairment). The iron-mediated noxious oxidative activity in the AD brain is also negatively related to the occurrence of the neuritic plaques.\(^\text{36,37}\) This is in line with the fact that the plaques do not form without iron and, accordingly, may have a protective effect in mopping up wayward iron in the brain of AD patients. As the iron content in the AD brain is, in general, not increased,\(^\text{38}\) the abnormal iron accumulation in neurofibrillary tangles and neuritic plaques must be evidence of a dysfunctional status of iron in the brain. On the same line, Quintana et al\(^\text{39}\) hypothesize that, in AD, dysfunctional ferritin (representing storage iron) may increase oxidative stress due to iron and myelin breakdown, which is also seen in old age. In this context, it is of interest that experiments with genetically engineered mice indicate that Cp plays an important role in protecting the CNS from iron-mediated free radical injury.\(^\text{40}\)

The authors determined the Cp concentration and oxidative activity in the serum of 44 and 26 AD patients, respectively, and in their age- and sex-matched controls. These patients had relatively mild disease (mean MMSE score 15.9). The Cp concentration was the same in both the patient and the control groups, but the Cp activity was significantly lower in the patient group.\(^\text{15}\) A second study of the same design was performed 12 years later on 40 AD patients with mild disease (mean MMSE score 17.9) and their healthy controls. The results of this study were the same as in the first study\(^\text{16}\) (see also Table 1). Taken together, the uniform results of the two studies indicate that the Cp activity in serum, but not the Cp concentration, is lowered in AD. At any rate, this applies to the mild stages of AD and can best be explained by decreased incorporation of copper in the Cp molecule, while the synthesis of apoceruloplasmin is the same in both patients and controls.

Brewer et al\(^\text{41}\) determined Cp concentration and activity in the serum of 31 AD patients with generally mild disease (mean MMSE score about 21.5) and their controls. In their studies, like the present authors’ study, they found that the Cp activity was lowered in the patients but not the Cp concentration. At the same time, free copper in plasma (not bound to Cp) was significantly increased in the patients. Brewer et al explained this by a lower degree of copper incorporation into the Cp molecule: “it lacks at least some of its coppers.”
Arnal et al determined Cp concentration in serum of AD patients on three different stages of the disease (from very mild to a severe stage; mean MMSE scores 11.6–22.2). In the most affected patients, the Cp concentration was significantly lower than in their controls but not in the two groups of patients that were less affected. Therefore, the possibility exists that the Cp concentration in serum may also be lower in the AD patients who were more heavily affected than in the study of Brewer et al or in the studies of the present authors. Arnal et al also found that free copper (not bound to Cp) in plasma increased in the patients and was negatively correlated with the MMSE score.

In experiments with an animal model (rabbits) of AD, Sparks and Schreurs found that trace amounts of copper in the drinking water of the animals accelerated AD’s pathological changes in their brains and induced learning deficits in the animals. Subsequently, Squitti et al have argued that the level of free copper in plasma of AD patients is a predictive value in assessing the progression of the disease. The present authors are not aware that Squitti et al have, in their studies, also determined the Cp oxidative activity in serum along with the Cp concentration. It is, therefore, difficult to estimate whether the free copper in plasma in their studies was independent of changes in the Cp activity. From the study of Brewer et al, it can be concluded that an increase of free copper in plasma and decreased Cp activity in the serum of AD patients are interrelated phenomena. Moreover, copper has been found to be significantly lower in the brain of AD patients. Until these differences have been sorted out, there seems little ground for instituting zinc therapy in AD patients in order to lower copper in serum as advocated by Hoogenraad.

Capo et al determined the levels of Cp oxidative activity in the cerebrospinal liquor from 10 AD patients and their controls. The results showed that Cp oxidative activity was significantly lower in the patients, whereas the copper concentration was the same in both groups. The results also showed that, normally, a relatively smaller part of the copper in the liquor is found bound to Cp than in serum. Although this finding is at variance with serum in this respect, the results indicate that the Cp oxidative activity is also lowered in this compartment in AD patients.

In contrast to iron, which is mostly transported bound to transferrin from the blood into the brain, copper most likely diffuses from blood to the brain in the ionized state. There is no lack of copper in the serum or cerebrospinal liquor of AD patients; thus, in the AD brain, the influx, or the efflux, of copper is seemingly compromised.

Clearly, the low amount of copper in the AD brain may lead to generally lesser synthesis in the brain of many copper enzymes besides ceruloplasmin. The superoxide dismutase 1 (SOD1), which scavenges the superoxide anion radical, is a copper enzyme with antioxidative function that is parallel to the activity of ceruloplasmin. However, judging from their activity in serum and red blood cells, respectively, separate processes of copper deprivation may prevail for Cp and SOD1. Thus, in the first AD study of the present authors, the activity of both enzymes was found significantly lowered in the patients, but independently. In the second AD study, which was performed 12 years later, determination of SOD1 was not included as the analytical kit used in the first study was no longer available.

In summary, iron is generally not increased in the AD brain. It accumulates in neuritic plaques and neurofibrillary tangles and can mediate tissue damage, resulting in axonal dysfunction and cell death. Cp oxidative activity in serum is lowered in AD. Defective incorporation of copper in Cp, besides presumptive lack of enzyme copper in AD brain, could be an essential feature in AD.

**Conclusion**

A correlation exists between increased iron in the substantia nigra and lowered Cp concentration and activity in the serum of PD patients. Variations in the Cp gene have, as previously mentioned, been associated with increased iron in the substantia nigra and PD. It is known that PD often has an hereditary basis. In an Icelandic study from 2000, it was found that the risk of acquiring PD is much greater in persons closely related to PD patients than in unrelated persons; at the same time, the risk to patients’ spouses was not increased. Accordingly, it should be one of the aims of future studies in this field to elucidate whether Cp gene variations or possible transcription defects are generally found in PD patients. Another intriguing and related research object is to delineate whether healthy persons with high amounts of iron in the substantia nigra are free of such deviations or defects.

The newly publicized findings that iron is generally not increased in the AD brain and that the brain is at the same time short of copper is of great importance. As there is no lack of copper in plasma in AD, the signs and symptoms of copper deprivation in the disease would most likely be confined to the CNS. To the knowledge of the present authors, copper deprivation has not been much studied in humans.
In this context, it should be mentioned that, in a 60-year-old study of Icelandic ewes suffering from heavy copper shortage, the most conspicuous pathological defects in the brains of their lambs were lesions in neuronal myelin and demyelination.\textsuperscript{51,52} Whether lesions in the iron-rich myelin sheath as envisaged by Texel et al\textsuperscript{53} might contribute to the pathological distribution of iron in the AD brain is at present an open question. At any rate, future studies on Cp in AD should also include research on other copper enzymes, which could further illuminate the pathogenesis of the disease. SOD1 was already mentioned above; another important copper enzyme is the multi-copper oxidase hephaestin. Hephæstin is a Cp homolog and is essential for the absorption of iron from the gastrointestinal tract, but it is also found with Cp in the cortical areas of the brain that are most affected in AD.\textsuperscript{31} Other proteins, like APP and the prion protein that are biologically connected with iron and copper and which may possess relevant enzymatic activities, should also be scrutinized in future studies on AD.\textsuperscript{35}

In contrast to PD, variations in the Cp gene have not been described in adult-onset AD.\textsuperscript{53} However, variations in the Cp gene or in the incorporation of copper in the Cp molecule might, if found, also be of importance in the etiology of AD.

In conclusion, defective Cp activity and concentration in serum are seemingly related to the occurrence of PD, and defective Cp activity in serum, along with defects in other copper enzymes, is possibly of importance in the etiology of AD.

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References


