Aceruloplasminemia: an inherited neurodegenerative disease with impairment of iron homeostasis1-3

Z Leah Harris, Leo WJ Klomp, and Jonathan D Gitlin

ABSTRACT Aceruloplasminemia is an autosomal recessive disorder characterized by progressive neurodegeneration of the retina and basal ganglia associated with specific inherited mutations in the ceruloplasmin gene. 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 in yeast. The presence of neurologic symptoms in aceruloplasminemia is unique among the known inherited and acquired disorders of iron metabolism; recent studies revealed an essential role for astrocyte-specific expression of ceruloplasmin in iron metabolism and neuronal survival in the central nervous system. Recognition of aceruloplasminemia provides new insights into the genetic and environmental determinants of copper metabolism and has important implications for our understanding of the role of copper in human neurodegenerative diseases. Am J Clin Nutr 1998; 67(suppl):972S–7S.

KEY WORDS Ceruloplasmin, copper, iron, neurodegeneration, human genetics, aceruloplasminemia

PHYSIOLOGIC FUNCTION OF CERULOPLASMIN

Ceruloplasmin is an abundant α₂-glycoprotein that contains > 95% of the copper found in the plasma of all vertebrate species. This protein is synthesized and secreted as a single polypeptide chain of 1046 amino acids with six atoms of copper incorporated per molecule during biosynthesis. Copper does not affect the rate of synthesis or secretion of apoceruloplasmin but a failure to incorporate copper during biosynthesis results in secretion of an apoprotein that is devoid of oxidase activity and rapidly degraded (1–3). In the inherited disorder of copper metabolism known as Wilson disease, an inability to transfer copper into a common intracellular pool for holoceruloplasmin biosynthesis and biliary copper excretion results in a marked decrease in the concentration of ceruloplasmin in the plasma secondary to the rapid turnover of the secreted apoprotein. Consistent with this concept, the Wilson disease gene has been cloned and shown to encode a cation transport P-type ATPase essential for copper transfer into the secretory pathway of the cell (4–6).

Ceruloplasmin is a multicopper oxidase and three distinct types of copper ions within the protein can be distinguished spectroscopically. Three of these copper atoms form a trinuclear cluster that is responsible for oxygen activation during the catalytic cycle of the enzyme (7). Recent determination of the crystal structure of ceruloplasmin supports these biophysical studies and delineates the amino acids in the amino and carboxyl terminal domains that are the essential copper ligands of the trinuclear cluster (8). Although ceruloplasmin can oxidize several substrates in vitro, early studies suggested a distinct role for ceruloplasmin as a ferroxidase. In these experiments, ceruloplasmin was shown to play a role in the mobilization and oxidation of iron from tissue stores with subsequent incorporation of ferric iron into transferrin (9). Further studies showed that animals made deficient in copper by dietary restriction develop an absence of circulating holoceruloplasmin and are thus unable to release tissue iron to the plasma (10). Support for a role of ceruloplasmin as a ferroxidase has also come from studies in Saccharomyces cerevisiae in which high-affinity iron uptake requires the presence of a homologous multicopper oxidase termed Fet3 (11, 12). This protein oxidizes iron in the extracellular environment, permitting high-affinity iron uptake in conjunction with a membrane permease (13). Consistent with the previously described role for the Wilson ATPase in the delivery of copper to ceruloplasmin, a homologous P-type ATPase in yeast (CCC2) delivers copper to Fet3, revealing a remarkable evolutionary conservation of the role of these copper proteins in iron homeostasis (14).

ACERULOPLASMINEMIA

The precise physiologic role of ceruloplasmin has been defined by the recognition of individuals with diabetes, retinal degeneration, and neurologic symptoms in association with a total absence of serum ceruloplasmin (15–17). Molecular genetic analysis of these patients identified specific inherited mutations in the ceruloplasmin gene (18–23). A typical pedigree from a family with aceruloplasminemia is shown in Figure 1. Also shown are the results of DNA amplification from exon 7 of the ceruloplasmin gene, which contains a five-base pair insertion in the affected individuals in this kindred. As can be seen from this analysis, aceruloplasminemia is inherited in a classic autosomal...
recessive fashion. Thus far, five distinct mutations have been defined in the ceruloplasmin gene in six separate kindreds with this disease (Table 1). Consistent with the autosomal recessive nature of this disorder, consanguinity has been observed in most cases. In all cases reported, these mutations result in an alteration of the ceruloplasmin open reading frame such that the amino acid ligands in the carboxyl terminal region essential for formation of the trinuclear copper cluster are eliminated. Because ceruloplasmin synthesized without this region would be unable to incorporate copper during biosynthesis, these data are consistent with the absence of detectable oxidase activity in the plasma of affected patients.

Clinical and pathologic studies in patients with aceruloplasminemia have shown both the histology of the hepatic parenchyma and hepatic copper content to be normal but liver iron concentrations to be markedly elevated (15–17). In addition, the iron content of the pancreas and other parenchymal organs is increased, with specific accumulation of iron in the islets of Langerhans and selective loss of pancreatic β cells. T2-weighted magnetic resonance imaging reveals an increased iron content in basal ganglia and examination of brain tissue reveals abundant iron deposition in microglia and neurons as well as selective neuronal loss (Figure 2). Similar findings are observed in the eye, with iron deposition and photoreceptor loss in the peripheral region of the retina. Taken together, these findings reveal a direct role for ceruloplasmin in human iron metabolism consistent with the early studies noted above.

The pathophysiology of aceruloplasminemia is best understood by examining ceruloplasmin within the context of the iron cycle, where ceruloplasmin functions to oxidize ferrous iron for transfer into transferrin; iron is subsequently delivered by transferrin to the bone marrow for hematopoiesis (Figure 3). The absence of ceruloplasmin leads to an accumulation of ferrous iron within both the reticuloendothelial system and the plasma. In this respect the pathophysiology of iron accumulation in parenchymal organs is analogous to that observed in atransferrinemia, hemochromatosis, and transfusion-related iron overload. Any situation in which transferrin is unavailable or unable to be saturated with iron or is oversaturated will lead to the rapid removal of ferrous iron from the circulation and its accumulation in liver and other tissues (26). Patients with aceruloplasminemia have hepatic iron and serum ferritin concentrations equivalent to those observed in persons with hemochromatosis, but are only mildly anemic because the nonenzymatic oxidation of iron permits some transfer of iron into transferrin. Symptoms of aceruloplasminemia are not observed in patients with Wilson disease because the extrahepatic production of holoceruloplasmin is sufficient to provide the 5% of the normal serum concentration necessary to sustain plasma iron turnover rates (10). The results of studies of copper metabolism in patients with aceruloplasminemia are normal, indicating that ceruloplasmin has no essential role in copper transport or metabolism in humans. Because copper metabolism is also normal in the offspring of these patients, it can be concluded that there is no essential role for maternal ceruloplasmin in placental or fetal copper transport or metabolism.

#### TABLE 1
Mutations in the human ceruloplasmin gene in aceruloplasminemia

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Exon</th>
<th>Predicted effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insertion or deletion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>607insA</td>
<td>3</td>
<td>Frameshift</td>
<td>23</td>
</tr>
<tr>
<td>1285 ins TACAC</td>
<td>7</td>
<td>Frameshift</td>
<td>18</td>
</tr>
<tr>
<td>2389delG</td>
<td>13</td>
<td>Frameshift</td>
<td>22</td>
</tr>
<tr>
<td>Nonsense</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trp858ter</td>
<td>15</td>
<td>Truncates protein</td>
<td>20, 21</td>
</tr>
<tr>
<td>Splice site</td>
<td>18</td>
<td>Frameshift, truncates protein</td>
<td>19</td>
</tr>
</tbody>
</table>

**NEUROLOGIC CONSEQUENCES OF ACERULOPLASMINEMIA**

Despite evidence of systemic iron overload, the clinical features of aceruloplasminemia are neurologic, resulting from progressive neurodegeneration of the retina and basal ganglia in association with iron accumulation in these tissues. This unique involvement of the central nervous system distinguishes aceruloplasminemia from other inherited and acquired disorders of iron metabolism and suggests that ceruloplasmin plays an essential role in normal brain iron homeostasis. Under normal circumstances, little, if any, ceruloplasmin crosses the blood-brain barrier and, thus, any proposed direct role for this protein in iron homeostasis in the central nervous system implies extrahepatic expression at this site. Consistent with this theory, in situ hybridization of murine and human central nervous system tissues reveals abundant cell-specific ceruloplasmin gene expression (27). As can be seen in Figure 4, 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 substantia nigra and other basal ganglia tissues. The expression of the ceruloplasmin gene in astrocytes observed by in situ hybridization is confirmed by in vitro studies that have identified ceruloplasmin gene expression in cultured astrocytes but not in neurons. Ceruloplasmin is synthesized and secreted from these astrocytes with size characteristics and kinetics identical to those observed in hepatocytes (Figure 5B).

The observation that ceruloplasmin is expressed in central nervous system tissues that are affected in patients with aceruloplasminemia suggests a direct role for the absence of this protein in the...
accumulation of iron and tissue injury (Figure 6). Under normal circumstances, iron exits the cerebral microvasculature after receptor-mediated endocytosis of transferrin and release of iron across the blood-brain barrier. As suggested by the data discussed above, ferrous iron is then oxidized by astrocyte-secreted ceruloplasmin and incorporated into transferrin synthesized by oligodendrocytes. In the absence of ceruloplasmin, ferrous iron is taken up rapidly by the brain parenchymal cells, analogous to what occurs in the periphery. The accumulation of ferrous iron within glia may then lead to cell-specific injury with resultant loss of glial-derived neurotrophic factors essential for neuronal survival. Alternatively, the accumulation of ferrous iron may result directly in oxidant-mediated tissue injury. The accumulation of iron in the brain in association with neurologic symptoms has been observed in other neurologic diseases, but the mechanisms of iron accumulation in the basal ganglia of such patients and the relation of this to clinical symptoms is unclear (28, 29). Nevertheless, a direct role for iron-mediated tissue injury in the brain is supported by studies on the role of this metal in oxidant-mediated ischemia-reperfusion injury after stroke and cerebral hemorrhage and by studies that revealed a marked increase in plasma lipid peroxidation in patients with aceruloplasminemia (24, 30). Last, it is possible that the inability to transfer iron into transferrin results in an iron-deficient state in neurons and that despite the accumulation of brain iron the neuronal loss is due to iron deficiency. Clarification of these issues must await the development of an animal model of aceruloplasminemia by homologous recombination.

**TREATMENT OF ACERULOPLASMINEMIA**

Aceruloplasminemia is a fatal disease and its early diagnosis and early treatment of patients are issues of paramount importance. Deferoxamine is a high-affinity iron chelator that combines with ferrous iron in a 1:1 molar ratio. Although the precise cellular location of iron chelation by this drug is unknown, 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 (31). Preliminary studies of this drug in patients with aceruloplasminemia indicate a reduction in body iron stores as well as amelioration of diabetes and a decrease in neurologic symptoms (32). If supported by further studies, the use of this drug may be an approach for preventive therapy in asymptomatic individuals before the onset of clinical symptoms or substantial iron accumulation in the central nervous system.
CONCLUSIONS

The presence of mutations in the ceruloplasmin gene in conjunction with clinical and pathologic findings suggest an essential role for ceruloplasmin in human biology and identify aceruloplasminemia as an autosomal recessive disorder of iron metabolism (25). The genetic characterization of aceruloplasminemia has provided initial insight into the specific role of ceruloplasmin in iron metabolism in the brain. Recent studies on the biochemical mechanisms of copper incorporation into this protein as well as data indicating 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 value in elucidating the mechanisms of neuronal loss in several neurodegenerative disorders in which abnormalities in iron metabolism have been shown (33, 34).

We gratefully acknowledge the collaboration of our many colleagues, Yoshitoma Takahashi, Hiroaki Miyajima, Ross MacGillivray, Anne Hughes, John Logan, and John Walshe, for sharing information and referring patients. We thank Hiroshi Morita for the micrograph of the putamen from a patient with aceruloplasminemia.

REFERENCES