Copper Metabolism and Copper Transport Disorders

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Abstract

Genetic disorders of copper metabolism are reviewed, particularly in Menkes disease and Wilson’s disease. The responsible genes for Menkes disease and Wilson’s disease are \textit{ATP7A} and \textit{ATP7B}, respectively, with the both proteins responsible for transporting copper from the cytosol to the Golgi apparatus in cells. However, the pathology of Menkes disease is completely different from that of Wilson’s disease, with Menkes disease characterized by a copper deficiency and Wilson’s disease by a toxic excess of copper. The reason for this difference is related to the particular cell types in which the ATP7A and ATP7B proteins are expressed. ATP7A is expressed in almost all cells, except hepatocytes, where ATP7B is expressed in hepatocytes.

Menkes disease is an X-linked recessive disorder characterized by copper deficiency. The typical features, such as neurological disturbances, connective tissue disorders and hair abnormalities, can be explained by the abnormally low activity of copper-dependent enzymes. The treatment is so far parenteral administrations of copper-histidine. When the treatment is started in patients more than 2 months of age, however, the neurological disturbances cannot be improved. Moreover, the treatment does not improve the connective tissue disorders. Thus an alternative treatment needs to be found.

Wilson’s disease is an autosomal recessive disorder characterized by the toxic effects of copper. The clinical symptoms mainly appear as diseases of the liver and nervous system. However, various other symptoms can also be observed and can sometimes make an early diagnosis difficult. All the patients should be treated with chelating agents or zinc. However, the treatments are ineffective in patients with fulminant hepatic failure. Liver transplantation is accepted for these patients. In these cases also, the disturbances are prevented by early treatments. Thus, early diagnosis is important. Screening in early infants should be established for early diagnosis of this disease.

Keywords: Menkes disease, occipital horn disease, Wilson’s disease, ATP7A, ATP7B, copper

Introduction

Copper is an essential trace element for all living organisms; it functions as an integral component of cuproenzymes, which include cytochrome C oxidase, l-lysyl oxidase, dopamine-ß-hydroxidase, superoxide dismu-

tase, tyrosinase, ascorbic acid oxidase and ceruloplasmin. In spite of copper being a required trace element, its oxidative potential when present in excess amounts can induce reactive free radical production and cause cellular damage as a result. Thus, the tight regulation of copper homeostasis, which is maintained by various mechanisms, including uptake, transport, storage and excretion of copper, is required. Disruptions to normal copper homeostasis are evident in two human genetic disorders, Wilson’s disease (WD) and Menkes disease (MD)\(^{1-3}\). Each disease results from the absence or dysfunction of homologous copper-transporting ATPases. The WD ATPase (ATP7B) has been recently implicated in relation to cancer. The ATP7B protein, whose site of expression is usually in hepatocytes, is expressed in various kinds of cancer cells. ATP7B expressed in malignant cells shows cisplatin-resistance\(^{4,5}\). Copper has been also reported to play roles...
in the pathogenesis of neurodegenerative diseases, including amyotrophic lateral sclerosis, Alzheimer’s disease and the prion-mediated encephalopathies. In this paper, genetic disorders of copper transport are reviewed, and some yet to be solved aspects of these diseases are discussed.

**Copper Homeostasis**

Figure 1 shows the general mechanism of copper metabolism in humans. The average daily intake is 2-5 mg of copper in healthy adults. About 40% of this is absorbed and an equivalent amount is returned to the gastrointestinal tract by biliary secretion. The liver is central to copper homeostasis.

Since the discovery in 1993 of a gene (ATP7A) responsible for MD, the molecular mechanism of copper metabolism in cells has been gradually elucidated (Fig. 2). Copper transporter 1 (Ctr1) is a high affinity copper transporter located in the cell plasma membrane that mediates copper uptake into cells in response to low cytosolic levels. Copper in the cytosol is delivered to Cu/Zn superoxide dismutase in the cytosol, to the Golgi apparatus and to mitochondria by Ccs2 (copper chaperone for superoxide dismutase 2), HAH1 (human ATX-1 homologue 1) and Cox17 (Copper chaperone for cytochrome c oxidase), respectively, the latter of which are generically named copper chaperones. Copper-transporting ATPases transport copper from the cytosol into the Golgi apparatus (Fig. 3). ATP7A is expressed in almost all cell types, except hepatocytes, where as the principal site of expression of ATP7B is in hepatocytes.

Various genetic disorders involving copper metabolism are characterized by either the depletion or accumu-
lation of copper. Some of these disorders in mammals are summarized in Table 1. Genetic disorders of copper metabolism in humans are manifested in the form of MD and WD (Table 2).

**Menkes disease (MD)**

The incidence of MD in Japan has been estimated to be 1 in 140,000 male births. MD is caused by a mutation in the ATP7A gene. Patients with MD exhibit a large variety of mutations. Perinatal diagnosis is made by mutation analysis when the mutation in the family has been identified.

In MD, copper transport from the cytosol to the Golgi apparatus in affected cells is disturbed, resulting in a reduction of copper efflux from the cell. Almost all cells in the intestine, kidney and blood brain barrier, but not hepatocytes, are affected. Orally administered copper accumulates in the intestine, resulting in the failure of copper absorption and thus giving rise to an overall copper deficiency. The characteristic features of MD are caused by a reduction in the activities of several copper-dependent enzymes (Fig. 4, Table 3). At present, the generally accepted treatment is subcutaneous injections of copper-histidine. If the treatment is initiated soon after birth, the onset of neurological degeneration that might otherwise be seen in untreated patients can be prevented. However, when the treatment is started later, the neurological degeneration cannot be prevented. These findings suggest that there is a critical period during neurological development in which a certain level of copper is essential. This period seems to be associated with maturation of the blood-brain barrier. Thus, early diagnosis is very important. We recently developed a screening test for MD

**Table 2** Characteristics of Menkes and Wilson’s diseases

<table>
<thead>
<tr>
<th></th>
<th>Menkes disease</th>
<th>Wilson’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance</td>
<td>X-linked recessive</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Prevalence</td>
<td>1/140,000 male births</td>
<td>1/35,000</td>
</tr>
<tr>
<td>Gene location</td>
<td>Xq13.3</td>
<td>13q14.3</td>
</tr>
<tr>
<td>Gene product</td>
<td>Cu-binding P-type ATPase (ATP7A)</td>
<td>Cu-binding P-type ATPase (ATP7B; 60% Identity with MD)</td>
</tr>
<tr>
<td>Expression in normal human</td>
<td>All tissues except liver</td>
<td>Liver, kidney and placenta</td>
</tr>
<tr>
<td>Mutations</td>
<td>No common mutation</td>
<td>R778L is a common mutation</td>
</tr>
<tr>
<td>Pathogenesis</td>
<td>Defect of intestinal Cu absorption, reduced activities of Cu-dependent enzymes</td>
<td>Defect of biliary Cu excretion and Cu incorporation into ceruloplasmin in the liver</td>
</tr>
<tr>
<td>Clinical findings</td>
<td>Onset is soon after birth, severe neurological degeneration, abnormal hairs, hypothermia, bone changes, cutis laxa, arterial rupture/thrombosis</td>
<td>Onset is during childhood, liver disease, loss of coordination, involuntary movements, dysarthria, Kayser-Fleischer rings</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td>Decreased serum Cu and ceruloplasmin, increased Cu concentration in the cultured fibroblasts, decreased liver Cu concentration</td>
<td>Decreased serum Cu and ceruloplasmin, increased urinary Cu excretion, increased liver Cu concentration</td>
</tr>
<tr>
<td>Treatment</td>
<td>Cu injections</td>
<td>Chelating agents (penicillamine, trientine), zinc</td>
</tr>
<tr>
<td>Animal model</td>
<td>Mottled mutant mice (macular, brindled, blotchy)</td>
<td>LEC rat</td>
</tr>
</tbody>
</table>

**Fig. 4** Disruption of copper metabolism in patients with Menkes disease

<table>
<thead>
<tr>
<th>Cuproenzyme</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome C oxidase (mitochondria)</td>
<td>Hypothermia</td>
</tr>
<tr>
<td>Tyrosinase (cytosol)</td>
<td>Hypopigmentation, Hair abnormalities</td>
</tr>
<tr>
<td>Dopamine β hydroxylase (secretory enzyme)</td>
<td>Hypotension</td>
</tr>
<tr>
<td>Lysyl oxidase (secretory enzyme)</td>
<td>Arterial abnormalities, Bladder diverticulum, Loose skin and joints osteoporosis (wormian bones, fractures)</td>
</tr>
</tbody>
</table>
Based on the ratio of homovanillic acid to vanillylmandelic acid in urine\(^{16}\).

Further to the above, the treatment with copper injections does not bring about an improvement in the connective tissue disorders because the injected copper cannot be transported into the Golgi apparatus. To ameliorate connective tissue disorders, copper thus needs to be delivered directly to the Golgi apparatus\(^{17}\). A combination therapy with copper injections and diethyldithiocarbamate (DEDTC) was studied using the macular mouse, an animal model of MD\(^{10}\). The combination therapy improved the copper concentration, cytochrome C oxidase activity and catecholamine metabolism in the brains of macular mice. These results suggest that DEDTC facilitates the passage of copper across the blood-brain barrier and across the Golgi membrane and that the combination therapy may be an effective treatment for the neurological degeneration and connective tissue disorders associated with MD.

### Occipital horn syndrome (OHS)

OHS also results from a mutation in the \(ATP7A\) gene and appears to be a mild phenotype of MD. Most patients with OHS have splice-site mutations, which produce the small amount of normal transcript as well as an abnormal transcript. The characteristic clinical features of this syndrome are connective tissue abnormalities\(^{19, 20}\).

### Wilson’s disease (WD)

WD is caused by a large number of different mutations in the \(ATP7B\) gene. Over 200 mutations have been reported and are listed in a database (http://www.medgen.med.ualberta.ca/database). The R778L mutation is mostly observed in Asian patients, while the H1069Q mutation is most common in European patients\(^{21-23}\).

In the normal liver, copper is supplied to endogenous enzymes, incorporated into ceruloplasmin via ATP7B and delivered to the blood, or excreted in the bile. In the liver of patients with WD, in which ATP7B is defect, biliary excretion of copper and the incorporation of copper into ceruloplasmin are disturbed, resulting in copper accumulation in the liver (Fig. 5). During the early stage of the disease copper distributes diffusely as metallothionein-copper in the hepatocyte cytosol. As the disease progresses, copper accumulates in the lysosome. The excessive copper in the lysosome probably cause the cause of the liver damage. The reduced incorporation of copper into ceruloplasmin decreases the serum level of ceruloplasmin bound copper as well as ceruloplasmin (holo-ceruloplasmin). At that time, the accumulated copper is released into the plasma as nonceruloplasmin bound copper, which is the cause of the elevation of urinary copper excretion and copper deposits in various tissues, such as the kidney, brain, cornea, muscle, bone and joints (Fig. 6).

The clinical symptoms appear mainly as liver and neurological diseases. However, various other symptoms are also observed which can sometimes make early diagnosis difficult. Diagnosis is usually made by low serum levels of copper and ceruloplasmin, high copper concentration in the liver and high urinary excretion of copper levels\(^{24, 25}\). DNA-based diagnosis is also available. Once a patient is diagnosed as having WD, all close relatives should be screened for WD. Treatment should be offered to presymptomatic patients, too. Patients with this dis-
ease should be treated with chelating agents and/or zinc\textsuperscript{26-28}. Zinc has been used in patients with WD as an orphan drug in the U.S.A, Europe and China. In Japan also, zinc will soon become available for the treatment of WD. The condition of patients with neurological diseases temporarily worsens during the first few weeks of the therapy with these agents. Tetrathiomolybdate has been reported not to induce the deterioration of neurological symptoms\textsuperscript{29}. In the case of patients with fulminant hepatitis or hemolysis, these treatments are often ineffective, thus rendering liver transplantation is the most appropriate option for these patients\textsuperscript{30}. Liver transplantation is also performed in some patients with neurological disorders\textsuperscript{31}. In these cases also, the disturbances are prevented by early treatment. Thus, early diagnosis and treatment are very important for WD. Owada et al. reported a mass screening method to detect presymptomatic patients with WD by measuring urinary holoceruloplasmin, and found two cases with WD from 48,819 children by the screening method\textsuperscript{32}. These findings suggest that early detection of WD is possible by measuring urinary holoceruloplasmin\textsuperscript{32,33}.

References
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