

Wilson disease and canine copper toxicosis^{1,2}

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ABSTRACT In this article we review the current clinical and research status of Wilson disease and canine copper toxicosis. One of the main clinical challenges in Wilson disease is for clinicians to recognize the possibility of Wilson disease when young patients present with liver disease, psychiatric disease, or a movement-disorder type of neurologic disease. Once the possibility of the disease is recognized, many copper-related tests are available that are quite accurate in making the diagnosis or ruling it out. It is important to remember that this is an inherited disease and that family members at risk should be screened, particularly siblings. The cloning of the Wilson disease gene opened up the possibility that a direct DNA test could be developed, allowing convenient screening of certain patients and family members. However, the large number of mutations already found, with no small set of mutations dominating the picture, have thwarted this approach. Once the diagnosis has been made, a variety of treatments are available. For maintenance therapy, therapy of presymptomatic patients, and therapy of pregnant patients, we use zinc. For initial therapy of patients with liver disease, we use a combination of zinc and trientine. For initial therapy of patients with neurologic disease we use tetrathiomolybdate. Canine copper toxicosis in Bedlington terriers is due to a gene different from the gene for Wilson disease. However, the disease is treatable with the same array of anticopper therapies that work in humans. Recently, we established linkage of the copper toxicosis gene to a microsatellite marker, which has made available a linkage test to breeders of Bedlington terriers. *Am J Clin Nutr* 1998;67(suppl):1087S–90S.

WILSON DISEASE

Introduction

Wilson disease is an autosomal recessive inherited disorder of copper metabolism (1, 2). Copper is an essential trace element but humans take in $\approx 25\%$ more than is required (1, 3). The mechanism for getting rid of excess copper involves biliary excretion into the gastrointestinal tract, where this excess copper, which we call regulatory copper, is then lost in the stool (4). In Wilson disease there is a failure of this excretory mechanism (5, 6) due to a mutation in a gene coding for an ATPase copper pump (7–9). As a result of failed excretion of regulatory copper, copper accumulates and causes toxicity, primarily in the liver and brain (1, 2).

Clinical presentation

Clinically, patients with Wilson disease present in one of three major ways (1, 2). Approximately one-third have liver disease,

usually in the mid-to-late teenage years or early twenties. The liver disease can present as a type of hepatitis, as chronic cirrhosis, or as liver failure. Another third have neurologic symptoms. The affected areas of the brain coordinate movement, and the neurologic form of Wilson disease is a movement disorder. One of the earliest problems is with speech. Patients typically develop hypokinetic and slurred speech. Other symptoms may involve difficulties in swallowing and controlling facial musculature—which lead to drooling—and tremors. They may have dystonia, which leads to spasms of limbs, face, or other areas, and can lead to permanent contractures. The remaining one-third of patients with Wilson disease have a psychiatric disturbance. Patients may exhibit any one of a series of abnormalities of behavior, including temper tantrums and other types of loss of emotional control, depression, and insomnia. They have difficulty focusing on tasks and therefore often begin to fail in school or are not able to carry out their normal work functions.

Diagnosis

The first key in the diagnosis of this condition is for physicians to recognize the possibility of Wilson disease. The neurologic symptoms are usually distinctive and tangible enough to lead to a referral to a neurologist, and most neurologists will include Wilson disease in the differential diagnosis of a movement disorder. However, large numbers of patients receive a diagnosis of chronic active hepatitis or cirrhosis (such as alcoholic cirrhosis) when they actually have Wilson disease. Many patients spend long periods of time in psychiatric clinics with a variety of psychiatric diagnoses before a diagnosis of Wilson disease is made, usually because of the inevitable development of neurologic symptoms. However, in patients with liver disease symptoms, a diagnosis of Wilson disease may never be made and the disease may progress and the patient may die.

When Wilson disease is considered a possible diagnosis, one of the best early tests is to measure 24-h urine copper concentrations (1), which are always elevated in symptomatic patients. The normal 24-h concentration is 0.3–0.8 $\mu\text{mol Cu/L}$ and symptomatic patients will always have $> 1.5 \mu\text{mol Cu/L}$. The urine should be collected into trace element-free containers and the laboratory should have the capability to assay copper in the

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appropriate range. Rarely, a false-positive urine copper value may result because of obstructive liver disease, such as primary biliary cirrhosis. A second useful test is a slit-lamp exam of the eyes by an ophthalmologist to check for corneal copper deposits, the so-called Kayser-Fleischer rings. These rings are a valuable diagnostic sign in patients exhibiting neurologic and psychiatric disease because they are almost always present in these patients if they have Wilson disease (1, 2). However, these rings are absent in about half of the patients who exhibit liver disease symptoms. A commonly used test is the measurement of serum ceruloplasmin. Ceruloplasmin is usually low in Wilson disease, but in $\approx 10\%$ of patients it can be normal. It is also somewhat low in $\approx 10\%$ of carriers of the gene, and so the value of a ceruloplasmin assay is mainly to raise suspicion, not to make a diagnosis.

Serum copper can be quite misleading because many physicians think it should always be elevated. To the contrary, it is usually abnormally low because 90% of the serum copper is normally attributable to ceruloplasmin. Because ceruloplasmin is usually low in Wilson disease, serum copper is also often lower than normal. For example, a normal plasma copper concentration is $\approx 15 \mu\text{mol/L}$, with $13.5 \mu\text{mol/L}$ being accounted for by ceruloplasmin. Thus, the normal nonceruloplasmin plasma copper concentration is $\approx 1.5 \mu\text{mol/L}$ and in patients with Wilson disease it is usually high, perhaps as high as $7.5 \mu\text{mol/L}$. This expanded pool of nonceruloplasmin plasma copper is the potentially toxic copper. In a patient with a ceruloplasmin concentration close to zero, total serum copper would be $7.5 \mu\text{mol/L}$, a low value. On the other hand, in a patient with a near normal ceruloplasmin concentration, the expanded nonceruloplasmin plasma copper pool will cause an elevation of total serum copper. As a result of these variations, the serum copper concentration can be low, normal, or high in patients with Wilson disease, and its measurement is not particularly useful for diagnostic purposes. Essentially, any useful information is related to the ceruloplasmin value.

The gold standard of diagnosis remains liver biopsy for the measurement of copper (1). The normal value is 20–50 $\mu\text{g/g}$ dry tissue wt, and in Wilson disease it is always $> 200 \mu\text{g/g}$ dry tissue wt. With the discovery and cloning of the gene it was hoped initially that a simple DNA test could be developed for diagnosis. However, there are already > 30 mutations causing Wilson disease that have been described (10, 11), making it very difficult to develop any kind of screening test based on DNA.

If a diagnosis is made in a family, it is extremely important to remember that Wilson disease is an inherited disease and preventive medicine should be practiced. Every full sibling of a patient with a new diagnosis is at 25% risk for also being homozygous for Wilson disease. If a diagnosis is made in these patients, they can be treated prophylactically and need never suffer illness from the disease (12, 13). There is little excuse for a second clinical case of Wilson disease emerging in a family once the diagnosis has been made in one family member. Again, measurement of 24-h urine copper is quite helpful in screening family members (1). In about two-thirds of affected siblings, the 24-h copper concentration will be elevated ($> 1.5 \mu\text{mol/L}$). However, sometimes presymptomatic affected individuals have urinary copper values that are only intermediately elevated (eg, 1.0–1.5 $\mu\text{mol/L}$) and not high enough to be diagnostic. Because gene carriers can also have intermediately elevated urinary copper concentrations, a liver biopsy should be made in such cases to establish whether the patient has Wilson disease.

In more distant relatives, the risk is lower than that for full siblings but is still substantially higher than the risk in the general population; therefore, screening may be done in them as well. For example, assuming a general population incidence of 1 in 40 000, a carrier frequency of 1% is obtained. On the basis of these numbers, children of Wilson disease patients have a 1 in 200 risk, nieces and nephews have a risk of 1 in 600, and cousins have a risk of 1 in 800.

Treatment

The treatment of Wilson disease has evolved considerably in the past 10–15 y. Beginning in 1956 with the introduction of penicillamine (14), this chelating agent, which causes excretion of excess copper in the urine, has been used predominantly. Although effective, this drug is also toxic (1). Subsequently, an alternative drug called trientine, sometimes shortened to trien, was developed for those patients who were intolerant of penicillamine (15). This drug acts also as a chelator and increases the urinary excretion of copper. It also probably aids in blocking the intestinal absorption of copper. Although this drug appears to be less toxic than penicillamine, it has not been used extensively enough for its entire spectrum of toxicities to be known. Those toxicities that have occurred seem to be similar to those of penicillamine.

Zinc therapy has been developed by two groups: Hoogenraad et al (16–18) in the Netherlands and our group in the United States (1, 13, 19–21). Zinc acts by a different mechanism than chelation. It induces intestinal cell metallothionein, which, because it has a high affinity for copper, binds copper coming into the intestinal cell and prevents its serosal transfer (19, 22–24). The accumulated copper is retained in the cell until the cell sloughs into the lumen of the bowel as the intestinal cell dies, with a 6-d turnover time. Thus, zinc produces a mucosal block of copper absorption. Not only does zinc affect the absorption of food copper, but it affects the reabsorption of endogenously secreted copper. Thus, the rather substantial quantities of copper in saliva and gastric juice and other intestinal secretions are not reabsorbed during zinc therapy, as they would be in its absence. It is this effect that allows the production of a significant negative copper balance and the slow removal of excess copper stores during zinc therapy.

We found that zinc is less effective if given with food, probably because it is complexed by phytates, fiber, and other substances in the food (1). Therefore, we give it 1 h before or after food and beverages, other than water, are consumed. The dose we use is 50 mg elemental Zn as the acetate salt, three times per day. We have found the acetate salt to be better tolerated than the sulfate salt.

We have used zinc for the maintenance phase of treatment and for treatment of presymptomatic patients from the time of diagnosis (13). Additionally, we strongly recommend zinc for treatment of pregnant Wilson disease patients. Because both trientine and penicillamine are teratogenic, and because zinc has been shown to be nonteratogenic (25), zinc is ideal for treatment during pregnancy.

For patients with acute fulminant hepatic failure, nothing may save their lives other than hepatic transplantation. However, if the liver failure is relatively mild and the patient is on the right side of the prognostic index of Nazer et al (26), medical treatment is usually effective. Medical treatment will also work well for patients in the hepatitis-cirrhosis phase of the disease—we



use a combination of trientine and zinc for these patients. We use trientine because it produces a somewhat more brisk negative copper balance than does zinc and is not as toxic as penicillamine. We use zinc not only because it prevents the absorption of copper from the intestinal tract, but by the process of induction of metallothionein in the liver it helps protect the liver against further copper damage (27).

The initial treatment of patients with neurologic disease is a problem, in our opinion, because presently available therapies are potentially harmful or inadequate. Penicillamine has been shown to often make these patients neurologically worse, and they often do not recover from that worsening (28). The mechanism is probably the mobilization of hepatic copper, resulting in further elevation of brain copper. Trientine has not been used enough in this setting to know whether it carries the same risk, but its mechanism of action is so similar that it might. Hoogenraad et al (16–18) used zinc for the initial therapy of these patients and reported good results (18). Our impression has been that zinc acts rather leisurely in its control of copper toxicity and that there is a risk of the disease progressing somewhat during the early phases of zinc therapy.

Because no currently available agent or combination is optimal, we developed ammonium tetrathiomolybdate as a therapy for this type of patient (29–31). The mechanism of action of tetrathiomolybdate is distinct from that of the other drugs (32, 33). It combines with copper and protein, forming a tripartite complex. When given with food, tetrathiomolybdate binds food copper with food protein and prevents copper absorption. When given between meals, tetrathiomolybdate is absorbed and complexes nonceruloplasmin plasma copper with albumin. This complex is unavailable for cellular uptake and is thus nontoxic. To take advantage of both mechanisms of action, we give tetrathiomolybdate six times per day, three doses with meals and three doses between meals. We use tetrathiomolybdate for 8 wk and are able to gain quick control of copper toxicity without loss of function in the overwhelming majority of patients. After the 8-wk treatment they are switched to zinc maintenance therapy.


CANINE COPPER TOXICOSIS

When we began our work with canine copper toxicosis in Bedlington terriers, we did so because we thought it would make an excellent model for both cloning the Wilson disease gene and for developing therapy. However, the gene appears to be different from the Wilson disease gene even though the affected dogs have a defect in biliary excretion of copper (34, 35). Presumably, the defect is somewhere else in the excretory pathway.

Canine copper toxicosis is an autosomal recessive disorder with a high frequency in Bedlington terriers. It is estimated that the disease allele frequency may be as high as 0.5 in both the American and British population of dogs. Affected animals develop liver disease and generally die between 2 and 6 y of age, unless treated. The same array of anticopper agents used in Wilson disease is effective in canine copper toxicosis (36).

This has been a difficult disease for breeders to eliminate because with such a high gene frequency, 75% of the dogs are either affected or are carriers. The disease is not diagnosed until 1 y of age, by an invasive liver biopsy and a quantitative liver copper assay, and carriers are not identifiable until they have produced an affected progeny. Although the disease is treatable in dogs, it is obvious that prevention by selective breeding of ani-

mals free of the gene is the ideal long-term solution to a veterinary disease such as this. Accordingly, we set out to find a linked marker to the disease gene. Over the past several years we have produced > 500 canine microsatellites, mostly cytosine adenine repeats, from the dog genome. With this number of markers, the dog chromosomes are essentially saturated and it is possible to find a linked marker to almost any canine disease gene of interest.

After evaluating 213 microsatellite markers, we found one we called 41.07 that is linked to the canine copper toxicosis gene in Bedlington terriers (35). Using this linkage, it is now possible to offer a linkage test to breeders to help in the process of eliminating the canine copper toxicosis gene. Using fluorescence in situ hybridization, we have shown that the location of the 41.07 marker is different from the Wilson disease gene in the dog, thus, confirming that these are distinct genes (unpublished observations, 1997). 

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