



WILSON'S DISEASE: FROM PATHOPHYSIOLOGY TO TREATMENT

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Abstract. Wilson disease is a genetic autosomal recessive disorder caused by metabolic errors due to a continuous accumulation and copper toxicity in many tissues, particularly the liver, brain, eye. The liver is the seat of both etiological and biochemical abnormality affected by copper deposition. Wilson disease is a treatable disease. With appropriate therapy the disease progression could be stopped and symptoms can often be improved. Treatment is aimed at removing excessively accumulated copper and preventing its further accumulation and is for lifetime. Diet without copper in Wilson disease is difficult to meet, almost all foods containing copper. Drugs used in the treatment of Wilson's disease are copper chelators class: D-penicillamine, trientine, ammonium tetrathiomolibdat. These drugs bind copper and promote the urinary excretion of copper. Teratiomolibdat is still under investigation in Wilson disease and may represent a therapeutic alternative for D-penicillamine in neurological damage caused by the disease. Long term therapy of Wilson's disease is based on the use of zinc salts. Zinc acts by blocking the intestinal absorption of copper and preventing the accumulation of deposits. The major advantage of zinc salts is the absence of adverse effects. New medications with antioxidant effect such as vitamin E or curcumin have not accumulated enough evidence to be put into practice. Patients with severe liver failure need liver transplantation. Interrupting chelation therapy causes aggravation of the disease so treatment should be followed for lifetime.

Keywords: Wilson disease, child, therapy

Introduction

Wilson's disease is an autosomal recessive genetic disorder caused by mutations on ATP7B gene, that encodes copper transporting proteins[1]. In healthy persons this gene is expressed in various tissues: liver, central nervous system, kidneys, cornea, mammary glands etc [2]. The major physiological role of ATP7B is in copper metabolism homeostasis. In the hepatocytes, ATP7B protein (a P-type ATP-ase) transports copper from cytosol into the lumen of Golgi network. At this point the copper is incorporated into secreted copper-dependent proteins like ceruloplasmin. The excess copper is embedded into vesicles, which subsequently merge with the canalicular membrane through the ATP7B and is excreted into the bile[3]. In Wilson's disease ATP7B expression, function and intracellular activity are disrupted by mutations,[4] which results in decreased excretion of copper and its accumulation in target organs.

Copper metabolism. Copper is essential to a variety of biological processes such as the mitochondrial

respiratory chain, synthesis of neurotransmitters, connective tissue formation and the iron metabolism, but is highly toxic when present in excessive amounts. Its toxicity is determined by the potential to facilitate the production of reactive oxygen species[5].

Copper homeostasis is maintained through a number of mechanisms involving the activity of transporters: ATP7A and ATP7B. Mutations in the genes responsible for encoding these transporters are the causes of copper imbalance and cellular copper overload. Copper is essential for the action of diverse enzymes. Normal dietary intake and absorption of copper exceed the metabolic needs[5]. An ordinary diet provides sufficient copper, between 1 and 10 mg/day depending on the amount of shellfish, meat, vegetables and chocolate consumed. The recommended daily intake is 0.9 mg/day. Although the intake is not regulated, intestinal absorption of copper is high (55-75%) Normal copper balance is maintained by regulation of excretion, rather than absorption, and the predominant route of copper excretion (approximately 985%) is hepatobiliary in nature. The renal pathway is responsible for less than 5% of copper excretion. Usually more than 85% of copper intake is excreted[6].

Copper is absorbed in the proximal small intestine through the hCTR1 expressed on enterocytes. After

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copper reaches the hepatocyte, it is incorporated into copper-containing enzymes and copper-binding proteins (CBPs), including ceruloplasmin, a serum ferroxidase, and to various aminoacids, histidine being the most important. Under this forms copper is transported via the portal circulation, into the liver where it is excreted in the bile[7].

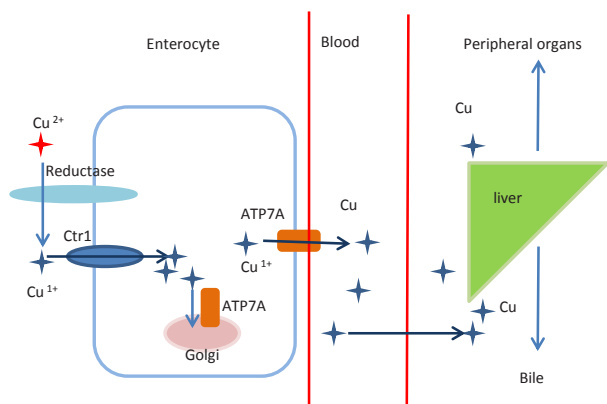


Fig.1. Copper metabolism: absorption, transportation and excretion.(adapted after Bull, Thomas et al [9])

Copper carried by albumin is in the form of Cu II and must be reduced to Cu I by reductases on the external surface of the hepatocyte membrane in order to be captured. In hepatocytes it is always bound to low-molecular-weight proteins called metallochaperones which transport copper to different molecules within

the cell[8]. ATOX 1 directs cytoplasmic copper into the trans-Golgi network in which it enters through ATP7B ATP-ase[6].

The ATP7B gene was first discovered in 1993 by Bull and his team[10] and by Tanzi et al[11]. Petruhin et al[12], published the first characterization of its gene structure in 1994. Since then, over 500 mutations[5] have been described and over 380 of them have been identified in Wilson's Disease patients world-wide (WD mutation database <http://www.wilsondisease.med.ualberta.ca/database.asp>)

Wilson's disease is a chronic, severe and progressive condition requiring lifelong chelation therapy. There is still no cure.

Untreated, Wilson's Disease may be fatal, most patients dying due to liver disease. Survival rate is improved with a right chelation therapy or by liver transplant. In general, the prognosis depends on the severity of liver disease and neurological disorders, as well as the patient's treatment adherence. Liver functions normalize after 1-2 years of treatment in most patients who present without hepatic cirrhosis or compensated cirrhosis, and they are maintained stable. At the other end of the spectrum, drug therapy is rarely effective in patients with acute liver failure, due to the length of time needed to remove toxic copper from the organism.

Wilson's Disease Diet

Copper is found in different amounts in a wide variety of foods. For this reason, Wilson's Disease can't be controlled with dietary restriction alone.

	Unrestricted consumption (less than 0.1 mg copper / portion)	Consumed with caution	
Meat and meat substitute	Beef, eggs, turkey and chicken	All fish except shellfish (3oz = 88g) Peanut butter (2 tablespoons = 30g)	Beef, lamb, pork, pheasant, duck, goose, octopus, salmon, organs (liver, kidney, heart, brain), shellfish (clams, oysters, shrimp, lobster, crab), tofu, soy meat, nuts and seeds
Vegetables	Most vegetables including fresh tomatoes	Bean sprouts (1cup = 236 g), beets (1/2 cup = 118 g), spinach (1/2 cup =118g cooked); double amount for the fresh ones, tomato, juice and other tomato products 1/2 cup, broccoli 1/2 cup mashed, asparagus 1/2 cup mashed	Mushrooms, vegetable juice cocktail
Fruits	Most of them Fruits dried at home are permitted	Mango (1/2), Papaya, Pear (one medium pear), Pineapple (1/2 cup)	Nectarine, commercially dried fruits including raisins, dates, prunes, avocado
Bread, cereals and starchy foods	Breads & pasta from refined flour, rice, cereals with <0.1 mg of copper per portion	Whole wheat bread (1 slice), whole wheat biscuits (6 pieces), potatoes (mashed 1/2 cup or small size), pumpkin (3/4 cup = 177 g) parsnips (2/3 cup mashed = 165 g), green beans (1/2 cup)	Dried beans (soy beans, dried peas; lentil lima beans, baked beans, garbanzo beans, pinto beans), wheatgerm, cereals with >0.2 mg of copper per portion
Fats	Butter, cream, margarine, mayonnaise, sour cream, oils, salad, dressings	Olives	
Milk and products	Most of milk products		Soy milk
Sweets and desserts	Jams, jellies and candies	Syrups 1 oz	Desserts that contain high amounts of ingredients rich in copper; candy with nuts, chocolate, or cocoa
Beverages	Coffee, tea, fruit juices, lemonade, soups	Carbonated beverages, dehydrated and canned soups	Mineral water, soy-based beverages, copper-fortified formulas

Table I. Wilson's Disease diet

This is usually not enough. The presence of copper in most foods makes its dietary exclusion impracticable. However, it is recommended to avoid foods rich in copper. The dietary intake of copper should be less than 1mg per day (normal daily requirement is approximately 2-5mg/day). In Table I food groups that can be administered or totally contraindicated to WD patients are presented, according to their content in copper. A low copper diet must contain all the nutrients necessary for normal development and functioning of the organism.

In Wilson's Disease some patients receive chelation treatment with D-penicillamine and may develop a deficiency of vitamin B-6 (pyridoxine), so a supplement of 25 mg daily is necessary.

Pharmacological therapy

Several drugs, copper chelators are available for the treatment of Wilson's Disease, including: D-penicillamine, trientine, zinc, tetrathiomolybdate and dimercaprol, of which only D-penicillamine and zinc are available in our country. These drugs are effective but sometimes have severe side effects.

Once the diagnosis has been established, treatment needs to be life-long[13].

D-penicillamine. D-penicillamine is considered the first line therapy for Wilson's Disease. Oral administration of D-penicillamine leads to complete reversibility or improvement of the hepatic abnormalities, neurological and psychiatric symptoms in most patients with Wilson's disease. The key of success is early diagnosis and treatment, so the clinical onset can be indefinitely postponed in asymptomatic patients if they receive continuous treatment. The mechanism of action of D-penicillamine remains controversial. Its use in Wilson's disease is based on its properties as in vitro copper chelator. The major effect of D-penicillamine in Wilson Disease is to promote the urinary excretion of copper[14]. The initiating doses are 5mg/kg/day and they will be progressively increased by 5mg/kg/day within 14 days until 20mg/kg/day with a maximum of 250 mg, in 2-3 divided doses. Administration is recommended one hour before main meals, because it is very well known that the absorption is inhibited by foods. If D-penicillamine is taken during a meal, its absorption is decreased by about 50%[15].

The association with pyridoxine is also useful (doses 25-50 mg/day) because D-penicillamine interferes with the action of pyridoxine. The maintenance dose in adults is 750-1000 mg/day divided in two or three doses.[5] D-penicillamine is rapidly absorbed from the gastrointestinal tract with a double-peaked curve for intestinal absorption[16]. Once absorbed, 80% of D-penicillamine circulates bound with plasma proteins. The excretion is predominantly via kidneys. The half-time of this drug is nearly 1.7-7 hours, with considerable individual variation[15,16,17].

The treatment efficacy can be monitored by determining the 24-hour urinary copper excretion. The maximum values are reached immediately after initiation of treatment and may reach up to 16 μmol (1000 μg) per 24 hours. Serum ceruloplasmin may decrease after initiation of the treatment and urinary

copper excretion should be maintained at about 250-500 μg /24 hours for a good compliance to treatment conditions.

After 2 days of D-penicillamine cessation, urinary copper excretion should be <100 μg /24 hours. Higher values may indicate non-adherence to therapy (in those patients non-ceruloplasmin-bound copper is elevated >15 $\mu\text{g/l}$)[18]. Also normalization of non-ceruloplasmin-bound copper is another sign of therapy effectiveness.

In patients with hepatic Wilson's disease, the improvement of clinical symptoms and liver function recovery is usually achieved within the first 2-6 months of treatment, but cytolytic syndrome may persist even longer than one year. Histological hepatic improvement was also observed, but regression of fibrosis and portal hypertension is less obtained.

In patients with neurologic Wilson's disease, after 4 weeks of chelatory treatment with D-Penicillamine, worsening of neurologic symptoms has been reported, with marked extrapyramidal phenomena in 20 % of cases. In this situation the D-Penicillamine dose is reduced to a third (250mg/day in adults), then it can be progressively increased with one third of the dose (250mg/day in adults) every 4-7 days up to a concentration of urinary copper at least 1000 $\mu\text{g/day}$, preferably 2000 $\mu\text{g/day}$. Neurological aggravation was also described in other treatments used in Wilson Disease (trientin, zinc), but less often.[19] Despite neurological worsening, treatment should be continued in order to obtain further improvement.

In patients with neurologic Wilson's disease, symptoms improvement is slower and can be observed even after 3 years. The most important sign of therapy effectiveness is clinical improvement and normalization of laboratory tests.

Tolerability of D-penicillamine may be enhanced by starting with incremental doses, 125-250 mg/day, increased by 250mg every 4-7 days until a maximum 1000-1500 mg/day in 2-4 divided dosages. [18] Administration of a dose of 1500 mg/day in a single dose, may result in rapid neurologic deterioration, as well as the direct resumption of treatment in patients who have stopped for a long period of time.

Many side effects are described in D-penicillamine treatment, but those that require cessation of therapy are rare. Thus, in the first 3 weeks the so-called hypersensitivity reactions may occur, characterized by fever, rash, lymphadenopathy, neutropenia, thrombocytopenia and proteinuria. Bone marrow toxicity includes severe thrombocytopenia or total aplasia. Under these conditions, D-penicillamine should be discontinued immediately. Late adverse reactions include nephrotoxicity, highlighted by proteinuria or the appearance of other cellular elements in the urine, also requiring discontinuation of treatment, Goodpasture syndrome (with higher dosages) and lupus-like syndrome with hematuria, proteinuria and positive antinuclear antibody; dermatological toxicity with atrophic skin changes, elastosis perforans serpiginosa[20], pemphigous and pemphigoid lesions, lichen planus and aphthous stomatitis. Very late side effects are rare: nephrotoxicity, myasthenia gravis[21], polymyositis, loss of taste, hypo-immunoglobulin A and

serous retinitis. Hepatic siderosis has been reported in patients with reduced levels of serum ceruloplasmin and non-ceruloplasmin-bound copper[22].

Trientine. Trientine (triethylene tetramin dihydrochlorid or 2,2,2-tetramine) was introduced in 1969 as an alternative to D-penicillamine, as a chelator with a polyamine structure. The lack of the sulfhydryl group causes the formation of stable links between the four nitrogen atoms and copper. Like D-penicillamine, trientine increases copper urinary excretion and reduces its digestive absorption.

Fewer data exists about the pharmacokinetics of trientine. Gastrointestinal absorption is low, and what is absorbed is metabolized and inactivated[23]. The amount that is urinary excreted is about 1% of the administered trientine and about 8 % of the trientine metabolite, acryltrien[24]. Trientine potency as copper chelator in comparison with D- penicillamine is controversial[25]. These two drugs may mobilize different pools of body copper[26]. Urinary copper excretion is lower than that of D- penicillamine and is not recommended as first-line drug.

Usual dosages of trientine are 900-2700 mg/day, in two or three divided doses. The maintenance dose is 900-1500 mg. In children the dose generally used is 20 mg/kgc/day, to the nearest 250 mg, given in two or three divided doses per day. This drug should be administered 1 hour before or 3 hours after meals. Trientine is an effective treatment for Wilson's disease[27]. Trientine has also proved very effective as initial therapy, even in patients with decompensated liver disease[28]. Generally, the adverse effects of D-penicillamine treatment disappear when it is replaced with trientine and do not reappear even if the treatment is prolonged. Trientine is also an iron chelator and administration of iron should be avoided because the resulting compounds are toxic. As with D- penicillamine , during initiation of treatment with trientine, more severe neurological symptoms may occur, but less frequently. As adverse effects in patients with Wilson's disease treated with trientine, have been reported lupus-like syndrome and reversible sideroblastic anemia. However, these patients were treated previously with D-penicillamine, so the true frequency of this reactions when trientine is used de novo is unknown.

The effectiveness of treatment is monitored by measuring the urinary excretion of copper in 24 hours after cessation of therapy for 2 days and measuring the non- ceruloplasmin copper[5].

Ammonium tetrathiomolybdate. Ammonium tetrathiomolybdate is a very strong copper chelator. In the gastrointestinal tract it prevents copper absorption. Some is absorbed systemically and can bind excess copper in the blood making it unavailable for cellular uptake[29]. Ammonium tetrathiomolybdate regulates copper transport to the specific metalloenzymes[30]. At low doses ammonium tetrathiomolybdate removes copper from metalloenzymes but at higher doses it forms an insoluble copper complex which is deposited in the liver[31]. It still remains an experimental treatment and it is not commercially available. Clinical trials were performed using the following dosages: 60-100 mg/day given in 2 divided doses to patients with Wilson's disease which didn't tolerate D-penicillamine

or trientine, demonstrating significant reduction of hepatic copper deposits .The advantage of this type of therapy is that it delays the appearance of neurological decompensation longer than other agents.

The most important side effect is bone marrow depression[32]. Other potential adverse effects are hepatotoxicity[33], neurological dysfunction with too aggressive chelating effect, antiangiogenic effects[34].

Zinc. Zinc was first used for Wilson's disease treatment by Schouwink in Holland in the early 1960's[35]. Zinc is not recommended as therapy for symptomatic Wilson disease onset due to the slowly installed effect. It has been suggested that it may be useful in pre-symptomatic disease, pregnant women or as maintenance therapy in patients who have previously used other chelators.

Zinc is a co-factor for more than 70 types of enzymes. The mechanism of action is different from that of D-penicillamine and trientine : zinc interferes with the absorption of copper from the gastrointestinal tract. Zinc induces enterocyte metallothionein, a cysteine-rich protein that is an endogenous chelator of metals. Metallothionein has higher affinity for copper than for zinc and thus binds preferentially copper present in enterocytes and inhibits its penetration into the portal circulation. Once bound, copper is not absorbed, but is eliminated into faeces as enterocytes are shed with normal turnover[36]. Because copper reaches into the gastrointestinal tract also from the saliva and intestinal gastric secretion, treatment with zinc may cause a negative balance for copper , thereby mobilizing copper from deposits[37]. Zinc may also act by inducing levels of hepatocellular metallothionein, thus binding excess copper and preventing hepatocellular injury[38].

In practice different zinc salts are used (sulphate, acetate or zinc gluconate) in doses of 150 mg of elemental zinc daily in adult or patients weighing more than 50kg. For children weighing less than 50 kg the dose is reduced to 75 mg. Administration is in three divided doses, 30 minutes before meals. Adherence to the administration of three divided doses can be a problem. The zinc salt used does not make a difference with respect to efficacy but may affect tolerability. Zinc medication taken with meals may interfere with absorption. There aren't studies that prove the advantage of using multi-chelation therapy. No additive effect of zinc was described with other chelators. However, in order to avoid the neutralization of zinc efficiency by chelators, times of administration must be different[5].

The therapeutic efficacy of zinc is appreciated by clinical and biochemical improvement. 24 hour urinary excretion of copper should be less than 100 µg/24 hour on stable treatment. In addition, the non-ceruloplasmin-bound copper concentration should decrease. Urinary excretion of zinc must be measured from time to time to check compliance.

Zinc has few side effects. Gastric irritation and headache are common, depending on the type of zinc salt used. Side effects occur rarely with zinc acetate. Zinc can also have immunosuppressive effects affecting leukocyte chemotaxis . In some cases, serum lipase and amylase may increase without imaging evidence of pancreatitis . Neurological adverse effects do not occur under treatment with zinc other than in exceptional

cases. Hepatic deposition of copper can continue under this therapy. Patients who don't respond to monotherapy with Zinc or presenting with recurrent cytolytic syndrome will benefit from additional or introduction of other chelation therapy. Oral zinc will be used as third-line therapy in patients who develop intolerance to D -penicillamine or trientine. Although zinc is used for maintenance treatment of Wilson disease, it was given also as first-line therapy in studies to asymptomatic patients. Apparently , the effectiveness is comparable to D- penicillamine, but tolerance is better.

Current guidelines recommend that all symptomatic patients with Wilson disease should receive a chelating agent like D-penicillamine or trientine. [18]Zinc may also have a role as first-line therapy in patients with neurological Wilson's disease.

Other treatments

Antioxidants, mainly Vitamin E may have a role as adjunctive treatment[39, 40]. Serum and hepatic levels of vitamin E have been found to be low in Wilson's disease[41]. Symptomatic improvement when Vitamin E was added to the treatment regimen was occasionally reported, but no rigorous studies have been, so far, conducted[5].

Other drugs used to treat patients with neurologic Wilson's disease include: anticholinergics, baclofen, gamma-aminobutyric acid antagonists (GABA), levodopa for pseudo-parkinsonian syndrome and dystonia; anti-epileptic for seizures; neuroleptics for psychiatric symptoms. You can also impose protein diet restriction , lactulose therapy in cases of hepatic encephalopathy .

Animal data suggest a role for amitriptyline in impending liver failure due to Wilson disease but no human data are available[42]. Curcumin is an ideal antioxidant and an effective scavenger of reactive oxygen species and can act like copper-chelating agent[43], but clinical data in patients with Wilson disease are not yet available.

Liver transplantation. Wilson disease mortality rate remains high despite medical therapy. Indication of liver transplantation in patients with Wilson disease are: fulminant hepatic failure associated with hemolysis and elevated copper level or advanced cirrhosis and liver failure that does not respond to chelation therapy and supportive measures, Survival rate of patients with liver transplantation for Wilson disease at one year is 78-87%. This procedure corrects the enzymatic defect completely and permanently. Liver transplantation is not generally recommended for the treatment of refractory extrahepatic manifestations (neurological damage), but in some situations in which patients received liver transplant, there was a net improvement in neurological and psychiatric symptoms.

Haemofiltration may be necessary in patients with hepatic failure.

MARS is an extracorporeal hepatic support system using a dialysis mode in which the patient's blood is dialysed through a membrane system impregnated with albumin. There are few reported cases and it is believed that this therapeutic method only provides support for liver transplantation[44].

Follow up. Wilson's disease patients should be closely monitored weekly during the first 4-6 weeks from the initiation of chelation therapy (24 hours urinary copper, liver and kidney function tests, blood counts) to observe proper excretion of copper, compliance to treatment and adverse reactions. The best method of assessing compliance and efficiency is to measure the non-ceruloplasmin copper evaluated by the formula: total serum copper (mg/dl) - 3x the serum ceruloplasmin (mg /dl).The benchmark for compliance and efficiency is less than 15µg/dl. Another method of evaluating the same parameters is by measuring urinary excretion of copper during chelation therapy, and the value should be 200-500 µg/dl, and with zinc therapy < 75 µg/dl[19]. Elevated values of copper urinary excretion translate into non-adherence to therapy and possible liver damage. Lower values of urinary copper excretion may suggest the use of large doses of medication and they need to be adjusted.

In patients with D- Penicillamine therapy evaluation and history should focus on possible signs that reveal worsening neurological and psychiatric symptoms (especially depression)

In the first year of treatment evaluation must be performed every 6 months, then annually. In patients with severe disease, the ophthalmic exam: assessment of Kayser Fleischer ring by slit-lamp ophthalmologic evaluation should be performed annually.

Long-term treatment of Wilson disease

Alcohol and hepatotoxic drugs are absolutely contraindicated. Zinc therapy can be assessed in terms of compliance by measuring urinary zinc which must have the value of 2 mg / 24 hours. Chelation lifetime therapy with D-penicillamine and zinc is essential as treatment discontinuation leads to acute fulminant liver failure and rapid and irreversible neurological damage.

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