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Benefits and drawbacks of current copper chelators in Wilson disease

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ABSTRACT

Background. Wilson disease (WD) is an autosomal-recessive disorder of copper metabolism, caused by mutations in the ATP7B gene, which codes for a membrane-bound copper-binding ATPase. This leads to progressive copper accumulation in the liver, with hepatic injury and subsequent copper release into the blood.
Aim. To analyze the efficacy and side-effects of the current copper chelating agents used in treatment of Wilson disease.
Material and methods. Retrospective study of 37 adult patients diagnosed with Wilson disease at the Gastroenterology and Neurology Departments of Fundeni Clinical Institute between 2012 and 2017. Patients were grouped into three categories: (a) those with isolated liver disease, (b) those with isolated neurologic (or psychiatric) involvement and (c) those with both liver and neurologic involvement.
Results. There were 54% females (n=20). Mean age at diagnosis was 23 ± 10 years-old. Liver cirrhosis was diagnosed in 54% of cases. Neurologic involvement was described in 65% of patients. Dysarthria was the most common neurologic feature (43%), followed by Parkinsonism (41%). D-Penicillamine was used as initial treatment in 89% of patients, but was interrupted in 27% of them (n=9) due to its adverse reactions. Keyser-Fleischer rings were absent in a significant proportion (40%) of patients with neurologic involvement. Both Trientine and D-Penicillamine were associated with improved or stationary liver fibrosis, however results were slightly better for Trientine. D-Penicillamine appeared to be a better option than Trientine in patients with neurologic involvement.
Conclusions. Based on our study, we recommend treatment with D-Penicillamine as first-line therapy in patients with neurologic involvement. However, due to the frequent side-effects of D-Penicillamine, Trientine could be considered first-line treatment in patients with isolated hepatic involvement.

Keywords: Wilson disease, Trientine, D-Penicillamine, copper chelator

Abbreviations (in alphabetical order):

CT – Computer tomography
KF – Keyser-Fleischer
INR – International Normalized Ratio
LT – Liver transplant
MRI – Magnetic resonance imaging
TE – Transient elastography
WD – Wilson disease

INTRODUCTION

Wilson disease (WD) is an autosomal-recessive disorder of copper metabolism, caused by mutations in the *ATP7B* gene, which codes for a membrane-bound copper-binding ATPase [1]. Over 600 mutations of this gene have been described [2]. The most common mutation in Central-Eastern European countries is the point mutation *H1069Q*, which affects between 50-80% of the patients [3]. Dietary copper is absorbed in the small intestine by the enterocytes, and then transported along the portal circulation to the liver, where it is taken up by the hepatocytes [4]. ATP7B is involved in the transport of copper (a) to the trans-Golgi network, where copper is incorporated into

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ceruloplasmin and also (b) to vesicles, which fuse with the apical membrane, thus excreting excess copper into the bile [5]. The resulting ATPase defect seen in Wilson disease will therefore lead to (a) impaired incorporation of copper into ceruloplasmin and (b) impaired copper excretion into the bile [6]. This leads to progressive copper accumulation in the liver, with hepatic injury and subsequent copper release into the blood. From there, copper deposition occurs in extrahepatic sites of the body, particularly in the nervous system and cornea. Consequently, hepatic disease, neuro-psychiatric disease and Keyser-Fleischer corneal rings comprise the main clinical features of Wilson disease [7].

Treatment of Wilson disease consists of (a) *dietary regimen* with avoidance of copper-rich foods (shellfish, mushrooms, liver, chocolate, and cocoa), (b) *copper-chelating agents* (D-Penicillamine or Trientine), which eliminate the excess copper from the body and (c) *Zinc salts*, which block copper intestinal absorption [1]. Liver transplantation is sought in patients with acute liver failure or decompensated cirrhosis unresponsive to medication [8,9], whereas Wilson disease with severe neurologic involvement is still a controversial indication for liver transplantation [10,11].

AIM

To analyze the efficacy and side-effects of the current copper chelating agents used in treatment of Wilson disease.

MATERIAL AND METHODS

We analyzed retrospectively the medical records of 37 adult patients diagnosed with Wilson disease at the Gastroenterology and Neurology Departments of Fundeni Clinical Institute between 2012 and 2017. Diagnosis of Wilson disease was established either by genetic testing or through a score higher than 3 on the Leipzig scoring system. Patients were grouped into three categories: (a) those with isolated liver disease, (b) those with isolated neurologic (or psychiatric) involvement and (c) those with both liver and neurologic involvement.

The degree of liver involvement was assessed through abdominal ultrasound, transient elastography, and laboratory parameters (serum albumin, total serum protein, fibrinogen, INR, prothrombin time, aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin and conjugated bilirubin). Laboratory work-up also included serum ceruloplasmin, serum copper and urinary copper excretion. Liver disease was defined by the presence of abnormal findings on clinical or paraclinical examinations. If liver cirrhosis was present, abdominal CT scan and/or abdominal MRI scan were also added to examination protocol, along with upper digestive endoscopy. Severity of liver cirrhosis was measured through the Child-Pugh and MELD scores.

Assessment of neurologic abnormalities was done by standard neurological examination and through non-contrast brain MRI. Patients were examined for the presence of signs of parkinsonism (e.g., bradykinesia, hypokinesia, rigidity, hypomimia and/or rest tremor), cerebellar disease (intention tremor, dysmetria), dysarthria, dysphagia, postural tremor, dystonia, pyramidal signs and gait abnormalities. Brain imaging was performed on a 1.5 Tesla MRI and protocol included the following sequences: T1/T2, T1SE, T2-FLAIR, DWI, SWI, 2D-TOF and 3D-TOF. Neurologic involvement was defined by the presence of abnormal finding on clinical or paraclinical examinations.

Ophthalmological assessment with slit lamp exam was performed in 25 patients. Patients' medical records were accessed using the hospital's medical database Hippocrates. Data collection and analysis were made using Microsoft Office Excel 2016. The study was approved by the Medical Ethics Committee of Fundeni Clinical Institute.

RESULTS

Demographics

There were 54% females (n=20). Mean age at diagnosis was 23 ± 10 years-old (range: 4-45 years-old). Mixed hepatic-neurologic involvement was described in 54% of patients, while isolated hepatic disease was present in 35%, and isolated neurologic involvement in 11%. Psychiatric symptoms were present in 24% of patients. A positive family history of Wilson disease was detected in 24% of cases. Genetic analysis performed in 7 of the patients revealed the following mutations: heterozygous *H1069Q* (n=3), homozygous *H1069Q* (n=2), compound heterozygous *H1069Q*/*G1341D* (n=1) and heterozygous *W779X* (n=1).

Treatment

D-Penicillamine was used as initial treatment in 89% of patients (n=33), but was interrupted in 27% of them (n=9) due to its adverse reactions. Neurological worsening was the most frequent side effect that required treatment interruption (see Figure 1). Of the nine patients in whom D-Penicillamine was interrupted, seven were switched on Trientine, one resumed treatment with D-Penicillamine after some time, and the last one received treatment with Zinc acetate only. Trientine was used as initial therapy in 5% of patients (n=2) and was interrupted and switched with D-Penicillamine in one (due to neuro-

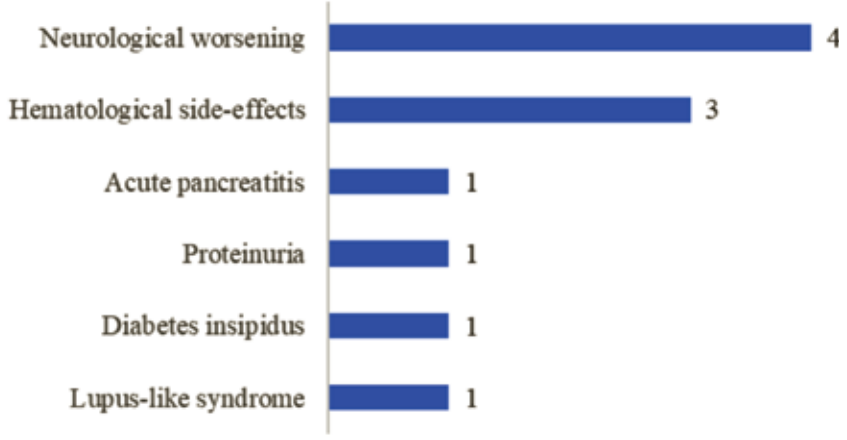

FIGURE 1. Main side-effects which required D-Penicillamine interruption in our patients

logical worsening, fatigue and irritability). About 57% of the patients received Zinc acetate, taken 50 mg three times daily, either in association with copper chelating agents or as monotherapy.

Liver involvement

Liver cirrhosis was diagnosed in 54% of cases, while other liver abnormalities were detected in another 35% of patients, i.e., liver steatosis on abdominal ultrasound or liver fibrosis on transient elastography. Fibrosis scores measured by transient elastography were monitored during follow-up in 15 patients. One patient who received only Zinc acetate (due to D-Penicillamine intolerance) presented worse fibrosis scores on follow-up elastography. Under Trientine (n=5), administered either *per primam*, or because of D-Penicillamine interruption, fibrosis scores were improved in 4 patients and stationary in one. Under D-Penicillamine (n=9), fibrosis scores were improved in 4 cases, stationary in 4, and worsened in one (see Figure 2).

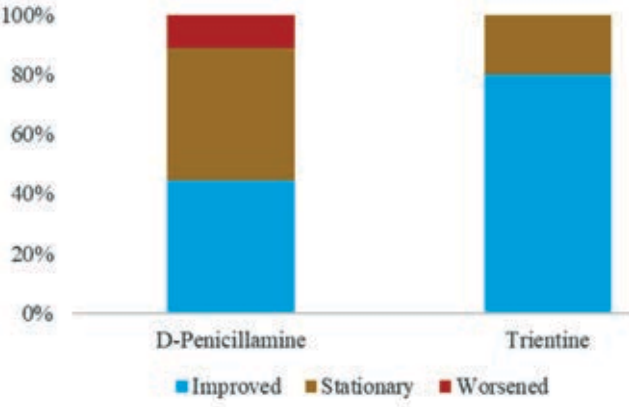

FIGURE 2. The effects of D-Penicillamine and Trientine on liver fibrosis scores in our patients

Neurologic involvement

Neurologic involvement was described in 65% of patients (n=24). Dysarthria was the most common neurologic feature (n=16), followed by parkinsonism (n=15), cerebellar syndrome (n=12), dysphonia (n=10), dystonia (n=6) and sialorrhea (n=3). Some kind of tremor (be it resting, postural and/or intention) was present in over one third of patients (n=13). Postural instability and gait disturbances were mentioned in 27% of patients (n=10) (see Figure 3). All patients admitted to the Neurology Department had neurologic involvement, as compared to 48% of the patients admitted to the Gastroenterology Department.

Neurological exam was repeated during follow-up in 15 patients (ten on D-Penicillamine and five on Trientine). On the long run, treatment with D-Penicillamine either improved neurologic symptoms or prevented neurologic worsening in almost all 10 patients (except for one, who admitted poor treatment compliance).

Trientine was associated with worsening of neurologic symptoms in two patients, improvement in one, and improvement followed by worsening in the last two cases. Overall, treatment with D-Penicillamine offered a better control of neurologic symptoms than Trientine.

Of interest, Keyser-Fleischer rings were absent in a significant proportion (40%) of patients with neurologic involvement, as opposed to the classic notion that they are virtually always present in the neurologic stage of the disease [1]. However, they were more likely to be absent in the hepatic stage of the disease (see Figure 4).

DISCUSSIONS

A summary of the agents used in the treatment of Wilson disease is given in Table 1.

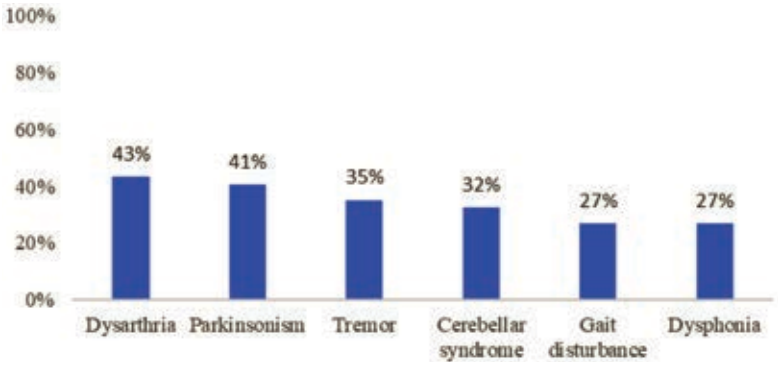

FIGURE 3. Main neurologic abnormalities seen in our patients with Wilson disease (and their frequencies)

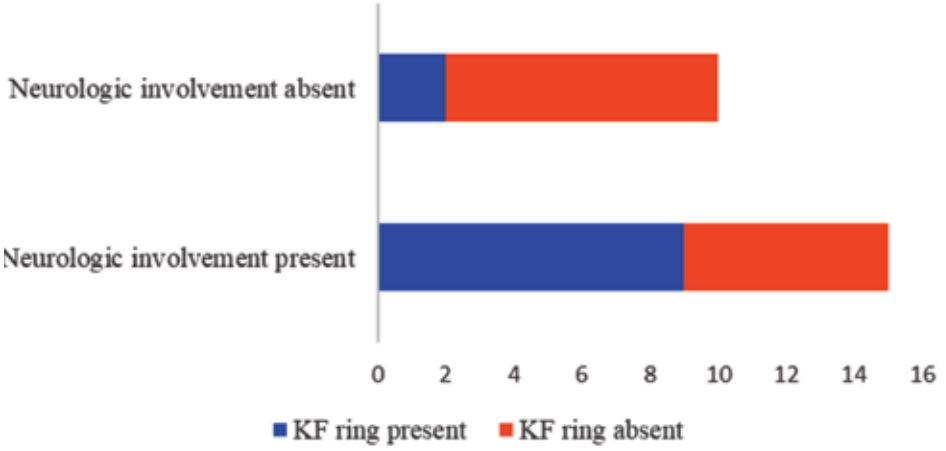

FIGURE 4. Prevalence of Keyser-Fleischer rings in patients with or without neurologic involvement

TABLE 1. Past and current medications used in the treatment of Wilson disease

Drug	Mechanism of action	Route of administration	Status
British antilewisite	Copper chelator	Intramuscular	No longer used
D-Penicillamine	Copper chelator	Oral	In use
Trientine	Copper chelator	Oral	In use
Zinc salts	Block intestinal absorption of copper	Oral	In use
Ammonium tetrathiomolybdate	Copper chelator	Oral	In study

British antilewisite (BAL, Dimercaprol)

The first agent successfully used in the treatment of Wilson disease was British antilewisite (2,3-dimercaptopropranol) back in 1951 [12,13]. British antilewisite is a heavy-metal chelating agent that was initially developed during World War II as an antidote against Lewisite (an arsenic-based chemical warfare agent). BAL was subsequently used in heavy-metal poisonings (especially with arsenic, mercury and gold salts), either accidental or iatrogenic in nature [14,15].

Its use in treating Wilson disease has been largely abandoned, due to its many disadvantages: (a) it required an intramuscular route of administration, which was painful; (b) it presented frequent side effects (fever, conjunctivitis, headache, dizziness, paresthesias, nausea and vomiting, local pain or abscesses at the injection site, liver damage, elevated blood pressure and tachycardia) and rare, but potentially serious side-effects, like seizures, stupor or coma; (c) it had an unpleasant mercaptan odor (of "rotten eggs") [16]. BAL can still be used occasionally in the initial treatment of severe Wilson disease, but only for a very short period. The recommended dosage is 2.5 - 5 mg/kg BW, two to five times daily, between 2 – 10 days [17].

D-Penicillamine

Of the currently used drugs, D-Penicillamine was described first in 1956, as an oral agent with a greater efficacy than BAL in increasing copper urinary excretion, and with no short-term adverse reactions

[18]. D-Penicillamine is a breakdown product of Penicillin. It was originally isolated from the urine of patients with liver disease receiving Penicillin and was later found to have chelating properties against copper [19]. D-Penicillamine is absorbed rapidly, but only partially (between 40-70%) from the intestine. It is worth mentioning that food, antacids, and iron interfere with its absorption [20]. The drug is available as capsules of 125 mg and 250 mg, and as tablets of 250 mg. The recommended dose in adults is between 750-1500 mg (divided three or four times daily), and the maximum dose allowed is 2000 mg daily [19,21]. Side-effects of D-Penicillamine are well-known and can be severe enough to require treatment interruption:

After treatment initiation, **neurologic worsening** (sometimes irreversible) may develop, and this may be seen in 10-50% of patients, presumably because cooper is massively mobilized from the liver into the blood circulation, and subsequently redistributed to the brain [1]. Supporting this theory is the observation that free copper becomes markedly increased in the striatum of mice after D-Penicillamine is administered [22]. Moreover, in one patient who presented neurologic deterioration after D-Penicillamine was initiated, new lesions were found on brain MRI, despite excellent hepatic decoppering (documented by liver biopsy) [23]. The frequency of early neurologic worsening was noted in approximately 11% of cases in one study, and involved only patients with neurologic signs at diagnosis. Mean time to development of neurologic worsening after treatment initiation was 2.3 ± 1.9 months [24]. Even patients with isolated hepatic disease or even with asymptomatic Wilson disease have developed neurologic signs after D-Penicillamine initiation [25,26].

Sensitivity reactions occur in up to 20% of patients [1], usually during the initial phases of the treatment. These include fever, rash, lymphadenopathy or hematologic side-effects (leukopenia or thrombocytopenia). In less than 1% of cases, **autoimmune reactions** may develop, such as lupus-like syndrome, myasthenic syndrome, polymyositis or dermatomyositis, membranous glomerulopathy, Goodpasture syndrome or pemphigus [27,28,29].

Physicians should monitor proteinuria with long-term D-Penicillamine use, as **nephrotoxicity** is a possible late side-effect, requiring immediate interruption of the drug [27,29]. Proteinuria most often develops in the first 12 months of treatment and resolves spontaneously after the drug is withdrawn (with a median duration of 8 months) [30]. Another serious side effect reported with long-term use is bone marrow toxicity, with subsequent thrombocytopenia or even aplasia.

Skin changes have also been encountered with prolonged D-Penicillamine use (e.g., pseudoxanthoma elasticum or cutis laxa), and may occur in up to one third of patients. D-Penicillamine has a negative effect on the cross-linking of collagen and elastin fibers. This may actually have a positive effect on liver fibrosis (by ameliorating it or preventing and delaying fibrosis). At the same time, this has a negative effect on wound healing, for which some authors recommend switching to Zinc or Trientine in the setting of planned surgery [27,28,29,31].

Supplementation of Pyridoxine (25 to 50 mg daily) is recommended during treatment with D-Penicillamine to avoid vitamin B6 deficiency [27,28].

Trientine

In 1969, Trientine (triethylenetetramine dihydrochloride) was used for the first time by Walshe to treat WD in a child with D-Penicillamine-induced nephrotoxicity [32,33]. Trientine is available as capsules of 250 mg, and the recommended dose for adults is between 750-1250 mg (divided two to four times daily), with a maximum dose of 2000 mg daily [34]. Like D-Penicillamine, Trientine should not be administered with foods [35]. Trientine is also known to cause neurologic deterioration at the beginning of treatment, although this happens less often than with D-Penicillamine [7,36,37]. A rare, but specific side-effect is *Trientine-induced colitis* (occasionally accompanied by ileitis or duodenitis), which improves after discontinuation of treatment [33,36]. By interfering with mitochondrial iron metabolism, Trientine can lead to *acquired sideroblastic anemia*, which also improves after dose reduction or cessation [38,39]. Trientine may produce gastro-intestinal adverse effects and *acute rhabdomyolysis*, as described in 4 patients with primary biliary cirrhosis who received copper-chelating therapy [40].

Regarding efficacy, Walshe (who introduced both chelating agents) found that Trientine successfully maintained or improved the clinical status in 20 previously treated patients who developed D-Penicillamine intolerance [41]. The same author pointed out that the cupruresis induced by both drugs is large in untreated patients, but less so in previously treated patients (especially for Trientine) [42]. Other authors have used Trientine in the setting of D-Penicillamine intolerance and found it to be an effective alternative, with no significant side-effects (apart from a decreased level of serum iron) [43,44]. Moreover, Trientine has been shown to prevent the abrupt clinical deterioration, sometimes fatal, seen after cessation of D-Penicillamine (because of fulminant hepatitis and liver failure) [45].

In a retrospective study of 380 patients comparing Trientine with D-Penicillamine (perhaps the largest on the subject), both agents showed similar efficacy, with hepatic improvement in more than 90% of cases and neurologic improvement in over 55% cases. Indeed, adverse events requiring treat-

ment interruption were more frequent with D-Penicillamine, while neurologic worsening occurred more frequently in patients treated with Trientine, as was the case in our retrospective study [46].

At the moment, Trientine is considered second-line therapy and is indicated in patients with Wilson disease and D-Penicillamine intolerance [34]. However, some authors have proposed that Trientine should be used in fact as first-line therapy, given the similar rate of efficacy, but the superior safety profile [47].

Both agents have been shown to be teratogenic in animals, however pregnancies in humans appeared to be safe with both agents, with a low rate of birth defects [48]. D-Penicillamine was associated with a lower rate of spontaneous abortions than Trientine (17% versus 28%), and both were lower than in untreated women (41%).

Ammonium tetrathiomolybdate

This is a compound that has been known to induce copper deficiency in ruminants since the 1940s [49]. It was not until 1992 however that tetrathiomolybdate was developed as an experimental drug to be used in the treatment of Wilson disease [50]. The drug is not yet commercially available in our country; however, studies have already been published regarding its efficacy and safety. Brewer, who was the first to study this drug, published over the years three papers regarding the efficacy and safety profile of Tetrathiomolybdate. In his studies, Tetrathiomolybdate was given to previously untreated patients with neurologic Wilson disease, during a period of 8 weeks, in doses varying between 120-410 mg per day [51,52,53]. He concluded that Tetrathiomolybdate was an excellent form of initial chelating therapy for patients with neurologic WD. The rate of neurologic deterioration (4%) was significantly lower than with D-Penicillamine, and other side-effects (bone marrow suppression or hepatotoxicity) occurred only with rapid escalation of doses. Brewer went further, and performed a randomized, double-blind, controlled study, which compared Tetrathiomolybdate with Trientine [54]. The rate of neurologic worsening was significantly lower with Tetrathiomolybdate than with Trientine, however side-effects (in the form of anemia and raised hepatic enzymes) were higher. Tetrathiomolybdate appears to strongly reduce the levels of free serum copper (which is thought to be responsible for the toxic effects seen in WD). In the study in which Tetrathiomolybdate and Trientine were compared, Trientine administration in some patients actually led to a spike in free serum copper (which was associated with neurologic worsening) [55]. Welshe was the first to observe that Trientine raises the serum copper concentrations (as opposed to D-Penicillamine which lowers them), suggesting that the copper chelating agents act by mobilizing copper from different body compartments [42].

CONCLUSIONS

In our study, D-Penicillamine was the most used copper chelating agent, however side-effects required treatment interruption in approximately one quarter of the patients. The most frequent adverse reaction was neurologic worsening. Both Trientine and D-Penicillamine were associated with improved or stationary liver fibrosis (as measured by transient elastography), however results were slightly better for Trientine. Only 60% of the patients with neurologic involvement presented Kayser-Fleischer rings. D-Penicillamine appeared to be a better option than Trientine in patients with neurologic involvement.

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