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# Wilson's Disease: Current Therapies, Its Controversies, and Potential New Therapeutics

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ABSTRACT

Wilson's Disease is a rare genetic disorder with the prevalence of 1 in every 30,000 people, due to the mutation of the ATP7B gene responsible for copper metabolism. The mutation causes copper accumulation in the body, especially in the liver and brain, which leads to hepatic, neurological, psychological symptoms. These symptoms, if not treated properly, may lead to death after several years. Several treatments including low copper diet, zinc salts treatment, chelating agents (penicillamine, trientine, ammonium tetrahimiolybdate), and liver transplant are currently available. Severe neurological deterioration and other side effects need new, more efficient, and safer therapeutics. Several new therapeutic agents including 4-phenylbutyrate, curcumin, chelating polymeric beads, and long term metabolic correction has been tested *in vitro* and *in vivo*. These new therapeutics may be a potential new treatment with less side effect and greater efficacy for Wilson's disease patients.

**Keywords:** 4-PBA, 4-phenylbutyrate, curcumin, microbeads, Wilson's disease

ABSTRAK

Penyakit Wilson adalah kelainan genetik langka, dengan prevalensinya 1 setiap 30.000 orang, disebabkan mutasi gen ATP7B yang bertanggung jawab untuk metabolisme tembaga. Mutasi menyebabkan akumulasi tembaga, terutama di hati dan otak, menyebabkan beberapa manifestasi klinis, neurologis, psikologis. Jika tidak diobati, dapat menyebabkan kematian setelah beberapa tahun. Beberapa cara pengobatan termasuk diet rendah tembaga, pengobatan garam seng, agen *chelating* (*penicillamine, trientine, ammonium tetrahimiolybdate*), dan transplantasi hati. Namun, adanya keterbatasan, gangguan neurologis parah, dan efek samping lain membutuhkan cara baru yang lebih efisien dan lebih aman. Beberapa terapi baru yang lebih potensial dengan efek samping lebih kecil, termasuk *4-phenylbutyrate*, kurkumin, manik-manik polimer khelasi, dan koreksi metabolik jangka panjang telah diuji secara *in vitro* dan *in vivo*. **Dias Rima Sutiono, Giardani Syafitri Sudiro. Penyakit Wilson: Terapi Masa Kini, Kontroversi, dan Terapi Potensial**

**Kata kunci:** 4-PBA, *4-Phenylbutyrate*, *curcumin*, manik-manik mikro, penyakit Wilson

## INTRODUCTION

Wilson's disease (WD) is a genetic disorder caused by the mutation of the ATP7B gene on chromosome 13.[1] This mutation causes an autosomal recessive disorder in copper (Cu) metabolism due to the dysfunction of Cu translocase in ATP7B protein highly expressed in kidney, liver, and placenta.[2,3] ATP7B encodes a transmembrane protein ATPase responsible for transporting Cu to either biliary excretion or ceruloplasmin production.[1,3] The protein itself will stay in the trans-golgi network (TGN) in normal Cu condition to help in the production of ceruloplasmin that is essential for iron metabolism. ATP7B will move towards the bile canaliculi during excess Cu concentration to help the dispose of Cu to the bile onto the feces.[2] Its dysfunction leads to the accumulation of Cu, especially in the liver and brain, and leads to Cu toxicity of the organs.[1,2] There have been more than 650 determined mutations of the ATP7B gene with sixty percent of them classified as missense mutations.[4]

Worldwide, WD is estimated to have the prevalence of 1 in every 30.000 people[5] with the ratio of mutation carriers of about 1 in 90 people.[4] The most common mutations of ATP7B gene are R778L in Southeast Asia, H1069Q/G in Europe and North America, and the combination of both in Indian population.[2,5] The phenotype of the disease may change according to the difference in mutational properties. Early detection and treatment are vital for the survival and even may lead to the prevention of neurological symptoms in patients with hepatic manifestation.[6] Untreated cases may lead to death within 2-5 years subsequent to the onset of neurological symptoms.[4]

## CLINICAL MANIFESTATIONS

The clinical manifestation of WD may appear at any age with the majority between 5 to 35

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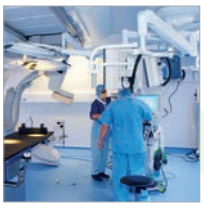



years of age. Onsets at 2 years old and 70 years old have also been reported, even though very rarely.[4] The most common clinical manifestations are in the form of hepatic, neurologic, and psychiatric conditions.[1,4] Since hepatic manifestations of WD are often nonspecific, any liver disease of unknown origin should be considered as WD. The manifestations may range from asymptomatic, small biochemical abnormalities, to cirrhosis and sometimes associated with hemolysis due to the escaped Cu from dead liver cells.[1]

Neurological symptoms may precede the onset of hepatic symptoms in 40-50% cases; in individuals with hepatic symptoms, neurological symptoms may develop in 2-5 years.[4] Variable neurologic manifestations are also observed, but can be roughly classified as: Akinetic-rigid syndrome with similarities to Parkinson's disease; Pseudosclerosis dominated by tremor; Ataxia; and Dystonic syndrome. Behavioral and psychiatric symptoms are common and may precede hepatic and neurologic symptoms.[1] WD may also present with exclusively psychiatric symptoms in around 10% of cases. This often leads to delayed diagnosis and treatment, which may endanger the patients.[7] The psychiatric symptoms range from slight personality changes and impulsiveness to paranoia, schizophrenia, or depression in different age groups. Severe cognitive deterioration is rare, but has been observed in WD patients.[1]

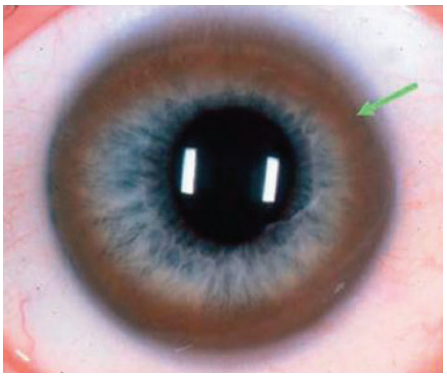

**Figure.** Brownish-green ring eye

Few physical signs associated with WD patients are sunflower cataracts and Kayser-Fleischer ring (KFR). Both signs are ophtamologic changes caused by deposits of Cu in different parts of the eye. KFRs are brownish-green ring caused by the deposit of Cu in Descemet's membrane of the cornea (**Figure**) while sunflower cataracts are caused by Cu deposit in the center of the lens.[1,4] Both ophthalmologic changes are often reversible sometime after treatments.[4,8]

## COMMON AVAILABLE TREATMENTS

Several treatment options are available for WD such as low Cu diet, zinc (Zn) salts treatment, chelating agents (penicillamine, trientine, ammonium tetrahimiolybdate), and liver transplant.[1] However, most are with dangerous side effects and obvious limitations.[4]

### Low Copper Diet

One of the simplest treatments of WD is low Cu diet by limiting or stopping the consumption of Cu. However, this method should not be considered as a sole treatment since it does not quickly and effectively chelate Cu.[4] Cu is also omnipresent in almost all food, making it impossible to avoid them completely.[9] The diet focusses to only avoiding foods high in Cu content, such as chocolate, liver, nuts, mushrooms, and shellfish.[3]

### Zinc Salts

Zn salts treatment reduces Cu uptake from the gastrointestinal (GI) tract by increasing metallothionein synthesis. Metallotihionein has higher affinity towards Cu than Zn; therefore, with the increase of Zn concentration, metallothionein will bind to Cu more aggressively and help Cu excretion through stool.[4] Despite its benefits, Zn salts has been reported to cause significant GI disorders. In fact, the doses, which is one hundred fold more than the daily intake of Zn, may cause mild Zn poisoning.[9,10] The aggressive Cu removal from the GI tract may also lead to symptomatic Cu deficiency that has been reported in WD patients under Zn therapy, usually with high daily Zn salt doses. Physical symptoms may includeocular motor abnormalities (nystagmus, conjugate ocular palsy, and saccades alterations), dementia, hypokalemic periodic palsy, oculogyriccrises, muscle cramps, distal dysesthesias, and respiratory dyskinesia with cough.[11]

### Chelating Agents

Penicillamine, trientine, and ammonium tetrahimiolybdate are all chelating agents used for the treatment of WD. Penicillamine works by binding free serum Cu or intracellular Cu into a penicillamine-Cu complex that will be excreted into the urine. Penicillamine is associated with various side effects including rashes, lymphadenopathy, neutropenia, thrombocytopenia, proteinuria, and nephrotoxicity and bone marrow suppression as a long term side effects.[4] Even though the occurrence is rare, epileptic status have been reported after several months or a year of treatment. One case, a 38-year-old woman, develops an immediate reaction in the form of epilepsy after first administration of penicillamine.[7] Due to its various side effects; penicillamine is no longer the preferred treatment for WD. Instead, trientine was prescribed to WD patients, especially those with intolerance towards penicillamine.

Trientine is a chemically distinct chelator from penicillamine that works by promoting urinary excretion of Cu.[1] Despite being claimed to be better than penicillamine, trientine have also been reported to cause several side effects, including hypersensitivity reactions, sideroblastic anemia, and hepatic siderosis, albeit more rarely.[4] Ammonium tetrathiomolybdate (TM) is a relatively novel and a very strong decoppering agent which binds Cu and prevent them from being absorbed by the GI tract or other cells.[1,4] TM remains an experimental drug with limited clinical experience.[1] In a double blind study, TM proves to be better for WD compared to trientine. Despite this, TM has been discovered to have side effects which include anemia and leukopenia[4] and possible adverse effects such as bone marrow depression, hepatotoxicity, and over-aggressive Cu removal.[1]

The most common problem with chelating agents is the worsening of neurological symptoms, albeit in different intensity and frequency.[1] This side effect is hypothesized to be due to chelating agent's mechanism of excreting excess Cu through urine. In order to pass the Cu through urine, Cu must first be transported through blood; this transportation may aid the distribution and accumulation of Cu in various organs, including brain. Recent large cohort study of WD patients under chelation therapy shows 50% patients are fully recovered or improved, 15% deteriorated, 8% needed liver transplant; and 7.4% died.[12] The worsening of existing symptoms and fatal liver damage will inevitably occur when the

treatment is stopped.[8]

### Liver Transplant

Liver transplant was regarded as the last resort for WD patients, especially those with acute liver failure or decompensated cirrhosis. Survival rate after liver transplant are mostly around 70% with higher rate in patients with chronic liver disease than on those with acute liver failure.[1] Patients with neurological symptoms may experience deterioration and high mortality rate after transplantation. This is due to the fact that liver transplantation does not help the restoration of neurological function through brain Cu elimination.[4] Another limitation for liver transplant is lack of available donor which may lead to the worsening, or even deathof WD patients, while waiting for a donor.

The limitations of the currently available treatments need new and better options. These treatments should be effective enough to eliminate Cu from organs and restore the organs' function. The treatments should also prevent neurological deterioration, a common side effect in almost all of the currently available treatments.

## POTENTIAL NEW THERAPEUTICS

4-Phenylbutyrate (4-PBA) and curcumin, chelating polymeric beads, and long term metabolic correction are some new therapeutics that have been researched and developed to better treat WD patients.

### 4-PBA and Curcumin

4-PBA is a clinically approved pharmacological folding chaperone, mostly used to restore protein expression in other liver diseases with mutated membrane proteins. Curcumin acts as an inhibitor of sarco(endo)plasmic Ca+-ATPases, reported to fix plasma membrane localization. The *in vitro* experiment was conducted using HEK293T and Human osteosarcoma cell line (U2OS). The substances were used to fix the misfolded proteins due to the mutation in ATP7B gene. The specific mutations of interest are R778L and H1069Q which are the two most common mutation in Asian and American-European respectively. The two mutations possess residual Cu transporting capacity that, if overexpressed, can be used to increase the ability of the WD patients to recover the functional Cu export.[13]

The results from the *in vitro* research suggested that the cells, which were incubated at 30°C after treatment by 4-PBA and curcumin, showed significant increase in the number of mutant ATP7B protein with residual Cu transport capacity. This treatment also leads to the re-localization of the mutant protein from endoplasmic reticulum (ER) to the TGN. The increase in expression of mutant ATP7B protein with residual Cu transport capacity and re-localization of the protein back to TGN suggested the possibility of utilizing 4-PBA and curcumin as a therapeutic agent to promote the restoration of the normal functional Cu export. Another advantage is the possibility to avoid neurological complication or deterioration due to the fact that both 4-PBA and curcumin are able to cross the blood-brain barrier.[13]

Curcumin by itself is considered to be a multifunctional against WD due to its antioxidant, copper-chelating, and superoxide dismutase (SOD) activities. Curcumin has the ability to scavenge both reactive oxygen species (ROS) and reactive nitrogen species (RNS), it can also inhibit lipid peroxidation and increase glutathione availability. Although in low concentrations, curcumin also has the ability to chelate Cu with relatively high affinity through the formation of Cu(II)-curcumin complexes. These complexes also possess SOD activity at the same time, useful for scavenging superoxide radicals. Hence, curcumin can be used to chelate Cu and relieve oxidative stress at the same time, which makes it superior to current therapeutic chelating agents.[14]

### Macroporous Polymeric Microbeads

Another potential therapeutics is macroporous polymeric microbeads that can specifically chelate Cu (II) ions. These microbeads are poly(glycidyl methacrylate-co-ethylene dimethacrylate)-based beads with the size of 20-40 µm. The microbeads contain polymers which include N,N-2-pyridylmethylamine (DPAB), triethylenetetraammine (TTAB), and 8-hydroxyquinoline (8HQB). These microbeads are responsible to adsorb all Cu released from food during digestion and Cu secreted by the body to the GI tract which will further shift the Cu balance to promote elimination.[7]

These polymeric beads have been tested *in vitro* and *in vivo* (in rats) for its efficacy and safety; the results support the initial prediction that the polymer will not be absorbed in the GI tract and will be eliminated completely through feces after successfully chelate Cu ions in the GI tract.[7,10] The polymeric beads did not alter food intake or bodyweight of the subject, indicating that the substances are nontoxic. At certain doses, all three polymeric beads are able to significantly reduce the level of Cu in kidneys and, in DPAB and TTAB, significantly reduce the Cu content in liver and brain of rats. The positive results from the study and the working mechanism of these polymeric beads which allows complete elimination through feces, which is the normal path for Cu elimination, ensures the possibility of this agent to be a potential new and better therapeutics for WD.[7]

### Long Term Metabolic Correction

Long term metabolic correction is one adaptation of gene therapy that ensures expression of transgene ATP7B over a long period of time. Adeno associated viral vectors (AAV) is a clinically tested vector that allows for a sustained therapeutic transgene expression in hemophilia B patients even 5 years after the administration of vector, with excellent tolerance. This treatment has been tested *in vivo* by administrating adeno associated vector serotype 8 (AAV8) with attached ATP7B cDNA placed under the control of liver-specific 1-antitripsin promoter to ensure efficient hepatic transduction, since an inefficient hepatic transduction was thought to be the problem with some tested long-term expression vectors, like lentivirus.[12]

The results yielded from the *in vivo* experiment indicate that AAV8 vectors are able to promote a sustained and efficient liver transduction, especially in mice with WD. The transduction of AAV8 vector does not alter the endogenous ATP7B gene. Moreover, a dose dependent serum holoceruloplasmin restoration, including its oxidase activity, was observed; even in WD mice with liver damage. The reduction of urinary Cu content and the increase in fecal elimination of Cu, this indicates that the normal metabolism of copper is restored in WD mice. Liver histology results show a dose dependent metal accumulation clearance in the liver with higher doses associated with hepatic metal content similar to those of healthy liver. The histology results also show the absence of almost all




histological changes common in WD mice, indicating complete or partial normalization of liver cells. These positive results indicate that AAV8 hepatic transduction can be a long term solution to WD patients, with a dose-dependent normalization of hepatic histology and function and no observed side effects.[12]

## CONCLUSION

Low copper diet, zinc salts treatment, chelating agents (penicillamine, trientine, ammonium tetrathiomolybdate), and liver transplant are currently available. Severe neurological deterioration and other side effects need new, more efficient, and safer therapeutics. Several new therapeutic agents including 4-phenylbutyrate, curcumin, chelating polymeric beads, and long term metabolic correction has been tested in vitro and in vivo. These new therapeutics may be a potential new treatment with less side effect and greater efficacy for Wilson's disease patients.

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