A comparative study of biliary trace elements and clinical phenotypes in Wilson’s disease

Ming-Shan Ren, Yu-Xin Fan, Ren-Min Yang, Yong-Zhu Han, Guo-Jun Wu, Yu-Rong Xin, Long Yu

Ming-Shan Ren, Ren-Min Yang, Yong-Zhu Han, Institute of Neurology, Teaching Hospital, Anhui College of T.C.M., Hefei 230031, Anhui Province, China

Yu-Xin Fan, Guo-Jun Wu, Yu-Rong Xin, Long Yu, National Laboratory of Genetic Engineering, Institute of Genetics, Fudan University, Shanghai 200433, China

Ming-Shan Ren, Associate Professor of Internal Medicine, MS in Neurology; Research Fellow of University of Rouen in Rouen, France, 1994-1995; having 18 papers and 1 book published.

Author contributions: All authors contributed equally to the work.

Supported by The Natural Science Foundation of Anhui Province, No. 97412001.

Correspondence to: Ming-Shan Ren, Associate Professor, Institute of Neurology, Teaching Hospital, Anhui College of T.C.M., Hefei 230031, Anhui Province, China

Telephone: +86-551-2816764-2107

Received: April 11, 1997

Revised: April 25, 1997

Accepted: June 28, 1997

Published online: December 15, 1997

Abstract

AIM: To further explore the etiological mechanism of Wilson’s disease (WD) by comparing the changes of biliary trace elements and its clinical phenotype.

METHODS: WD patients with different types and conditions (n = 20), non-WD patients with chronic liver damage (n = 22), and healthy volunteers (n = 10; used as controls) were studied. Biliary samples were taken by duodenal drainage. Atom absorption spectrophotometer was used to assay the copper and zinc content of each sample.

RESULTS: In WD, the copper content and copper/zinc ratio of biliary juice were evidently lower than those of non-WD patients with chronic liver damage and of healthy controls (F = 14.76, 25.4; 14.92, 26.2 respectively; P < 0.01), while the biliary zinc level had no significant difference from the two non-WD control groups (P > 0.05).

There were significant differences in biliary copper excretion among patients with different types and conditions (F = 3.75, P < 0.05; F = 6.20, P < 0.01).

CONCLUSION: Copper excretion by liver and the biliary system decreases obviously in WD, which plays a key role in the phenotypic copper retention, and the biliary copper retention is closely related with the severity of hepatic injury and illness.

Key words: Wilson’s disease; Copper; Zinc; Duodenal drainage; Bile

INTRODUCTION

Wilson’s disease (WD) is an autosomal recessive disorder of copper transport, first described by Kinneir Wilson in 1992 as hepatotentricular degeneration. The disorder has a worldwide frequency of between 1/35000 and 1/100000 and a corresponding carrier frequency of 1/90. Its symptoms develop from the toxic build up of copper primarily in the liver, and subsequently in the brain, kidney, cornea and other tissues. The resulting liver cirrhosis and/or neurological damage are fatal if not treated with copper chelating agents[1,2].

The gene responsible for WD was independently identified by three research teams in 1993[3-5], and is located on chromosome 13q14.3. The gene codes for a putative intracellular copper transport protein (ATP7B, a member of the cation-transporting P-type ATPAses family), spans > 80 kb of genomic DNA and consists of 22 exons[6]. Because of a defective ATP7B, the patients with WD have two fundamental disturbances of copper metabolism: A reduction in the rate of incorporation of copper into ceruloplasmin and a reduction in biliary excretion of copper. Although the role of copper in its pathogenesis has been known for several decades, few studies have been carried out to investigate the exact etiological mechanism of copper retention. Our study was designed to further explore the etiological mechanism of WD by observing the biliary trace element content of WD patients and non-WD patients in combination with its clinical phenotype.

MATERIALS AND METHODS

Subjects

Twenty in-patients with WD were chosen for this study, consisting of 13 men and 7 women with a mean age of 25-year-old. Their diagnoses all met the criteria proposed by Houwen et al[7]. Control subjects were chosen from among patients in the Department of Internal Medicine and healthy volunteers, all of who were carefully screened by clinical and copper metabolism examination for the
absence of clinical evidence of WD and then divided into two groups. The first control group consisted of 14 males and 8 females with mean age of 34-year-old, including 12 cases of chronic hepatitis, 6 of cirrhosis and 4 of primary biliary cirrhosis; the second control group of 10 healthy volunteers consisted of 5 men and 5 women with a mean age of 30-year-old.

**Laboratory studies**

All subjects maintained their regular diet throughout the study and underwent duodenal drainage to obtain a biliary sample after admission to the hospital. Eight of 20 patients with WD had no medicinal histories were asked to stop taking the above-mentioned medicines for 4 wk prior to the sampling. None of the control subjects had taken any agents that may affect the metabolism of internal trace elements during the 4 wk before sampling. The biliary samples were all collected at 09:00 am. The Hitachi-208 atom absorption spectrophotometer was used to assay the copper and zinc content of each sample. All values are presented as $\bar{x} \pm s$.

**Typing and grading**

As WD patients have genetic heterogeneity, they were classified according to the following clinical symptoms: Neurological type, 11 cases with predominant neurological symptoms; psychiatric type, 5 cases with mental symptoms; and hepatic type, 4 cases with liver symptoms\[10\]. The severity of disease was graded using the modified Goldstein method: Grade I, 7 cases with mild extrapyramidal system symptoms, no liver symptoms or obstacles to daily life; Grade II, 7 cases with obvious extrapyramidal system symptoms, no liver symptoms or obstacles to daily life; Grade III, 7 cases with obvious extrapyramidal system symptoms, no liver symptoms or obstacles to daily life; and Grade IV, 2 cases with serious extrapyramidal system symptoms, bedridden, obvious hepatoesplenomegaly or complications with ascites and liver function injury. Typing and grading were accomplished independently by two experienced neurologists in our department, neither of whom were aware of the results of biliary trace elements.

**RESULTS**

The biliary copper content and copper/zinc ratio of WD patients were notably lower than those of the first and second control groups and the differences were significant ($F = 14.76, 25.4; 14.92, 26.2; P < 0.01$). The comparison of biliary zinc content among the three groups showed no statistical significance ($F = 1.76, 1.98, P > 0.05$), indicating that the internal copper deposit of WD is directly related with the decrease of copper excretion by the liver and biliary system (Table 1).

The biliary trace elements of WD patients with different types are shown in Table 2. The biliary copper content in cases of neurological or psychiatric types was significantly higher than that in the hepatic type ($F = 3.75, P < 0.05$). In contrast, the biliary zinc content and copper/zinc ratio were similar among the three types ($F = 0.246, 0.855, P > 0.05$), showing that biliary copper excretion is consistent with the severity of hepatic injury of WD (Table 2).

The relationship between biliary trace elements and the severity of WD is shown in Table 3. The severity of the disease was classified into three groups (Grade I, Grade II and Grade III-IV). The biliary copper content of Grade II-IV WD patients was significantly lower than that of Grade I-II patients ($F = 6.20, P < 0.01$), but the biliary zinc content and copper/zinc ratio showed no significant differences ($F = 1.171, 1.081, P > 0.05$) among the 3 groups, illustrating that the severity of WD had a direct influence on biliary copper excretion but had no effect on biliary zinc excretion (Table 3).

**DISCUSSION**

Wilson's disease has various clinical manifestations caused by large amounts of deposition of internal copper resulting from abnormal copper metabolism. Patients with untreated WD are in positive copper balance. At present, there are many hypotheses to explain the etiological mechanism of copper retention, and the biliary copper excretion disturbance is considered as one of the main intrinsic mechanisms leading to the copper retention of WD\[11\]; however, the evidence for this remains deficient and there are few published studies of biliary copper excretion for WD. Many assumptions have failed to further explain its relations with the observed clinical conditions of the WD patients. An actual demonstration of its involvement in abnormal copper metabolism of WD remains to be further explored.

The biliary copper excretion speed for normal individuals averages 500-1300 μg/d, basically counteracting the copper absorption in gastrointestinal tract, while the excretion rate in urine and sweat is very small. Copper balance is maintained mainly through biliary excretion. In view of this, considerable emphasis has been laid on the fact that biliary copper excretion abnormality may underlie the disorder. Indeed, many investigators have been trying to elucidate the exact point of breakdown in the copper metabolic chain of WD. Gibbs and Walsh\[12\] used 64-Cu to observe directly the excretion process of the biliary tracts of two WD patients with biliary tract fistula and non-WD patients, and found that the biliary copper content of the common bile duct and the copper excretion of the biliary tract for the former were much lower than those for the latter. Courtoy et al\[13\] found that intravenously administered polymeric IgA in rats was bound to a receptor in the liver parenchymal cells and then transported via the endosomes to the bile canaliculus where it was excreted. Lyengar et al\[14\] further provided evidence that a high molecular weight copper-binding substance existing in the hepatic cells of normal subjects was absent in patients with WD by studying the cholecytokinin-stimulated biliary secretions. Afterwards, Hoof et al\[15\] pointed out that there was a similar mechanism for the transport of a copper protein to the human bile and that the genetic defect of this metabolic pathway in WD will lead to insufficient copper excretion.

Our results demonstrate that the biliary copper content and copper/zinc ratio of the patients with WD are significantly lower than those of non-WD patients with chronic liver damage and of healthy volunteers ($P < 0.01$, respectively). Additionally, we observed that the severity of liver lesions and patient conditions correlate well
with the decrease of biliary copper excretion, while the biliary zinc excretion has nothing to do with WD \( (P > 0.05) \). All these findings strongly suggest that liver and biliary pathways play important roles in the copper excretion dysfunction of WD and could, therefore, participate directly in the pathophysiology of copper retention. We think that due to a non-functional gene, the patients with WD lack a copper-transporting P-type ATPase (ATP7B) that would otherwise control endosome-mediated copper excretion to the bile canaliculus in the hepatic cells. Consequently, the copper will be routed to the lysosomes, where it will accumulate and fail to be discharged into the bile, leading to obvious decrease of biliary copper excretion and internal copper accumulation. The copper trapped in the hepatic cells further damages the endosomes, resulting in the vicious circle of disruption in copper transport and obvious hepatic damage\[14\].

This vicious circle may be the reason why healthy individuals and non-WD patients with chronic liver damage and other forms of biliary retention, such as chronic hepatitis or primary biliary cirrhosis, do not have the same outcome, and why the severity of hepatic damage in WD correlates well with the decrease of biliary copper excretion. Therefore, our findings, on one hand, have provided the experimental basis for our final understanding of WD pathogenesis, and, on the other hand, have indicated that if the biliary copper excretion can be promoted therapeutically, satisfactory results will be yielded both in maintaining the negative balance of copper metabolism and alleviating pathological damage to organs.

REFERENCES

1 Thomas GR, Bull PC, Roberts EA, Walshe JM, Cox DW. Haplotype studies in Wilson disease. *Am J Hum Genet* 1994; 54: 71-78 [PMID: 8279472]

2 Chelly J, Monaco AP. Cloning the Wilson disease gene. *Nat Genet* 1993; 5: 317-318 [PMID: 8298634 DOI: 10.1038/ng1295-317]

3 Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nat Genet* 1993; 5: 327-337 [PMID: 8298639]

4 Yamaguchi V, Henry ME, Gallin JD. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson disease. *Biochem Biophys Res Commun* 1993; 197: 271-277 [PMID: 8250934 DOI: 10.1006/bbrc.1993.2471]

5 Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasse W, Ross B, Romano DM, Parano E, Pavone L, Bressenotwicz LM. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nat Genet* 1993; 5: 344-350 [PMID: 8298641 DOI: 10.1038/ng1293-344]

6 Thomas GR, Roberts EA, Walshe JM, Cox DW. Haplotypes and mutations in Wilson disease. *Am J Hum Genet* 1995; 56: 1315-1319 [PMID: 7762553]

7 Houwen RH, Roberts EA, Thomas GR, Cox DW. DNA markers for the diagnosis of Wilson disease. *J Hepatol* 1993; 17: 269-276 [PMID: 8100247]

8 Walshe JM. Wilsons disease. In: Vinken PJ, Bruyn GW, Klawans HL, eds. Handbook of clinical neurology. Amsterdam: Elsevier 1986; 223-228

9 Yang YF. Normal copper metabolism and the etiological mechanism of Wilson disease. Zhongguo Shenjing Jingshen Jibing Zazhi 1984; 19: 60-62

10 Gibbs K, Walshe JM. Biliary excretion of copper in Wilson's disease. *Lancet* 1980; 2: 538-539 [PMID: 6105588 DOI: 10.1016/S0140-6736 (80)91863-2]

11 Courtoy PJ, Linet J, Quintant J, Schneider YJ, Vaerman JP, Baudhuin P. Transport of IgA into rat bile: ultrastructural demonstration. *Ann n Y Acad Sci* 1983; 409: 799-802 [DOI: 10.1111/j.1749-6632.1983.tb26927.x]

12 Iyengar V, Brewer GJ, Dick RD, Chung OY. Studies of cholecystokinin-stimulated biliary secretions reveal a high molecular weight copper-binding substance in normal subjects that is absent in patients with Wilson's disease. *J Lab Clin Med* 1988; 111: 267-274 [PMID: 3125292]

13 Van Hoof F, Den Tandt W, Scharpe S. Wilson's disease: hypothesis of a deficiency of copper excretion via the endosome to the bile. *Arch Neurol* 1992; 49: 800 [PMID: 1524511 DOI: 10.1001/archneur.1992.00530320020007]

14 Thomas GR, Forbes JR, Roberts EA, Walshe JM, Cox DW. The Wilson disease gene: spectrum of mutations and their consequences. *Nat Genet* 1995; 9: 210-217 [PMID: 7626145 DOI: 10.1038/ng0295-210]
