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ORIGINAL RESEARCH

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

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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

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INTRODUCTION

Wilson's disease (WD) is an autosomal recessive disorder of copper transport, first described by Kinnear Wilson in 1992 as hepatolenticular 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 ATPase 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

Table 1 Values of biliary trace elements of the Wilson's disease group and control groups ($\bar{x} \pm s$, μmol/L)

Group	n	Copper	Zinc	Copper/Zinc
Wilson's disease	20	4.42 ± 0.44	55.94 ± 3.14	0.13 ± 0.02
1st control	22	41.70 ± 1.97[a]	34.46 ± 1.85	1.54 ± 0.27[a]
2nd control	10	42.01 ± 2.63[a]	34.30 ± 2.84	1.56 ± 0.24[a]

[a]P < 0.01 *vs* Wilson's disease group.

Table 2 Comparison of biliary trace elements for different types of Wilson's disease ($\bar{x} \pm s$, μmol/L)

Type	n	Copper	Zinc	Copper/Zinc
Neurological	11	5.02 ± 0.61[b]	62.58 ± 22.84	0.13 ± 0.06
Psychiatric	5	4.78 ± 0.80[b]	57.41 ± 26.36	0.17 ± 0.14
Hepatic	4	2.31 ± 0.17	35.85 ± 11.51	0.10 ± 0.07

[b]P < 0.05 *vs* hepatic type.

Table 3 Variations of biliary trace elements in different severity of Wilson's disease ($\bar{x} \pm s$, μmol/L)

Grade	n	Copper	Zinc	Copper/Zinc
Ⅰ	7	5.67 ± 0.86[c]	82.31 ± 33.71	0.10 ± 0.05
Ⅱ	7	4.72 ± 0.55[c]	51.99 ± 19.57	0.17 ± 0.12
Ⅲ-Ⅳ	6	2.61 ± 0.22	29.76 ± 8.26	0.12 ± 0.07

[c]P < 0.01 *vs* Ⅲ-Ⅳ grades.

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 treatment before hospitalization, and the remaining 12 had histories of treatment with penicillamine, zinc sulfate, dimercaprol (BAL) and sodium dimercaptosuccinate (DMS); all of the patients with treatment 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 $\bar{x} \pm s$. The F-test for variation analysis was employed to determine the statistical significance of differences between the means. P values of < 0.05 were considered significant.

Typing and grading

As WD patients have genetic heterogeneity, they were classified according to the following clinical symptoms: Neurological type, 11 cases with predominantly neurological symptoms; psychiatric type, 5 cases with mental symptoms; and hepatic type, 4 cases with liver symptoms[8]. 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 Ⅲ, 7 cases with obvious extrapyramidal system symptoms, no liver symptom or mild hepatosplenomegaly with normal liver function, and no hypersplenia; Grade Ⅲ, 4 cases with serious extrapyramidal system symptoms, obvious hepatosplenomegaly and/or liver function injury; and Grade Ⅳ, 2 cases with serious extrapyramidal system symptoms, bedridden, obvious hepatosplenomegaly or complicated with ascites and liver function injury. Typing and grading were accomplished independently by two experienced neurologists in our department, neither of who 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 Ⅲ-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[9]; 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 copper excretion. In view of this, considerable emphases have 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 Walshe[10] 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*[11] 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[12] 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 cholecystokinin-stimulated biliary secretions. Afterwards, Hoof *et al*[13] 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.

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