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Impaired homocysteine metabolism in patients with alcoholic liver disease in Taiwan

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ABSTRACT

Impaired homocysteine metabolism plays an important role in alcoholic liver disease (ALD); however, there are limited data about its relationship with the risk and severity of patients with ALD in Taiwan. To understand plasma homocysteine and related vitamin concentrations in patients with ALD in Taiwan, we recruited 50 male patients with ALD from Cathay General Hospital, with 49 age-and gender-matched healthy adults as the control group. The Institutional Review Board for Human Studies approved the study, and informed consent was obtained from all patients prior to blood collection. Significantly higher plasma homocysteine concentrations but lower folate concentrations were obtained from patients with ALD. In addition, patients with ALD showed a significant lower erythrocyte reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio but higher plasma thiobarbituric acid-reactive substance (TBARS) concentration, which indicated that oxidative stress was occurring in patients with ALD. A negative correlation between plasma folate and homocysteine was observed in all subjects. There was also a negative correlation between plasma homocysteine and the erythrocyte GSH/GSSG ratio which indicated impaired homocysteine metabolism may have disrupted the antioxidative status. In addition, patients in Child-Pugh Class B and C showed higher plasma vitamin B_{12} concentrations than did patients without cirrhosis and patients in Child-Pugh Class A. These findings show that impaired homocysteine metabolism was observed in patients with ALD in Taiwan. In addition, the plasma vitamin B_{12} concentration may reflect the degree of liver injury.

Introduction

Multiple studies have shown that chronic alcohol administration leads to complex pathophysiological mechanisms that contribute to the development and progression of alcoholic liver disease (ALD), and among the mechanisms, abnormal homocysteine metabolism plays a key role. Homocysteine can be metabolized by methionine synthase (MS), cystathionine β-synthase (CBS), and betaine-homocysteine methyltransferase (BHMT) (Lee et al., 2004; Lu, Huang, Yang, & Tsukamoto, 1999).

Higher plasma homocysteine concentrations contributed to fat accumulation, inflammation, and severe injury to hepatocytes under chronic ethanol consumption in an animal study (Song, Zhou, Deaciuc, Chen, & McClain, 2008). In addition, our previous study further suggested that combined treatment with folate and vitamin B_{12}, cofactors of MS, could alleviate alcoholic liver injury by normalizing the plasma homocysteine level (Chen et al., 2011). Taken together, homocysteine itself may play an important role in the development of ALD, and normalization of plasma homocysteine levels may help decrease liver injury under chronic ethanol exposure.

In 1993, hyperhomocysteinemia in chronic alcoholics was first reported by Hultberg and colleagues (Hultberg, Berglund, Andersson, & Frank, 1993). Abnormal hepatic homocysteine and glutathione metabolism in patients with alcoholic hepatitis were also summarized by Lee and colleagues (Lee et al., 2004). A cell study indicated that hepatocytes isolated from rats fed Lieber-DeCarli ethanol diet for 4 weeks and then incubated *in vitro* released twice as much homocysteine into the medium as control rats (Kharbanda, 2009). In an animal study, Ji and Kaplowitz (2003) stated that betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in mice under intragastric ethanol infusion. Halsted and colleagues reported that folate deficiency disrupts hepatic homocysteine metabolism and promotes liver

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injury in micropig under long-term ethanol feeding (Halsted et al., 2002). Although more studies showed the relation between hyperhomocysteinemia and ALD, there are only limited clinical data in Asians, especially in Taiwanese. The Nutrition and Health Survey in Taiwan (NAHSIT 2005–2008) showed lower serum folate concentration in adults than that found in NAHSIT 1 (1993–1996). We believed the lower folate and vitamin B_{12} concentrations might disrupt homocysteine metabolism, especially in patients with ALD. The goals of our study were to establish homocysteine-related clinical data of patients with ALD in Taiwan and compare changes in homocysteine and its related vitamin concentrations among different degrees of liver injury.

Methods

Subjects

Fifty male patients with ALD were enrolled from Cathay General Hospital, and 49 age- and gender-matched healthy adults served as a control group. Venous blood (10 mL) of all patients and healthy controls was drawn into an EDTA-containing tube after an 8-h overnight fast. Afterward, plasma samples were obtained after centrifugation at 1200 × *g* for 15 min at 4 °C. Erythrocytes were obtained by washing these samples twice with ice-cold saline (0.9% NaCl). The Institutional Review Board for Human Studies approved this study. Patient consent was obtained prior to blood collection.

Biochemical analysis

Laboratory evaluations

An autoanalyzer (SYNCHRON CX System, Hitachi 7170, Tokyo, Japan) was used to analyze plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, and total bilirubin (bilirubin T), triglyceride (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), uric acid, and albumin concentrations in the patient and control groups.

Plasma folate, vitamin B_{12}, and homocysteine concentrations

Beckman Coulter ACCESS® (Brea, CA, USA) automated chemiluminescence was used to measure plasma folate and vitamin B_{12} concentrations. The plasma homocysteine concentration was obtained with an ABBOTT Imx analyzer (Abbott Park, IL, USA). The AxSYM® homocysteine assay is based on fluorescence polarization immunoassay (FPIA) technology (Shipchandler & Moore, 1995).

Erythrocyte reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio

The GSH concentration was measured spectrophotometrically at 405 nm according to the method of Tietze (1969), and the GSSG concentration was measured spectrophotometrically at 405 nm according to the method of Griffith (1980). Finally, the ratio of GSH/GSSG was calculated as (GSH − 2[GSSG])/(GSSG).

Plasma thiobarbituric acid-reactive substance (TBARS) concentration

Lipid peroxidation was quantitatively measured by measuring the TBARS concentration in plasma using the method of Ohkawa and colleagues (Ohkawa, Ohishi, & Yagi, 1979).

Child-Pugh classification

To investigate vitamin concentrations and the antioxidative status between different degrees of liver injury, patients were classified according to the Child-Pugh score. The Child-Pugh classification estimates the severity of cirrhosis based on biochemical and clinical indices including plasma albumin and bilirubin concentrations, prothrombin time, and the degree of ascites and hepatic encephalopathy.

Table 1
Characteristics and clinical data of the control group (Con) and patients with alcoholic liver disease (ALD).[a]

	Con	ALD
Number	49	50
Male	49	50
Female	0	0
Age (years)	36.3 ± 1.1	43.5 ± 3.3
Duration of drinking (years)	0	21.0 (2–40)
Height (cm)	171.0 ± 0.7	168 ± 1.0
Weight (kg)	69.4 ± 1.5	71 ± 3.4
Body-mass index	23.5 ± 0.4	23.5 ± 0.6
Aspartate aminotransferase (U/L)	22.6 ± 0.7	70.6 ± 7.7*
Alanine aminotransferase (U/L)	25.6 ± 1.5	60.0 ± 10.0*
Total bilirubin (mg/dL)	0.7 ± 0.0	2.0 ± 0.2*
Triglycerides (mg/dL)	93.9 ± 5.8	151.9 ± 19.0
Total cholesterol (mg/dL)	175.7 ± 2.7	165.6 ± 8.3
High-density lipoprotein-cholesterol (mg/dL)	47.7 ± 1.4	35.9 ± 2.2*
Low-density lipoprotein-cholesterol (mg/dL)	114.0 ± 3.0	86.7 ± 5.8*
Uric acid (mg/dL)	6.2 ± 0.2	6.1 ± 0.3
Albumin (g/dL)	4.7 ± 0.0	3.6 ± 0.1*

Data from 50 patients with ALD were compared with 49 normal controls (Con).
*$p < 0.05$ compared to the control group (by Student's *t* test).
[a] Values are expressed as the mean and SEM.

Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM). Student's *t* test was used to compare differences of means between the control and ALD groups using EXCEL software (Redmond, WA, USA). Statistical significance was assigned at the $p < 0.05$ level. To evaluate data among different stages of liver disease in this study, a one-way analysis of variance (ANOVA) with Fisher's *post hoc* test was used. SAS software (version 8.2, SAS Institute, Cary, NC, USA) was used to analyze all data. Differences were considered statistically significant at $p < 0.05$. Pearson's correlation coefficients were used to evaluate the degree of association among the plasma folate concentration, plasma homocysteine concentration, and erythrocyte GSH/GSSG ratio.

Results

The main characteristics, history of alcohol abuse, liver function test, and nutritional status of the control group and patients with ALD are summarized in Table 1. There were no significant differences in age, body-mass index (BMI), or plasma uric acid concentration between the two groups. As expected, the plasma AST activity, ALT activity, and bilirubin T concentration were significantly higher in patients with ALD than those in the control group ($p < 0.05$). The plasma TG concentration in patients with ALD was significantly higher than that in the control group ($p < 0.05$). In contrast, plasma HDL-C and LDL-C concentrations in patients with ALD were significantly lower than those in the control group ($p < 0.05$). In addition, the plasma albumin concentration in patients with ALD was significantly lower than that in the control group ($p < 0.05$).

As shown in Table 2, plasma homocysteine and vitamin B_{12} concentrations were significantly higher in patients with ALD than those in the control group ($p < 0.05$). In contrast, the plasma folate concentration was significantly lower than that in the control group ($p < 0.05$). To investigate the antioxidative status in patients with ALD, the erythrocyte GSH/GSSG ratio and plasma TBARS concentration are summarized in Table 3. As shown in Table 3, the erythrocyte GSH/GSSG ratio was significantly lower in patients with ALD