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Dietary amino acid taurine ameliorates liver injury in chronic hepatitis patients

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Summary. The effect of dietary amino acid taurine on the liver function of chronic hepatitis patients was investigated. The 24 chronic hepatitis patients with 2–5 times over normal activities of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) were selected and equally divided into taurine treatment and control groups. In taurine treatment group, each patient took 2 g taurine 3 times a day for three months, and then stopped treatment for 1 month. Patients taking placebo without taurine for 4 months served as a control group. ALT and AST activities and levels of cholesterol, triglyceride and thiobarbituric acid relative substances of serum plasma in the taurine group were all decreased at the end of three month treatment. The study suggested that dietary amino acid taurine may ameliorate liver injury for chronic hepatitis patients.

Keywords: Alanine aminotransferase – Antioxidation – Hapatitis patient – Liver function – Taurine

Introduction

More than 350 million people worldwide are infected with hepatitis B (De Clercq, 1999). The hepatitis caused by virus A, B, C, D, and E is also a serious disease for people in Taiwan. The ratio of adults infected with hepatitis B and C is 15–20% and 1–2%, respectively. Hepatitis B and C may easily induce hepatocellular carcinoma and death in later stages. However, diseases can be prevented by vaccination, drug control, and early diagnosis and treatment (Chen et al., 1996; Chang et al., 1997; Lee, 1997). The symptoms of hepatitis B and C patient include fatigue, jaundice, nausea, fever, vomiting, liver dysfunction, hepatocyte necrosis, hepatocyte apoptosis and coma. In the stage of liver dysfunction and hepatocyte necrosis, the associated plasma membrane leakage can be detected by analysis of plasma for lipid oxidation and liver cytosol-derived enzymes including alanine aminotransferase (ALT) and asparate aminotransferase (AST) (Moslen, 1996; Tsai et al., 1997). Chronic hepatitis patients usually have higher number of ALT and AST, and higher level of oxidative indicator such as thiobartituric acid relative substances (TBARS) as compared to healthy person.

ALT is found predominantly in the liver with lesser amount in the kidneys, heart and skeletal muscle. Liver dysfunction caused by injury or disease will increase the release of hepatocellular enzyme from liver parenchyma into the blood stream, elevating serum ALT levels. This enzyme is sensitive and specific to the hepatocellular disease. For the hepatocellular disease other than viral hepatitis, the ALT/AST ratio (DeRitis ratio) is less than 1; however, for the viral hepatitis the ratio is greater than 1. The ratio is a helpful index used in diagnosis of the viral hepatitis (Pagana and Pagana, 1998).

Amino acid taurine is a sulfur-containing amino acid that conjugates with bile acids in the human liver (Jacobsen and Smith, 1968). It is an essential amino acid to the cat (Hayes and Carey, 1975; Knopf et al., 1978). The physiological functions of taurine include bile acid conjugation, detoxification, osmoregulation, antioxidation, preventing lipid peroxidation, cell membrance stabilization, neuromodulation and calcium flux regulator (Thurston et al., 1980, 1981; Takihara et al., 1985; Hamaguchi et al., 1988; Huxtable, 1992; Balkan et al., 2002; Nandhini et al., 2002). Dietary source of taurine is rich and may come from fish products (100–1000 mg/100 g), especially in mollusks and fish liver (Konosu and

Yamaguchi, 1982; Sakaguchi and Urata, 1989). Previous studies (Hwang et al., 1998, 2000; Wang et al., 1998; Hwang and Wang, 2001) indicated that dietary taurine could protect liver of rat from injuries induced by oxidized oils and heavy metals. However, little information on the effect of dietary amino acid taurine to improve the chronic hepatitis patients is available. The objective of this study was to determine the benefit of dietary taurine on liver function of the chronic hepatitis patients.

Materials and methods

Materials

Taurine was purchased from Dokui Chemical Company (Taipei, Taiwan), its purity was 99.5%. The grade was edible.

Experiment design

Twenty-four hepatitis C patients with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) 2–5 times higher than normal level were selected at a local hospital in Taoyuan, Taiwan. The protocol for human subject was approved by the ethical committee of Taoyuan Veteran Hospital. In taurine-feeding group, patients consisted of 7 male and 5 female with an average age of 58 years old (46–75) and an average body weight of 63 kg (48–75), respectively. In the control group, patients included each 6 male and female with an average of 58 years (41–78) and an average body weight of 59 kg (45–73), respectively. All patients had signed concert form for treatment for 4 months (reference on human subject). In taurine testing group, 2 g taurine three times per day was administrated after meal for 3 months and then stopped treatment for 1 month. In control group, patients took placebo without taurine.

Each month blood samples of all patients were collected for analysis of blood profiles including red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb) and platelet, by using a Cell Hematology Analyzer (DYN 500, Sequoi-Turner, USA). The plasma samples of blood were collected by centrifugation ($2000 \times g$, 15 min) and examined for the levels of thiobarbituric acid relative substances (TBARS), cholesterol and triglyceride (TG) and the activities of AST and ALT.

Lipid peroxidation activities in plasma were assayed by measurements of malondialdehyde (MDA), an end-product of peroxidized fatty acids, and thiobarbituric acid (TBA) reaction product. The sample of 20 µl plasma was mixed with 1.0 ml 0.4% TBA in 0.2 N HCl and 0.15 ml 0.2% dibutylated hydroxytoluene (BHT) in 95% ethanol. The samples were then incubated in a 90 °C water bath for 45 min. After incubation, the TBAMDA adduct was extracted with isobutanol. The isobutanol extract was mixed with methanol (2:1) and the supernatant was examined by using the HPLC system at an excitation 515 nm and an emission 550 nm on a Hitachi Fluorescence Detector (Mido, Japan) (Tatum et al., 1990).

The levels of cholesterol and TG and the activities of AST and ALT in the plasma were assayed with enzymatic kits by using (a) the enzymatic kit for triglyceride (Vitalab Selectra, E. Merck, Dieren, Netherlands). Triglyceride was activated using lipase to form glycerol and fatty acids, glycerol and ATP activated by glycerol kinase to form L-α-glycerol-3-phosphate, and with O_2 to form H_2O_2. H_2O_2, 4-amino antipyrine and phenol were activated by peroxidase to form quinoneimine and determined by using 500 nm absorbance. (b) The enzymatic kit for cholesterol: cholesterol ester was activated by cholesterol esterase to form cholesterol. Cholesterol was activated by cholesterol oxidase to form H_2O_2, and H_2O_2, 4-amino antipyrine and phenol were activated by peroxidase to form quinoneimine which was determined by using 500 nm absorbance. (c) The enzymatic kit for AST activity: L-asparate and 2-oxoglutarate were firstly activated by AST to form oxaloacetate and glutamate; and oxaloacetate and NADH were then activated by malate dehydrogenase (MDH) to form malate and NAD^+. The level of NADH was determined by using 340 nm absorbance. (d) The enzymatic kit for ALT activity: L-alanine and α-ketoglutarate were activated by ALT to form glutamate and pyruvate. Pyruvate and NADH were then activated by lactate dehydrogenase (LDH) to form lactate and NAD^+. The level of NADH was determined as above described.

Statistical analysis

The ratios of ALT/AST in patients have been checked to be greater than 1 and diagnosed as a viral hepatitis. Two groups of experimental patients were well separated to prevent the sex and age differences. All data obtained from experiment were analyzed using one-way ANOVA (Puri and Mullen, 1980). Duncan's new multiple range test was used for significant difference among treatments at $P < 0.05$.

Results

After a three-month treatment with dietary taurine, all indicators of patient blood were not affected ($P > 0.05$). The data were similar to those in control group. The results of blood profiles were fitted in the range of healthy persons: 5.48–5.90 (normal range are 4.8–10.8) $\times$ 10^3 cells/µl for WBC, 4.54–4.75 (4.2–6.1) $\times$ 10^6 cells/µl l for RBC, 14.1–14.8 (14–18) g/dl for Hb, and 167–178 (130–400) $\times$ 10^3 cells/µl for platelet (Pagana and Pagan, 1998). No significant difference on data of blood profile in chronic hepatitis patients regardless of taurine and control groups. Results indicate that the blood profiles

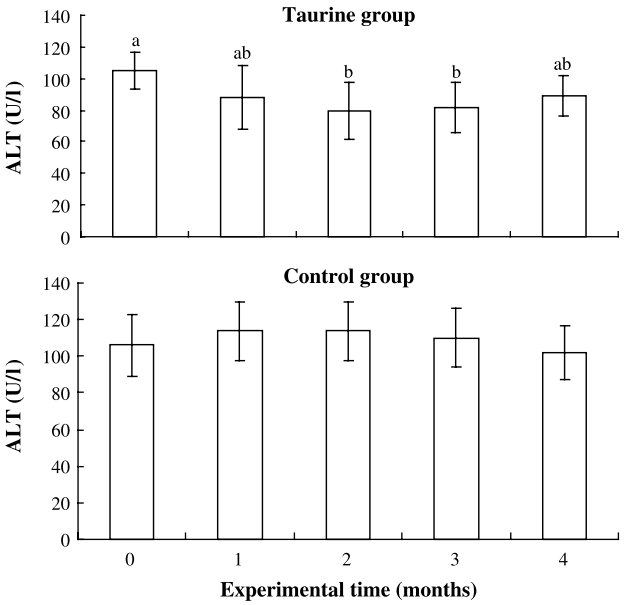

Fig. 1. Activity of alanine aminotransferase (*ALT*) of serum plasma in chronic hepatitis patients as affected by dietary taurine. The value (mean ± SD) in the same group with different superscripts (*a*, *b*) are significantly different ($P < 0.05$)

of hepatitis C patients are the same as the healthy person and dietary supplement with taurine would not make any improvement.

Liver enzyme ALT activity of chronic hepatitis patients treated with dietary taurine is shown in Fig. 1. After treating with dietary taurine for 2 months, ALT activity of plasma in the chronic hepatitis patients was significantly decreased ($P<0.05$). However, once stopping treatment at the end of third month, the amelioration of taurine to clinic symptoms of chronic hepatitis patients was reduced and ALT activity was elevated. No significant difference on the ALT activity of chronic hepatitis patients in control group was observed throughout the entire experiment period.

The effect of dietary taurine on AST activity of chronic hepatitis patients is shown in Fig. 2. At the end of three months treatment, AST activity of plasma in the chronic hepatitis patients was significantly decreased ($P<0.05$). Similar to results on ALT, once stopping treatment, AST activity was also elevated. No significant difference on the AST activity of chronic hepatitis patients in control group was observed.

Cholesterol level of chronic hepatitis patients treated with dietary taurine is shown in Fig. 3. After treating with dietary taurine for 3 months, cholesterol level of plasma in the chronic hepatitis patients was significantly decreased ($P<0.05$). However, stopping treatment at the fourth month, cholesterol level was elevated. No significant difference on cholesterol level in chronic hepatitis patients without taurine supplement was observed.

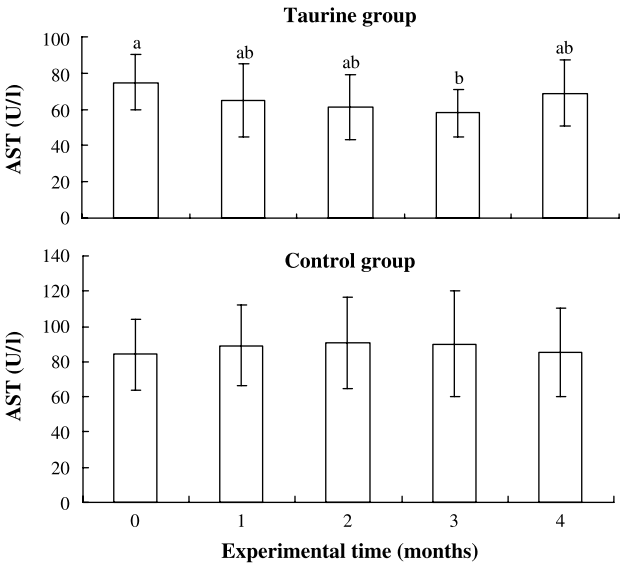

Fig. 2. Activity of aspartate aminotransferase (*AST*) of serum plasma in chronic hepatitis patients as affected by dietary taurine treatment. The value in the same group with different superscripts (*a*, *b*) are significantly different ($P<0.05$)

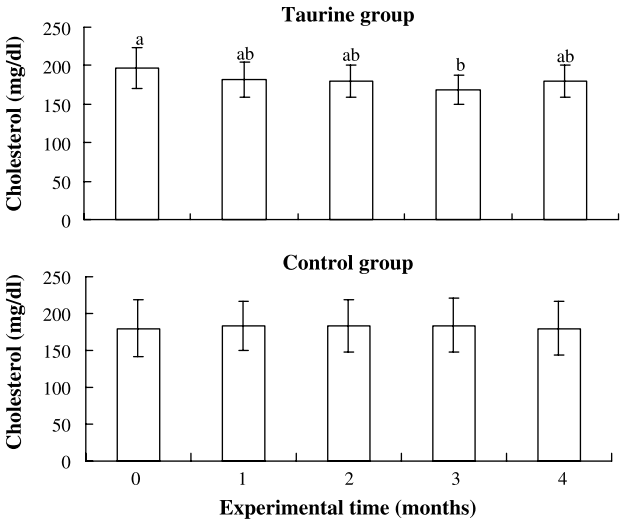

Fig. 3. Level of cholesterol of serum plasma in chronic hepatitis patients as affected by dietary taurine treatment. The value in the same group with different superscripts (*a*, *b*) are significantly different ($P<0.05$)

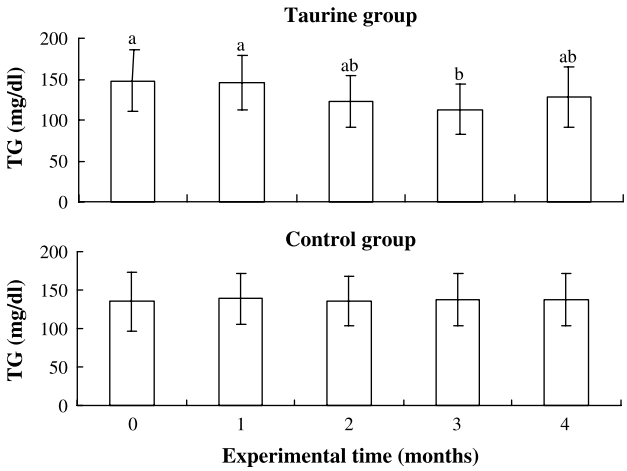

Fig. 4. Level of triglyceride (*TG*) of serum plasma in chronic hepatitis patients as affected by dietary taurine treatment. The value in the same group with different superscripts (*a*, *b*) are significantly different ($P<0.05$)

The effect of dietary taurine on TG level of chronic hepatitis patients is shown in Fig. 4. After treating with dietary taurine for 3 months, TG level of plasma in the chronic hepatitis patients was also significantly decreased ($P<0.05$). Once stopping treatment, TG level was elevated as that of cholesterol level. During the experimental period, no significant changes on TG level in chronic hepatitis patients with placebo was observed.

TBARS value of chronic hepatitis patients during dietary taurine treatment is shown in Fig. 5. At end of two month dietary taurine treatment, TBARS level of plasma in the chronic hepatitis patients was significantly decreased ($P<0.05$). Interestingly, ever stopping treatment,

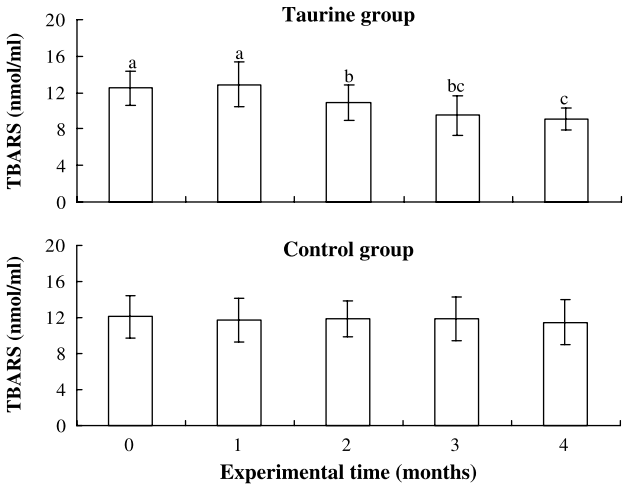

Fig. 5. Thiobarbituric acid relative substances (*TBARS*) value of serum plasma in chronic hepatitis patients as affected by dietary taurine treatment. The value in the same group with different superscripts (*a*, *b*, *c*) are significantly different ($P < 0.05$)

TBARS values were kept decreased. No significant difference on TBARS value in chronic hepatitis patients without taurine supplement was found. Results suggested that the peroxidation of blood plasma in hepatitis patients could be availably ameliorated by dietary taurine.

Discussion

Blood profiles, activities of ALT and AST and levels of TG, cholesterol and TBARS in the plasma of chronic hepatitis patients without supplement of taurine during experimental period of 4 months were not changed. However the chronic hepatitis patients were treated with dietary taurine, the clinical symptoms including AST and ALT activities, TG and cholesterol levels, and TBARS values were significantly affected. ALT and AST activities in plasma serve as biomarkers for liver functions (Ronald and Koretz, 1992). The ranges in healthy persons are as follows: 10–40 U/l for ALT and 5–45 U/l for AST (Pegama and Pegama, 1998). Both ALT and AST activities in chronic hepatitis patients were higher than those of healthy persons. ALT activity, however, was higher than AST activity in the chronic hepatitis patents, indicating that hepatitis virus might injure liver function. Dietary taurine significantly reduced the enzymatic activities of ALT and AST in the plasma of chronic hepatitis patients, suggesting that the liver injury by hepatitis virus could be ameliorated by taurine. According to report by Wright et al. (1986), the function of taurine for preserving liver cells was presented by the high concentration of taurine existing in cell membrane.

The value of TBARS an end product of lipid peroxidation in the plasma is an additional indicator of liver injury. This value in the plasma of hepatitis patients was significantly reduced when the patients were treated with the dietary taurine. The results was in agreement with the previous studies (Alvarez and Storey, 1983; Tadolini et al., 1995; Hwang et al., 1998, 2000; Wang et al., 1998). Therefore, it is reasonable to assume that taurine may act as a powerful scavenger in reducing lipid peroxidation induced by drugs (Alvarez and Storey, 1983; Waters et al., 2001), heavy metals (Hwang et al., 1998; Hwang and Wang, 2001) and oxidized oil (Hwang et al., 2000).

Although the levels of TG and cholesterol in the plasma of chronic hepatitis patients were not higher than those of healthy persons (120–200 mg/dl for cholesterol, 35–170 mg/dl for TG), these in the plasma of hepatitis patients were significantly reduced when the patients were treated with the supplement of taurine. These results are the same as those reported previously (Huang et al., 1988; Mizushima et al., 1996; Dawson et al., 1999). The reduction of TG and cholesterol levels in the plasma may induce the decrease of lipid peroxidation, resulting in inhibiting production of TBARS. Judging from the above data taurine plays an important role in the properties of antioxidation and has some improvements on the liver function of chronic hepatitis patients. Results suggested that the peroxidation of blood plasma in hepatitis patients could be availably ameliorated by dietary amino acid taurine.

This experiment was conducted by selecting 24 hepatitis patients, so sample number of each group was only 12. In addition, the ages of patients distributed too huge and the each group involved of men and women. These factors may induce the standard deviation of each indicator becoming large. In the primary study, results indicated that less 0.5 g of taurine per day did not show significant ameliorating effect on hepatitis patients. So high dose of taurine has healthy benefits on chronic hepatitis patients, but low dose has not. Therefore taurine can not play a role as therapeutic drug. On the other hand, the LD_{50} value of taurine tested by orally administration into ICR (Institute of Caner Research) strain male mice was more than 15 g/kg. It means that taurine is no toxic substance when people take it. Meanwhile, previous papers (Hwang et al., 1998, 2000) reported that the diet with 5% taurine had no toxicity on rats. Therefore, taurine is a safe dietary nutrient to benefit chronic hepatitis patients.

Acknowledgement

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