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ORIGINAL ARTICLE

Effect of taurine on alcoholic liver disease in rats

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Abstract To investigate the effect of taurine on alcoholic liver disease in rats, male Wistar rats were administered alcohol intragastrically for 3 months. The effect of β-alanine-mediated taurine depletion and taurine administration on the development of alcoholic liver disease was examined. It was found that taurine administration produced lower levels of aspartate aminotransferase and alkaline aminotransferase than that of the untreated group. In addition, the levels of hepatic total protein, glutathione and superoxide dismutase were higher in the taurine treated groups than those in the untreated control or the taurine depleted groups, while hepatic malondialdehyde content exhibited the negative effect. Moreover, the concentrations of hepatic hydroxyproline, serum hyaluronic acid, interleukin-2, interleukin-6, tumor necrosis factor-α and laminin were all decreased in the taurine treated groups. The pathological changes showed that the percentage of fatty degeneration and inflammation in the taurine groups were lower than that of the control, taurine depleted and automatic recovery groups. These in vivo findings demonstrate that hepatic disease caused by chronic alcohol consumption can be prevented and cured by administration of taurine.

Keywords Alcoholic liver disease · Effect · Protection · Rat · Taurine

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Introduction

The liver is considered to be the main organ capable of oxidizing alcohol. The chronic consumption of alcoholic beverage is the major cause of liver injury and the development of serious liver disease. Alcohol abuse is a leading cause of morbidity and mortality throughout the world. It is estimated that in the United States as many as 10% of the men and 3% of the women may suffer from persistent problems related to the use of alcohol (Worman 1998). In China, the incidence of alcoholic liver disease (ALD) has increased. Alcohol abuse has been considered the second leading cause of hepatic lesion after virus hepatitis (Lu et al. 2002). It has been reported that the mortality rate among youths caused by ALD constitute 80% of total hepatic-linked mortality (Liu and Zhang 2004).

Taurine, a sulfur-containing β-amino acid, is the major free intracellular amino acid present in many tissues of human and animals (Huxtable 1992). There is significant evidence that taurine exerts physiologic and pharmacologic functions, such as maintaining structure and function normally in the hematological system, immune system, reproductive system, visual system, cardiovascular system and nervous system. Since taurine is synthesized mainly in the liver, the effects of taurine on the alimentary system have assumed particular attention. Kerial et al. (1998) have demonstrated a protective effect of taurine against hepatic steatosis and lipid peroxidation when co-administered with alcohol for 28 days. In China, it is reported by Chen et al. (1999) that taurine protects against liver damage caused by multiple insults except for alcohol.

In this study, we investigated the effects of taurine on ALD, and elucidated the mechanism underlying the cytoprotection.

Materials and methods

Experimental animals and treatments

Six-week-old male Wistar rats weighing 125–150 g were maintained under a controlled condition of light (12 h of light, 12 h of dark) and temperature (23 ± 2°C), and were given free access to food (commercial standard rat chow) and water.

Chemicals

Sixty percent alcohol was purchased from Shenyang Lao-Long kou distillery. Taurine and pyrazole were purchased from Sigma Chemical Company (St. Louis, MO, USA). Kits of total protein (TP), reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), alanine aminotransferase (ALT), aspartate aminotransfer (AST), nitrogen monoxidum (NO), inducible nitric oxide synthase (iNOS) and hydroxyproline (Hyp) were purchased from Nanjing Jiancheng Bioengineering Institute. Interleukin-2 (IL-2), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) radioimmunoassay kits were purchased from Beijing Chemclin Biotech Co., Ltd. Hyaluronic acid (HA) and laminin (LN) radioimmunoassay kits were purchased from Beijing North Institute of Biological Technology (BNIBT in short) (Pan Jia Mao A20, Feng Tai District).

Experimental design

The experiment was divided into preventive experiment and curative experiment. The preventive experiment aims to elucidate whether taurine can prevent the occurrence of ALD at the same time of alcohol administration. Fifty wistar rats were randomly divided into five groups ($n = 10$). The control group (C) was fed commercial standard rat chow and received an intragastric administration of physiologic saline every morning. Alcohol and pyrazole at dosages of 8 g/(kg day) and 24 mg/(kg day), respectively, were co-administered intragastrically to rats of the model group (M) every morning. These rats were fed high-fat diet (commercial standard rat chow dipped in corn oil). Other groups were treated with alcohol and pyrazole in addition to being maintained on tap water containing 1% β-alanine to cause endogenous taurine depletion (D). Still other rats (preventive group) were maintained on tap water containing either 1 or 2% taurine respectively (PI, PII). Twelve weeks later, blood and hepatic tissue were collected after the rats were euthanatized for biochemical analysis and histological tests.

The curative experiment aims to elucidate whether taurine can reverse ALD after the liver has been injured by chronic alcohol consumption, therefore taurine was given after the ALD model was established. ALD model rats were randomly divided into three groups ($n = 10$). The rats were allowed free access to commercial standard rat chow. The rats in the automatic recovery group (A) were given tap water, while the taurine treatment groups (TI, TII) were given water containing either 1 or 2% taurine respectively. After 4 weeks of taurine treatment following termination of alcohol administration, the rats were euthanatized and blood and hepatic tissue were collected for biochemical analysis and histological tests.

Relative weight of the liver

After the rats were euthanatized, the livers were weighed and the relative weights of the livers (the ratio of the liver weight to the body weight) were calculated.

Biochemical analysis

Blood samples were collected from the jugular vein. After standing at room temperature for 45 min, serum was separated by centrifuging at 1,500 rpm for 15 min at 4°C. Serum transaminase (ALT and AST) activities were determined by colorimetry using kits on spectrophotometry.

Liver tissues were taken and rapidly homogenized in ice-cold saline. Tissue homogenates were centrifuged at 3,000 rpm for 10 min at 4°C to remove crude fractions. Then the supernatants were collected. Lipid peroxidation, measured as GSH, MDA and SOD, were determined by colorimetry using kits on spectrophotometry according to the procedure provided, respectively. Hepatic content of TP was also determined by colorimetry using coomassie brilliant blue as the visualization reagent.

Inflammatory factors, which can stimulate the inflammatory reaction, measured as serum concentrations of IL-2, IL-6 and TNF-α were determined by a radioimmunoassay based on reagent kits. Hepatic fibrosis indexes, measured as Hyp, HA and LN, were also determined by radioimmunoassay based on reagent kits labelled ^{125}I.

The hepatic content of NO was determined by nitratase method based on the reagent kit. The hepatic content of iNOS was determined based on the reagent kit using spectrophotometry.

Histological analysis

Hepatic tissues from the left hepatic lobe were fixed in 10% (v/v) phosphate buffered formalin solution (pH 7.0) and embedded in paraffin wax. Sections were cut into 5 μm and stained with haematoxylin and eosin. The adipohepatic percentage was the ratio of adipohepatic area to the area of

total visual field. The degree of fatty degeneration according to the proportion of hepatocytes containing fat droplets was classified.

Statistic analysis

Data were presented as the mean ± SE and significant differences were determined by Duncan's multiple range test using SPSS 12.0 statistical analysis software. P values less than 0.05 were considered significant.

Results

Relative weight of the liver

Figure 1 showed that the taurine-treated and normal animals exhibited smaller hepatic indices than the M and D groups following 3 months of alcohol and pyrazole consumption, especially when compared to the PI and PII groups with the D group. Four weeks after addition of taurine to the water, the relative weight of the liver significantly decreased in the TII group compared with the A group ($P < 0.05$).

Serum analysis

As shown in Fig. 1, serum concentrations of ALT and AST were significantly raised by alcohol treatment ($P < 0.05$), especially when endogenous taurine was depleted by β-alanine administration ($P < 0.01$). Furthermore, there were significant differences between the taurine groups and the D group. Therefore the levels of serum ALT and AST were significantly decreased when taurine was co-administered with alcohol and was maintained for 4 weeks after alcohol withdrawal ($P < 0.05$).

As shown in Fig. 2, serum concentrations of IL-2, IL-6 and TNF-α in the M and the D groups were all remarkably higher than values of the C group ($P < 0.05$). Especially when compared with the D group, 1 and 2% taurine could significantly decrease the levels of IL-2 and TNF-α.((s for IL-6, the difference was significant between the D group and PII group ($P < 0.05$). The above indices were remarkably decreased when 2% taurine was co-administered with alcohol and was maintained for 4 weeks after alcohol withdrawal ($P < 0.05$).

Hepatic analysis

As shown in Fig. 3, hepatic contents of TP and GSH were significantly decreased by alcohol treatment, but they were all significantly increased when 2% taurine was co-administered with alcohol and was maintained for 4 weeks after alcohol withdrawal. Hepatic levels of MDA were significantly increased by alcohol treatment, especially in the D group ($P < 0.01$). However, 2% taurine administered during alcohol consumption significantly lowered the hepatic content of MDA when compared with the M group, as well as in the curative experiment. Alcohol treatment also caused a decrease in the hepatic concentration of SOD. However, administration of 2% taurine significantly increased the hepatic concentration of SOD compared with the M group

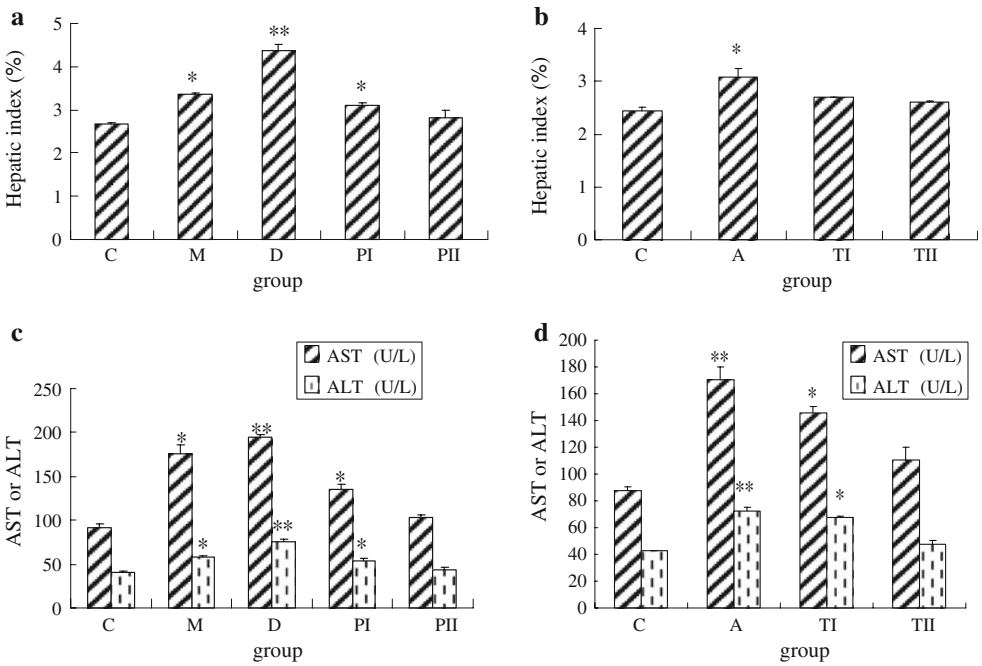

Fig. 1 Effects of taurine on relative weight of the liver and serum ALT and AST. **a** Showing preventive effects of taurine on relative weight of the liver. **b** Showing curative effects of taurine on relative weight of the liver. **c** Showing preventive effects of taurine on serum concentrations of ALT and AST. **d** Showing curative effects of taurine on serum concentrations of ALT and AST. Values are mean ± SE ($n = 5$). *$P < 0.05$, compared to the C group. **$P < 0.01$ compared to the C group

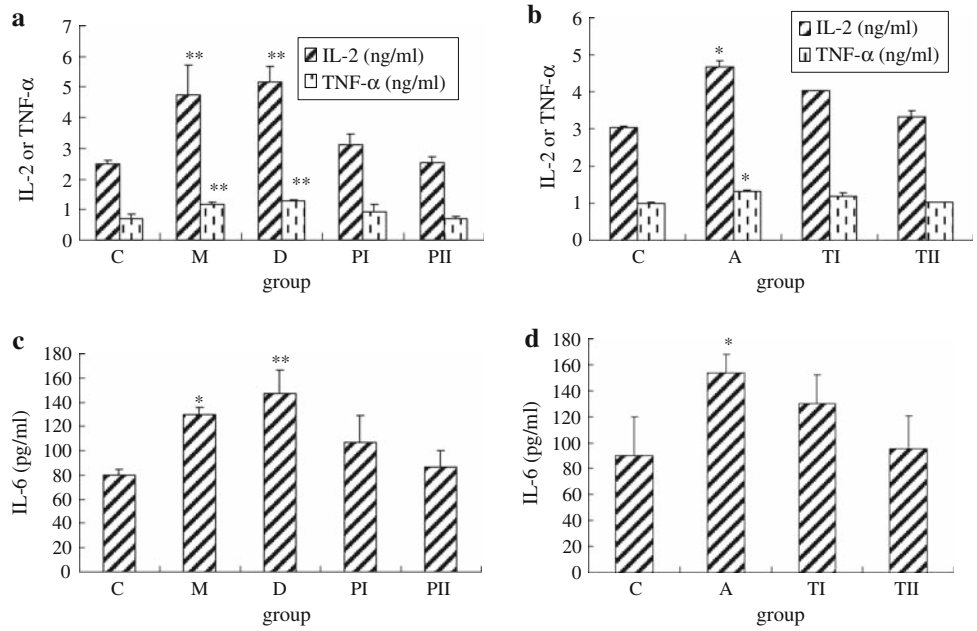

Fig. 2 Effects of taurine on correlation cytokine of rat. **a** Showing preventive effects of taurine on serum concentrations of IL-2 and TNF-α. **b** Showing curative effects of taurine on serum concentrations of IL-2 and TNF-α. **c** Showing preventive effects of taurine on serum concentrations of IL-6. **d** Showing curative effects of taurine on serum concentrations of IL-6. Values are mean ± SE ($n = 5$). *$P < 0.05$, compared to the C group. **$P < 0.01$ compared to the C group

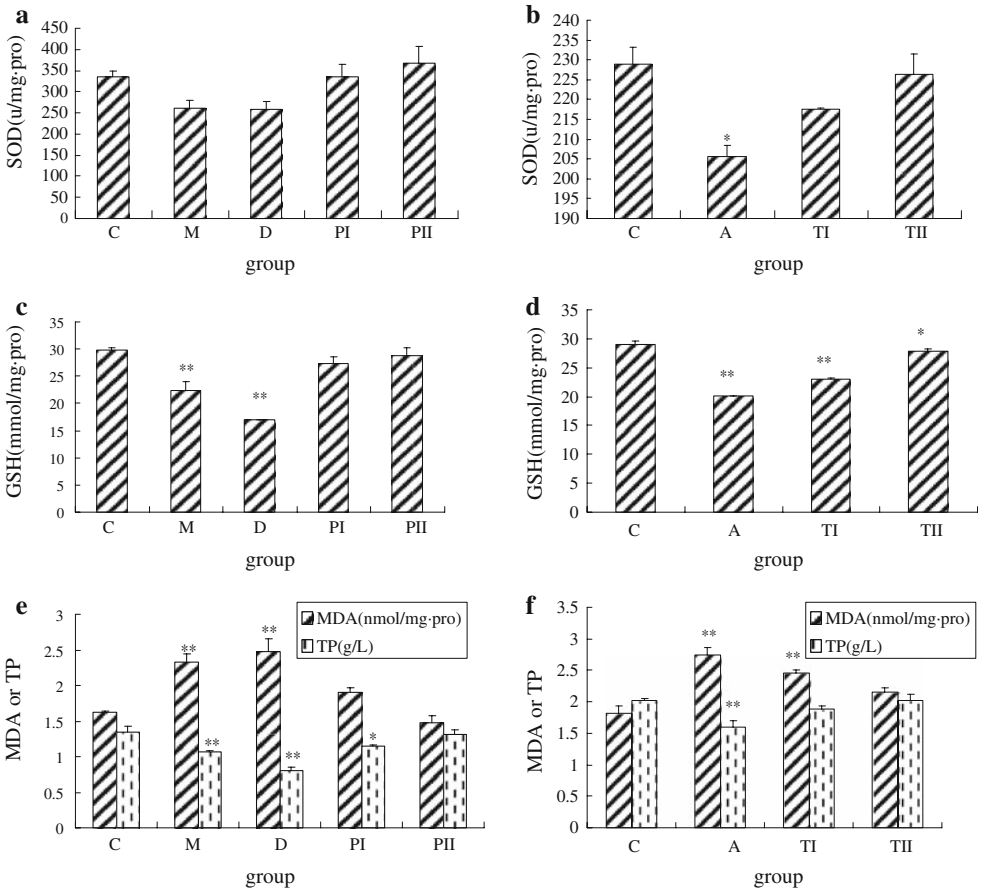

Fig. 3 Effects of taurine on hepatic lipid peroxidation in rats. **a** Showing preventive effects of taurine on hepatic SOD. **b** Showing curative effects of taurine on hepatic SOD. **c** Showing preventive effects of taurine on hepatic GSH. **d** Showing curative effects of taurine on hepatic GSH. **e** Showing preventive effects of taurine on hepatic MDA and TP. **f** Showing curative effects of taurine on hepatic MDA and TP. Values are mean ± SE ($n = 5$). *$P < 0.05$, compared to the C group. **$P < 0.01$ compared to the C group

($P < 0.05$). Furthermore, there were significant differences in the levels of TP, GSH, MDA and SOD when comparing the D group with the taurine groups.

As shown in Fig. 4, the hepatic levels of NO and iNOS were significantly raised by alcohol treatment ($P < 0.05$), especially in the D group ($P < 0.01$). When 2% taurine

was co-administered with alcohol and was maintained for 4 weeks after alcohol withdrawal, hepatic levels of NO and iNOS were significantly decreased compared with the M group ($P < 0.05$).

Serum concentrations of HA and LN were significantly raised by alcohol treatment ($P < 0.05$), however, hepatic concentrations of Hyp was only partially elevated compared with the C group. But all three indices were significantly raised when endogenous taurine was depleted by β-alanine administration ($P < 0.05$). The results showed that the levels of hepatic Hyp, serum HA and serum LN were significantly decreased when 1 or 2% taurine was co-administered with alcohol and was maintained for 4 weeks after alcohol withdrawal (Fig. 4).

Histological analysis

The results of hepatic histological examination showed that the livers of rats treated with alcohol and β-alanine were enlarged with hyperaemic surfaces, tight teguments and granulations on the surface. Optical microscopic examination showed that the rats treated with alcohol had developed significant steatosis and contained inflammatory cell infiltrations with significant engorged hepatic cells, loose cytoplasm and many fat vacuoles in the cell plasma. The nuclei were located on the periphery of the cells (Fig. 5c). The liver cell cords of the M and D groups lacked normal orderly structure, with the collagenous fibers enlarged and the hepatic lobules separated (Fig. 5d, f). The degrees of steatosis, inflammation and fibroplasia were remarkably reduced when treated with taurine preventively (Fig. 5g–j) and curatively (Fig. 5m–p). But in the A group, there were also fat vacuoles localized in the cytoplasm, the cell nuclei were dispersed to the periphery of the cells and the central veins were in disorder (Fig. 5k, l).

Table 1 shows a hepatic steatosis classification chart and adipohepatic percentage data. In the preventive experiment, the adipohepatic percentage of the M group was

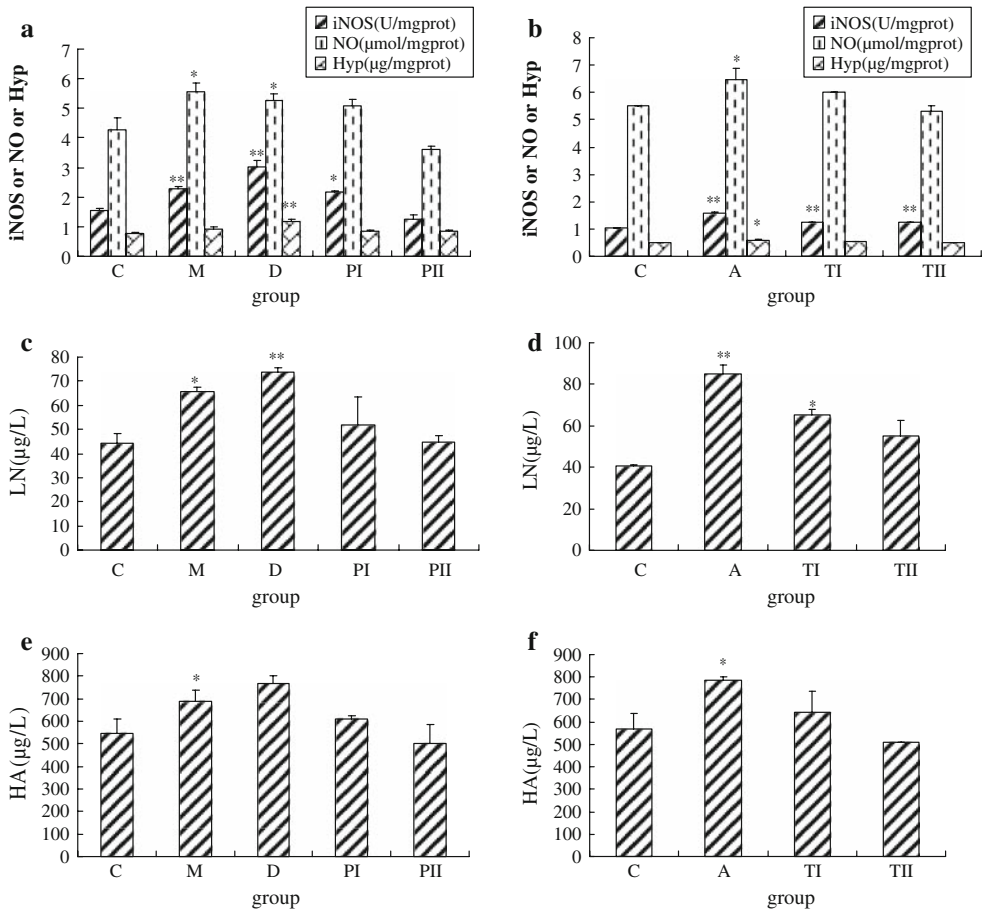

Fig. 4 Effects of taurine on hepatic content of NO, iNOS and the fibrosis index in rats. **a** Showing preventive effects of taurine on hepatic iNOS and NO. **b** Showing curative effects of taurine on hepatic iNOS and NO. **c** Showing preventive effects of taurine on serum concentrations of LN. **d** Showing curative effects of taurine on serum concentrations of LN. **e** Showing preventive effects of taurine on serum concentrations of HA. **f** Showing curative effects of taurine on serum concentrations of HA. Values are mean ± SE ($n = 5$). *$P < 0.05$, compared to the C group. **$P < 0.01$ compared to the C group

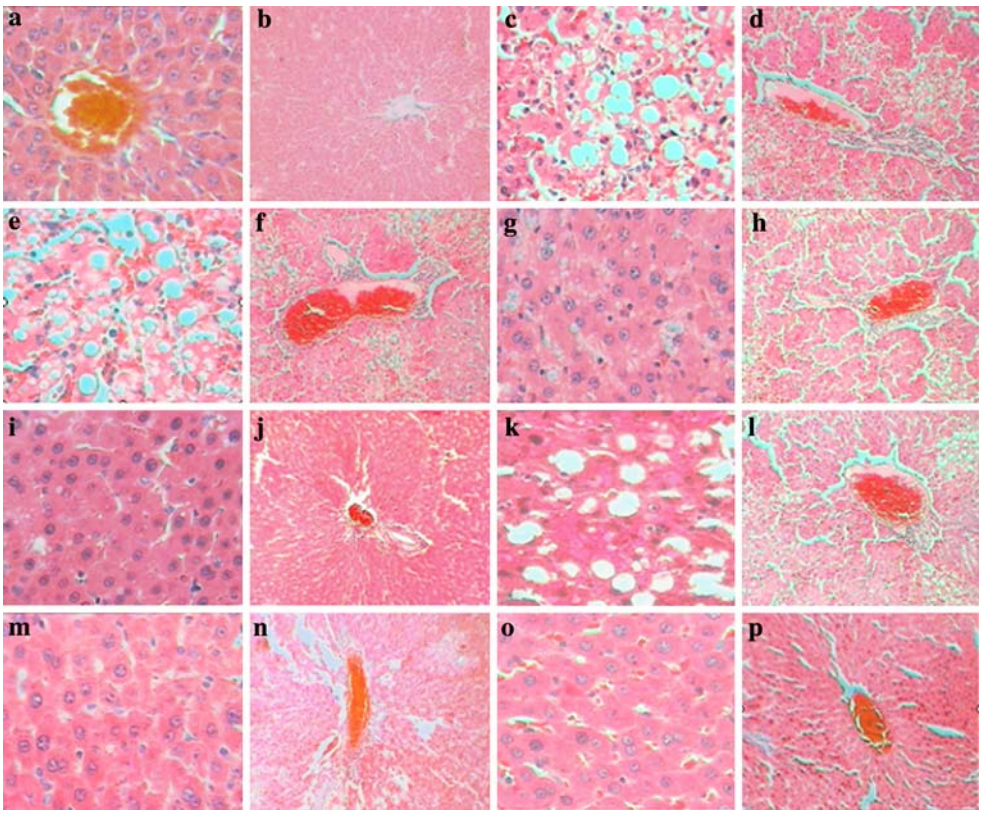

Fig. 5 Histological changes of the liver. These photomicrographs of hepatic cells and central veins show different degrees of steatosis, thrombosis, inflammatory cell infiltration and collagenous fiber hyperplasia in each group. **a**, **b** C group. **c**, **d** M group. **e**, **f** D group. **g**, **h** PI group. **I**, **j** PII group. **k**, **l** A group. **m**, **n** TI group. **o**, **p** TII group. HE stain. **a**, **c**, **e**, **g**, **i**, **k**, **m**, **o** ×400. **b**, **d**, **f**, **h**, **j**, **l**, **n**, **p** ×100

significantly larger than that of the C group ($P < 0.05$). However, when the rats were treated with taurine at the time of alcohol consumption, the adipohepatic percentage was significantly reduced compared with the M group ($P < 0.05$). In the curative experiment, the adipohepatic percentage was also significantly reduced by taurine administration when compared with the A group ($P < 0.05$).

Table 1 Effects of taurine on pathologic changes of the liver

Group	Steatosis classification	Adipohepatic percentage
Preventive experiment		
C	1	0.04 ± 0.04 a
M	6	27.36 ± 0.16 b
D	6	37.38 ± 2.42 c
PI	5	19.06 ± 0.27 d
PII	4	9.28 ± 0.30 e
Curative experiment		
C	1	0.04 ± 0.04 a
A	5	18.74 ± 0.24 b
TI	4	5.97 ± 0.47 c
TII	1	0.56 ± 0.07 a

Results represent means ± SE ($n = 5$). Superscripts represent significant difference between two groups within the same experiment ($P < 0.05$)

Discussion

As is known, ALD includes alcoholic fatty liver, alcoholic hepatitis, alcoholic fibrosis and alcoholic cirrhosis which can occur alone or together. The co-administration of alcohol and pyrazole combined with a high fat diet leads to ALD, associated with prolonged alcohol metabolism, hepatic cell membrane dysfunction and liver injury. This study describes a model of ALD exhibiting the damage mentioned above (Zhou et al. 1986).

Taurine is a β-type sulfur amino acid that is widely distributed in the tissues, cells and body fluids of human beings and animals. As a conditional essential amino acid of human beings and most animals, taurine has been used for many years in the treatment of hepatopathologies such as chronic active hepatitis. Recent research showed the effect of taurine on hepatic steatosis caused by alcohol (Kerial et al. 1999). But whether or not taurine can prevent or cure alcoholic hepatitis and alcoholic fibrosis has received little attention. This study aims toward evaluating

both preventive and curative effects of taurine on ALD completely.

The results indicating that the relative weight of the liver and serum concentrations of ALT and AST significantly increased while the hepatic content of total protein significantly decreased in the M and D groups may result from alcohol-mediated lipid accumulation, hepatic cell hypertrophy and membrane damage. Under those conditions, hepatic ALT and AST are released into blood. It is known that protein is mainly synthesized in liver, and when the liver is injured protein synthesis is adversely affected. Through this mechanism alcohol could cause a decrease in hepatic protein content, as shown in the present experiments. However, taurine administration would appear to reduce the degree of fat deposition, inhibit hepatic cellular swelling and attenuate the increase in the relative weight of the liver mediated by alcohol. Based on the sensitive index of liver dysfunction, namely, serum ALT, AST and hepatic TP, taurine treatment partially prevents ALD.

Many of the enzymes involved in the synthesis of triglycerides and phospholipids are associated with the endoplasmic reticulum. Chronic alcohol feeding leads to proliferation of smooth surfaced endoplasmic reticulum, so the lipid-synthesizing capacity of the liver could be enhanced. As alcohol is also metabolized by the microsomal enzyme system, it interferes with lipid metabolism. Microsomal alcohol oxidation may interfere with lipid metabolism by generating oxygen radicals such as O^{2-} and HO^{-}, which initiate a cascade of lipid peroxidation and damage cell membranes (Polavarapu et al. 1998). There is evidence that taurine exhibits antioxidant activity, regulates calcium, modulates osmotic pressure and stabilizes membranes (Trachtman et al. 1993; Wang et al. 2002). In this study, both alcohol and β-alanine significantly increased hepatic lipid peroxidation, while taurine significantly increased hepatic levels of GSH and SOD while decreasing hepatic levels of MDA. The results indicate that taurine may protect against free radical damage, elevate cytoprotective factors (reducing agent) and reduce damaging factors (oxidative products and radicals) (Banks et al. 1991). Thus, taurine reduces alcohol-induced hepatic steatosis and lipid peroxidation (Kerial et al. 1999).

NO plays an important role in the process of alcohol hepatic damage. Under physiological conditions, NO catalyzed by endothelial NOS (eNOS) regulates the microcirculation so it could inhibit oxidative stress and reduce the capability of generating free radicals formed by peroxidases. However, when the liver is damaged by alcohol, large amounts of NO are formed by inducible NOS (iNOS). This kind of NO could aggravate oxidative damage and disturb the microcirculation. There is evidence that taurine has no significant effect on the expression of iNOS in damaged lung tissue caused by pneumosilicosis. However, this study found that hepatic content of NO and iNOS was significantly elevated by alcohol, but taurine treatment could reduce the synthesis of NO by inhibiting iNOS expression.

Alcohol treatment resulted in alcoholic hepatic fibrosis, increasing interstitial extracellular matrix (ECM) through a reduction in the degradation of ECM. Because Hyp is the main constituent of collagen protein, the degree of hepatic fibrosis can be estimated by hepatic Hyp content. Meanwhile, liver fibrosis could lead to abnormal proliferation and insufficient degradation of protein constituents, such as collagen protein and proteoglycan. In this study, the abnormal increase of serum HA, LN and hepatic Hyp is consistent with the previous conclusion that taurine can inhibit hepatic fibrosis caused by CCl_4 (Chen et al. 1999; Wei et al. 2004). The results indicate that taurine can inhibit the proliferation of hepatic stellate cells and collagenoblasts, the synthesis of collagen and the deposition of hepatic ECM. In this way, taurine minimizes the degree of hepatic fibrosis. In addition, the values of serum IL-2, IL-6 and TNF-α obtained from this experiment showed that taurine administration inhibited the abnormal increase in cytokine content, indicating that taurine could minimize ALD by regulating the immunological network.

Histological observations showed that taurine is capable of not only preventing but actually reversing the pathomorphological changes of ALD, such as changes in fat deposition and inflammatory cell infiltration.

Conclusion

In conclusion, this study demonstrated that, through the regulation of fat metabolism in the liver, stabilization of membrane permeability, improvement in the capacity of anti-oxidation, elimination of oxyradicals, inhibition of abnormal proliferation of cytokine and the deposition of collagen, taurine not only prevents but also cures ALD.

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