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Betaine or taurine administration prevents fibrosis and lipid peroxidation induced by rat liver by ethanol plus carbon tetrachloride intoxication

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Summary. The aim of this study was to investigate the effect of betaine or taurine on liver fibrogenesis and lipid peroxidation in rats. Fibrosis was induced by treatment of rats with drinking water containing 5% ethanol and CCl_4 ($2\times$ weekly, 0.2 ml/kg, i.p.) for 4 weeks. Ethanol plus CCl_4 treatment caused increased lipid peroxidation and disturbed antioxidant system in the liver. Histopathological findings suggested that the development of liver fibrosis was prevented in rats treated with betaine or taurine (1% v/v in drinking water) together with ethanol plus CCl_4 for 4 weeks. When hepatic taurine content was depleted with β-alanine (3% v/v in drinking water), portal-central fibrosis induced by ethanol + CCl_4 treatment was observed to proceed cirrhotic structure. Betaine or taurine was also found to decrease serum transaminase activities and hepatic lipid peroxidation without any change in hepatic antioxidant system in rats with hepatic fibrosis. In conclusion, the administration of betaine or taurine prevented the development of liver fibrosis probably associated with decreased oxidative stress.

Keywords: Betaine – Taurine – Taurine depletion – Liver fibrosis – Oxidative stress – Carbon tetrachloride – Ethanol

Introduction

Liver fibrosis is a very complex phenomenon. During liver fibrosis, the synthesis and deposition of extracellular matrix proteins are increased and this accumulation of connective tissue inhibits normal functions of the liver. Fibrotic process involves many hepatocellular factors, including Kupffer cells, hepatic stellate cells and various chemical mediators that are produced by these cells (Friedman, 2003). It was demonstrated that reactive oxygen species play an important role in the activation of Kupffer and hepatic stellate cells and that there is a strong relationship between oxidative stress and fibrogenesis (Poli and Parola, 1997; Parola and Robino, 2001). Indeed, enhanced lipid peroxidation and/or deranged antioxidant system is found to be associated with experimental (Yalçın et al., 1985; Gasso et al., 1996; Alptekin et al., 1997; Deulofeu et al., 2000; Bruck et al., 2001; Balkan et al., 2001) and human cirrhosis (Suematsu and Abe, 1982; Loguercio et al., 1996). Therefore, therapeutic potentials of several compounds such as S-adenosylmethionine (SAM), polyenylphosphadylcholine, antioxidant vitamins, flavonoids, free radical scavengers and sulfhydryl compounds have been tested in liver fibrosis (Parola and Robino, 2001; Gebhard, 2002; Friedman, 2003).

Betaine and taurine have essential roles in sulfur-amino acid metabolism (Barak et al., 1996; Hansen, 2001). It has been proposed that betaine and taurine may have hepatoprotective effects (Barak et al., 1996; Hansen, 2001). However, there is inadequate knowledge on the role of betaine or taurine treatment in the prevention of liver fibrosis. In this study, we wanted to investigate the effect of betaine or taurine on the development of liver fibrosis and changes in prooxidant and antioxidant status of the liver in ethanol-carbon tetrachloride (CCl_4)-induced cirrhosis model.

Materials and methods

Animals and treatments

Wistar rats weighing 180–220 g were fed a standard rat chow ad libitum and two experimental models were applied. In the first model, the animals were divided into six groups of 8 animals each. Animals in group 1 were

used as the control group. Animals in group 2 and 3 received drinking water containing betaine (1% v/v) or taurine (1% v/v) for 4 weeks, respectively. Animals in group 4 were given drinking water containing ethanol 5% (v/v) together with two doses of CCl_4 (0.2 ml/kg, i.p.) weekly for 4 weeks. Animals group in 5 received betaine, as in group 2 and ethanol plus CCl_4 as in group 4. Animals in group 6 received taurine, as in group 3 and ethanol plus CCl_4 as in group 4.

In order to examine the effect of taurine depletion by β-alanine on liver fibrosis , the animals were divided into 4 groups of 6 animals each. The first group was controls. The second group was given β-alanine in drinking water (3%, v/v) for 4 weeks to deplete taurine. The third group was given ethanol and CCl_4 similar to group 4 in the first model. Finally, the fourth group was given β-alanine plus ethanol and CCl_4.

Betaine and taurine was obtained from Ambresco, Ohio, USA and Acros Organics, New Jersey, USA, respectively. Other chemicals were supplied by Sigma Chemical Co., St Louis, USA.

Methods

Blood was collected in tubes containing EDTA. Plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined by using Roche autoanalyser. The livers were rapidly removed, washed in 0.9% NaCl and kept in ice. To determine hepatic taurine content, the liver was homogenized in ten volumes of 3% sulfosalicylic acid. After centrifugation, the supernatant was filtered through 0.45 μm filter and finally taurine was determined with Hewleet Packard amino acid analyzer (Chen et al., 2004). Hepatic betaine was analyzed using the method of Barak and Tuma (1979). In brief, the liver was homogenized in 15% trichloroacetic acid. Betaine was reacted with potassium triiodide to form betaine periodide. The precipitate of betaine periodide was dissolved in ethylene dichloride and measured spectrophotometrically at 365 nm. Some portions of liver were also homogenized in ice-cold 0.15 M KCl (l0%, w/w). The degree of lipid peroxidation was also assessed by two different methods in the liver. First, the levels of malondialdehyde (MDA) were measured by thiobarbituric acid test (Ohkawa et al., 1979). The breakdown product of 1,1,3,3-tetraethoxypropane was used as a standard. Second, diene conjugate (DC) levels were determined in hepatic lipid extracts at 233 nm spectrophotometrically and calculated using a molar extinction coefficient of $2.52 \times 10^4\,M^{-1}\,cm^{-1}$ (Buege and Aust, 1978). Liver glutathione (GSH) levels were measured with 5,5-dithiobis-(2-nitrobenzoate) at 412 nm (Beutler et al., 1979). α-tocopherol and ascorbic acid levels were measured in liver homogenates

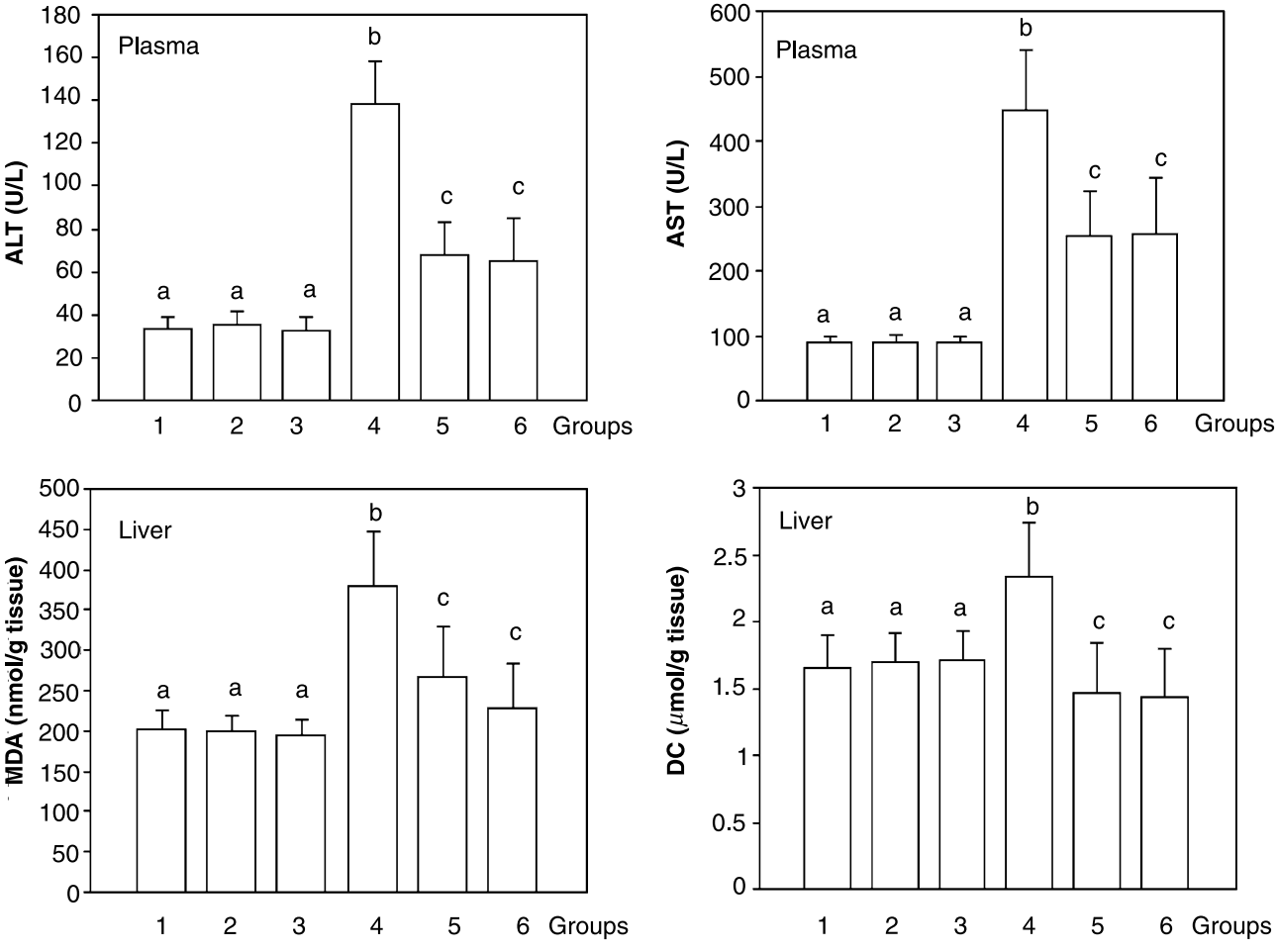

Fig. 1. The effect of betaine or taurine treatment on plasma alanine transaminase (*ALT*) and aspartate transaminase (*AST*) activities and hepatic malondialdehyde (*MDA*) and diene conjugate levels (*DC*) in normal and ethanol plus carbon tetrachloride treated rats (Mean ± SD; n = 8 each). Groups: *1* control; *2* betaine; *3* taurine; *4* ethanol + CCl_4; *5* ethanol + CCl_4 + betaine; *6* ethanol + CCl_4 + taurine. Values not sharing a common superscript letter are significantly different by ANOVA test; $p < 0.05$

by the method of Desai (1978) and Omaye et al., (1979), respectively. Hepatic superoxide dismutase (SOD) activities were assayed by its ability to increase the effect of riboflavin-sensitized photooxidation of orthodianisidine in postmitochondrial fractions (Mylorie et al., 1986) Glutathione peroxidase (GSH-Px) (Lawrence and Burk, 1976) and glutathione transferase (GST) (Habig and Jacoby, 1981) activities were measured using cumene hydroperoxide and 1-chloro-2,4-dinitrobenzene using as substrates, respectively, in postmitochondrial fractions. Protein levels were determined using bicinchoninic acid (Smith et al., 1985).

Histopathological analyses

Livers were dissected and fixed in 10% buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological studies.

Statistical analyses

The results were expressed as mean ± SD. Statistical analysis was performed by a one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post-hoc test.

Results

The results are shown in Fig. 1 and Tables 1– 4. According to this;

a) Ethanol plus CCl_4 treatment caused significant increases in plasma ALT and AST activities and hepatic MDA and DC levels (Fig. 1). This treatment decreased hepatic betaine and taurine contents (Tables 1, 2). Significant decreases in GSH, α-tocopherol and ascorbic

Table 1. The effect of betaine supplementation on hepatic betaine contents in control and ethanol plus carbon tetrachloride treated rats (Mean ± SD; n = 8 each)

Groups	Betaine (μmol/g tissue)
Control	4.81 ± 0.52^{a}
Betaine	8.56 ± 0.93^{b}
Ethanol + CCl_4	3.46 ± 0.79^{c}
Ethanol + CCl_4 + Betaine	7.38 ± 0.67^{d}

Values not sharing a common superscript letter are significantly different by ANOVA test; $p < 0.05$

Table 2. The effect of taurine supplementation on hepatic taurine contents in control and ethanol plus carbon tetrachloride treated rats (Mean ± SD; n = 8 each)

Groups	Taurine (μmol/g tissue)
Control	4.37 ± 0.86^{a}
Taurine	10.0 ± 1.56^{b}
Ethanol + CCl_4	2.86 ± 0.40^{c}
Ethanol + CCl_4 + Taurine	8.76 ± 1.12^{bd}

Values not sharing a common superscript letter are significantly different by ANOVA test; $p < 0.05$

Table 3. The effect of betaine or taurine treatment on glutathione (GSH), α-tocopherol and ascorbic acid levels and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione transferase (GST) activities in the livers of normal and ethanol plus carbon tetrachloride treated rats (Mean ± SD; n = 8 each)

Parameters	Control	Betaine	Taurine	Ethanol + CCl_4	Ethanol + CCl_4 + Betaine	Ethanol + CCl_4 + Taurine
GSH (nmol/g tissue)	6.48 ± 0.41^{a}	6.47 ± 0.47^{a}	6.50 ± 0.39^{a}	5.52 ± 0.42^{b}	5.71 ± 0.54^{b}	5.83 ± 0.26^{b}
α-Tocopherol (nmol/g tissue)	42.9 ± 5.08^{a}	42.1 ± 3.35^{a}	40.9 ± 3.26^{a}	36.0 ± 3.92^{b}	40.7 ± 3.30^{ab}	42.4 ± 6.40^{a}
Ascorbic acid (nmol/g tissue)	53.0 ± 8.50^{a}	55.7 ± 6.90^{a}	55.5 ± 7.52^{a}	39.7 ± 4.52^{b}	39.7 ± 3.24^{b}	42.5 ± 4.81^{b}
SOD (U/mg protein)	21.0 ± 2.16^{a}	19.9 ± 2.31^{a}	19.75 ± 1.69^{a}	18.6 ± 3.17^{a}	19.2 ± 4.45^{a}	17.4 ± 4.31^{a}
GSH-Px (nmol/mg protein/min)	333.2 ± 38.6^{a}	334.6 ± 37.8^{a}	336.2 ± 58.8^{a}	213.8 ± 33.7^{b}	200.8 ± 21.1^{b}	209.0 ± 21.2^{b}
GST (nmol/mg protein/min)	300.7 ± 33.7^{a}	323.7 ± 39.6^{a}	314.7 ± 34.8^{a}	182.1 ± 47.0^{b}	199.9 ± 14.5^{b}	206.6 ± 24.0^{b}

Values not sharing a common superscript letter are significantly different by ANOVA test; $p < 0.05$

Table 4. The effect of β-alanine treatment on hepatic taurine contents and plasma alanine transaminase (ALT) and aspartat transaminase (AST) activities in control and ethanol plus carbon tetrachloride treated rats (Mean $\pm$ SD; n = 6 each)

Groups	Taurine (μmol/g tissue)	ALT (U/L)	AST (U/L)
Control	4.21 ± 0.95^{a}	32.5 ± 5.50^{a}	91.7 ± 5.68^{a}
β-Alanine	1.53 ± 0.20^{b}	33.7 ± 6.74^{a}	86.8 ± 6.52^{a}
Ethanol + CCl_4	2.89 ± 0.35^{c}	141.2 ± 39.7^{b}	455.0 ± 109.3^{b}
β-Alanine + Ethanol + CCl_4	1.28 ± 0.39^{bd}	232.5 ± 56.5^{c}	606.7 ± 114.1^{c}

Values not sharing a common superscript letter are significantly different by ANOVA test; $p < 0.05$

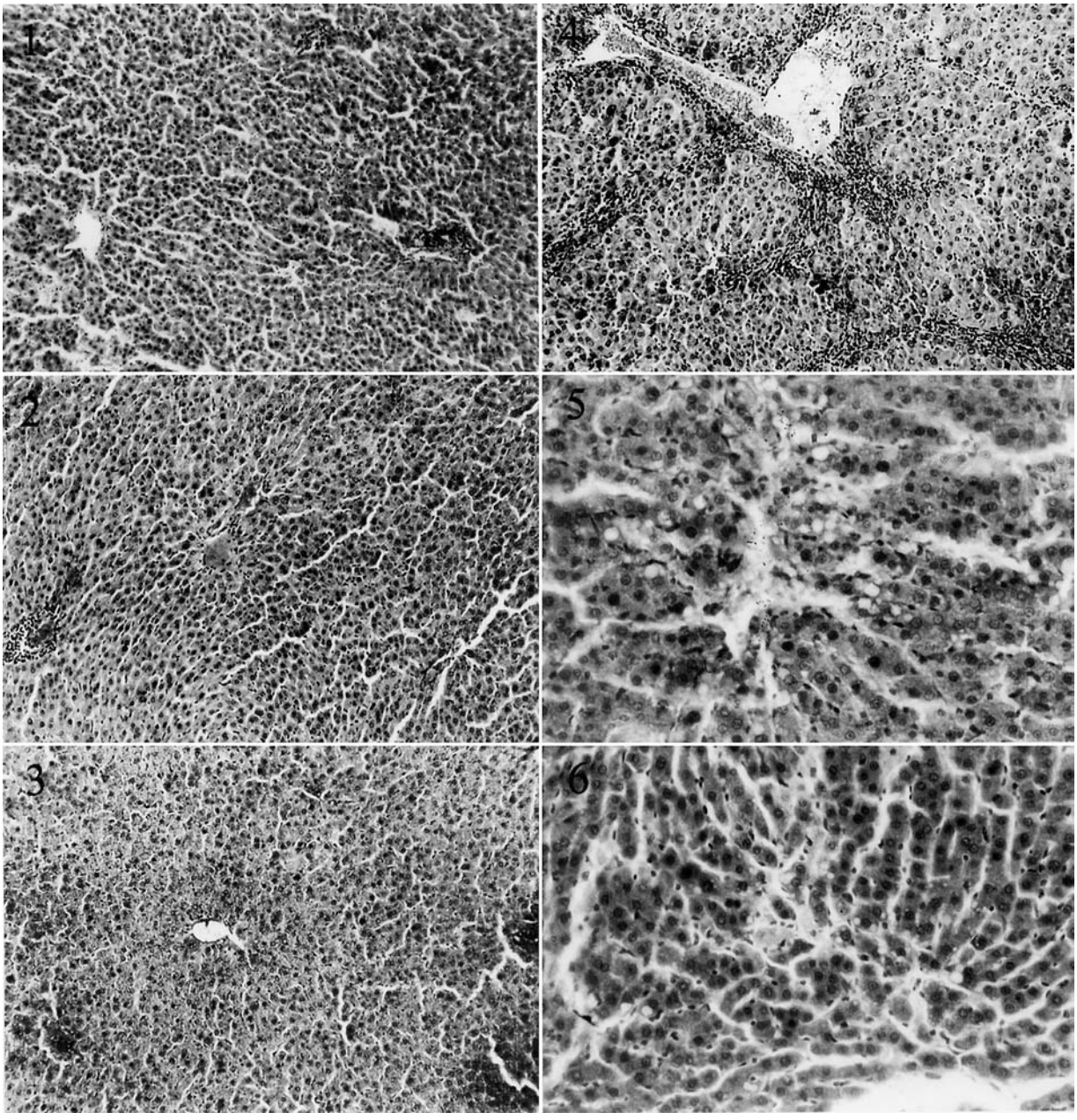

Fig. 2. Hematoxylin and eosin staining of liver sections from: **1** control; **2** betaine; **3** taurine; **4** ethanol + CCl_4; **5** betaine + ethanol + CCl_4; **6** taurine + ethanol + CCl_4

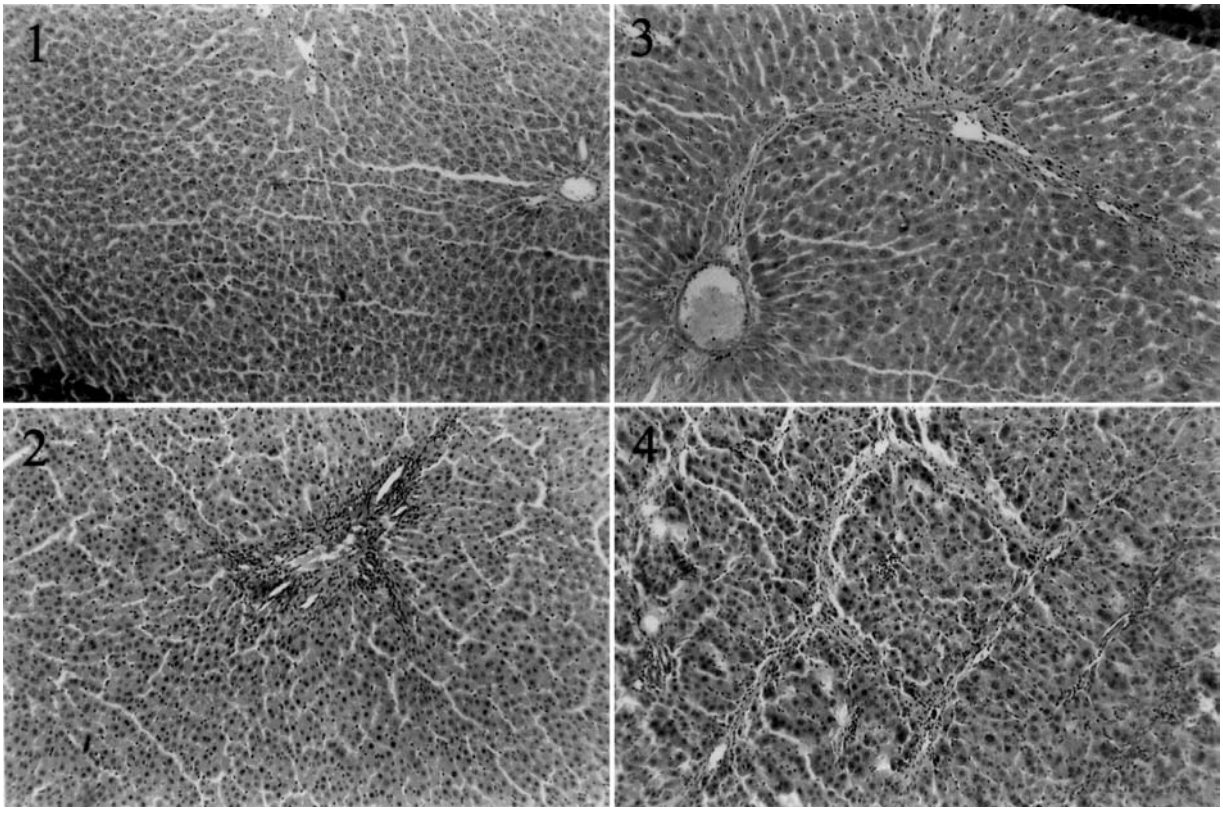

Fig. 3. Hematoxylin and eosin staining of liver sections from: **1** control; **2** β-alanine; **3** ethanol + CCl_4; **4** β-alanine + ethanol + CCl_4

acid levels and GSH-Px and GST activities were observed, but hepatic SOD activity remained unchanged as compared with those in controls (Table 3).

b) Betaine or taurine treatment together with ethanol plus CCl_4 in rats caused decreases in hepatic MDA and DC levels and serum transaminase activities (Fig. 1) as well as increasing hepatic betaine and taurine levels (Tables 1, 2). No changes in GSH and ascorbic acid levels and antioxidant enzyme activities were observed with betaine or taurine treatment in ethanol plus CCl_4 treated rats. However, α-tocopherol levels were observed to increase in ethanol plus CCl_4-treated rats with taurine treatment, but not with betaine (Table 3).

c) Betaine or taurine treatment to normal rats did not have any effect on plasma transaminase activities and lipid peroxide levels and antioxidant system in the liver as compared to controls (Fig. 1 and Table 3).

Histopathological assessment of rats showed the following changes (Fig. 2). In the control, betaine and taurine groups, normal liver structure was seen. In the ethanol plus CCl_4 treated group, there was pseudonodular structure surrounded by broad fibrous bands. Taurine or betaine treatment totally protected against ethanol plus CCl_4 induced liver fibrosis. However, we detected light macro- and microvesicular steatosis in the livers with betaine or taurine treatment in ethanol plus CCl_4 treated rats.

On the other hand, β-alanine treatment has been observed to significantly decrease hepatic taurine content, but had no effect on plasma ALT and AST activities (Table 4). In β-alanine + ethanol + CCl_4 group, plasma ALT and AST activities were higher than those observed in ethanol + CCl_4 group. In β-alanine treated group, normal liver structure was seen, but rare mononuclear cells in portal area were present. In ethanol + CCl_4-treated rats, portal-central fibrosis was proceeded to complete cirrhosis by β-alanine administration (Fig. 3).

Discussion

Animal models of liver fibrosis are important for research into the underlying mechanisms or treatments associated with this disease. Currently, cirrhosis is mainly induced either by the ligation of common bile duct or by application of hepatotoxins such as CCl_4 injection or thioacetamide (Wu and Norton, 1996). In our study, experimental

fibrosis was induced by low dose of CCl_4 and drinking a 5% ethanol solution within 4 weeks in rats. It has been reported that hepatic histological changes in ethanol-CCl_4 induced fibrosis model were similar to those found in human alcoholic cirrhosis (Siegers et al., 1986). In our study, CCl_4-ethanol treatment to rats resulted in hepatic fibrosis as assessed by biochemical and histopathological findings. Histopathological examination of the livers at the end of 4-weeks treatment period revealed pseudonodular structure surrounded by broad fibrous bands. We found that MDA and DC levels increased, GSH, α-tocopherol and ascorbic acid levels and GSH-Px and GST activities decreased, but SOD activity remained unchanged in cirrhotic liver. These findings are in accordance with other studies which showed increased oxidative stress accompanied liver cirrhosis (Yalçın et al., 1985; Gasso et al., 1996; Deulofeu et al., 2000; Bruck et al., 2001; Balkan et al., 2001). In our study, we investigated whether or not betaine or taurine treatment prevents the development of ethanol-CCl_4-induced liver fibrosis.

Betaine (trimethylglycine), is a metabolite of choline and is produced by choline oxidase in the liver. In addition, plants are rich dietary source of betaine (Barak et al., 1996). Betaine administration is also found to reduce hepatic steatosis caused by either CCl_4 (Junnila et al., 2000) or ethanol (Balkan et al., 2004; Barak et al., 1993). The hepatoprotective effect of betaine has been reported to be related to its increasing effect of SAM levels (Barak et al., 1996). As it is known, betaine participates in the synthesis of methionine from homocysteine and restores SAM levels, which has essential roles in phospholipid metabolism and membrane structure (Barak et al., 1996). It has been reported that SAM treatment prevents liver fibrosis by restorating hepatocyte membranes, enhancing antioxidant reserve, especially glutathione levels and decreasing lipid peroxide levels (Gasso et al., 1996; Mato et al., 1999; Deulofeu et al., 2000). Betaine treatment is also found to decrease lipid peroxidation in ethanol treated rats (Balkan et al., 2004; Kanbak et al., 2001). However, there is no knowledge in the literature about the effect of betaine, a SAM precursor, on liver fibrosis.

Taurine (2-aminoethane sulfonic acid), a nonprotein amino acid, is one of the most abundant amino acids present in tissues. It is both synthesized from cysteine and methionine and ingested directly with certain foodstuffs (Hansen, 2001). Taurine has been suggested to have a number of protective properties including protection against hepatic damage (Hansen, 2001). Some investigators have demonstrated that taurine reduced thioacetamide (Doğru-Abbasoğlu et al., 2001) or acetaminophen (Waters et al., 2001) induced hepatic necrosis and ethanol (Balkan et al., 2002) induced hepatic steatosis. Our recent studies have shown that taurine treatment has also a protective effect against thioacetamide-induced cirrhosis (Balkan et al., 2001). It has been postulated that the hepatoprotective effect of taurine may be related to its antioxidant effect (Redmond et al., 1996; Hansen, 2001; Waters et al., 2001; Doğru-Abbasoğlu et al., 2001; Balkan et al., 2001).

On the other hand, it has been reported that both betaine and taurine act as an osmolyte in Kupffer cells and that these compounds may play an important role in the modulation of Kupffer cell functions such as phagocytosis and eicosanoid and tumor necrosis factor α formation (Warskulat et al., 1997). Recently, it has been reported that both taurine and betaine have inhibitory effects on Kupffer cells (Kim and Kim, 2002). Kupffer cells are known to be critical mediators of the inflammatory and fibrogenic responses in liver cirrhosis (Friedman, 2003). Indeed, in our study, when hepatic taurine content was depleted with β-alanine, portal-central fibrosis induced by ethanol + CCl_4 treatment was observed to proceed to complete cirrhotic structure. On the other hand, the inhibition of Kupffer cells with gadolinium chloride is found to prevent CCl_4-induced liver cirrhosis (Muriel and Escobar, 2003). These findings clearly indicates that Kupffer cells may play an important role in cirrhotic process.

In our study, taurine or betaine is found to prevent the development of hepatic fibrosis according to histological and biochemical findings. Nodule structure or broad fibrotic bands were not observed in the liver following taurine or betaine treatment together with ethanol plus CCl_4. These treatments caused significant decreases in plasma transaminase activities compared to the ethanol-CCl_4 group. In addition, these compounds decreased lipid peroxidation parameters such as MDA and DC without affecting antioxidant system excluding α-tocopherol levels in cirrhotic rat liver. Although the exact mechanism underlying protective effects of betaine and taurine treatments in liver fibrosis are not completely known, observed the protection may be related to the decrease in oxidative stress in the liver. In addition, inhibitory effect of taurine or betaine on Kupffer cell functions may play an additional role in the prevention of liver fibrosis. However, further studies are needed to solve this problem.

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