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Taurine: Protective properties against ethanol-induced hepatic steatosis and lipid peroxidation during chronic ethanol consumption in rats

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Summary. Alcohol was administered chronically to female Sprague Dawley rats in a nutritionally adequate totally liquid diet for 28 days. This resulted in hepatic steatosis and lipid peroxidation. Taurine, when co-administered with alcohol, reduced the hepatic steatosis and completely prevented lipid peroxidation. The protective properties of taurine in preventing fatty liver were also demonstrated histologically. Although alcohol was found not to affect the urinary excretion of taurine (a non-invasive marker of liver damage), levels of serum and liver taurine were markedly raised in animals receiving alcohol + taurine compared to animals given taurine alone. The ethanol-inducible form of cytochrome P-450 (CYP2E1) was significantly induced by alcohol; the activity was significantly lower than controls and barely detectable in animals fed the liquid alcohol diet containing taurine. In addition, alcohol significantly increased homocysteine excretion into urine throughout the 28 day period of ethanol administration; however, taurine did not prevent this increase. There was evidence of slight cholestasis in animals treated with alcohol and alcohol + taurine, as indicated by raised serum bile acids and alkaline phosphatase (ALP). The protective effects of taurine were attributed to the potential of bile acids, especially taurine conjugated bile acids (taurocholic acid) to inhibit the activity of some microsomal enzymes (CYP2E1). These *in vivo* findings demonstrate for the first time that hepatic steatosis and lipid peroxidation, occurring as a result of chronic alcohol consumption, can be ameliorated by administration of taurine to rats.

Keywords: Amino acids – Taurine – Ethanol – Protection – Hepatic steatosis – Lipid peroxidation – CYP2E1 – Homocysteine – Methionine synthase

Introduction

Alcohol-induced liver damage

Although ethanol is produced by yeast and bacterial fermentation in the gut of mammals, the interest in its toxicity is primarily as an exogenous compound, which is readily absorbed from the gastrointestinal tract. About 2–10% of the ethanol absorbed is eliminated through the kidneys and lungs, the rest is oxidized almost exclusively in the liver (Lieber, 1997a). There are three main pathways of ethanol metabolism (Fig. 1) which are located in different subcellular compartments of the hepatocyte: the alcohol dehydrogenase pathway (ADH) of the cytosol; the microsomal ethanol oxidizing system (MEOS) in the endoplasmic reticulum; and catalase in the peroxisomes (Lieber, 1993). Each of these pathways produces specific metabolic and toxic disturbances, and all three result in the production of acetaldehyde, a highly toxic metabolite which, in turn, is converted to acetate (Fig. 1). The MEOS involves an ethanol-inducible form of cytochrome P-450 (CYP2E1) that not only oxidizes ethanol, but also generates oxygen radicals and activates many xenobiotics to hepatotoxic and carcinogenic metabolites (Farinati et al., 1989). The induction of CYP2E1 is responsible for tolerance to alcohol seen in alcoholics (Lieber, 1997a). A great deal is known about ethanol metabolism, and the pathological consequences of ethanol ingestion have been described in detail (Harrison and Burt, 1993). However, the key intermediate

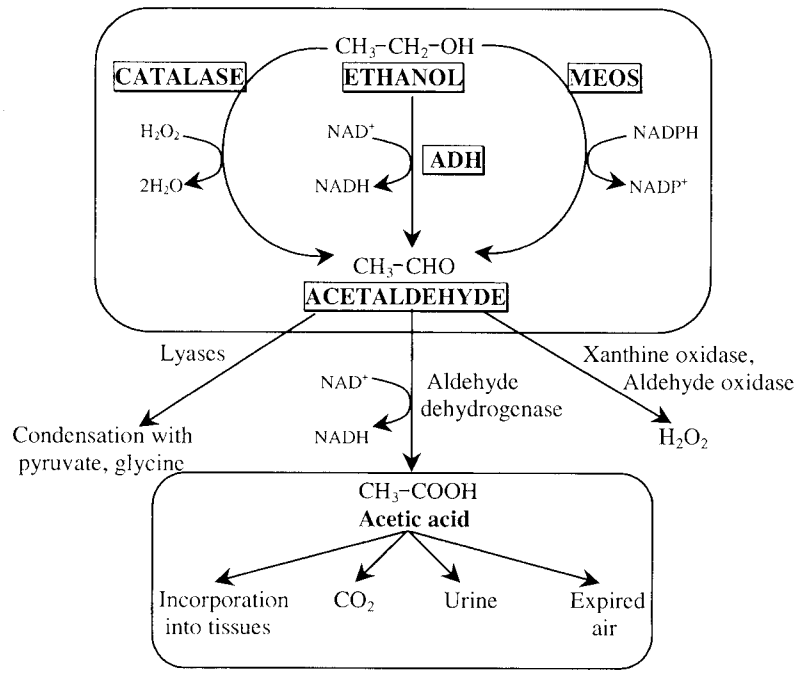

Fig. 1. The metabolism of ethanol. *ADH* alcohol dehydrogenase; *MEOS* microsomal ethanol oxidizing system

pathological processes that link initial metabolism of ethanol to its pathological effect are not fully described. As alcohol is an important cause of fatty liver (steatosis), cirrhosis and cancer, much research has been devoted to elucidating this mechanism (Lieber, 1993). The liver plays a central role in the metabolism of lipids, and there are several documented interactions of ethanol with lipid metabolism (Lieber and Savolainen, 1984). Alcohol-induced steatosis is the most common and most easily recognizable change of alcoholic liver disease and is present in more than 80% of alcoholics (Harrison and Burt, 1993). Recent reports have indicated that changes in methionine metabolism or methylation in the liver may have an important role in alcohol toxicity (Fig. 2). Although ethanol itself does not inhibit methionine synthase, acetaldehyde is known to inhibit highly purified methionine synthase *in vitro* (Kenyon et al., 1998). Inhibition of methionine synthase activity in the liver, induces the enzyme betaine homocysteine methyltransferase (BHMT), which also recycles homocysteine to methionine. However, betaine is derived from

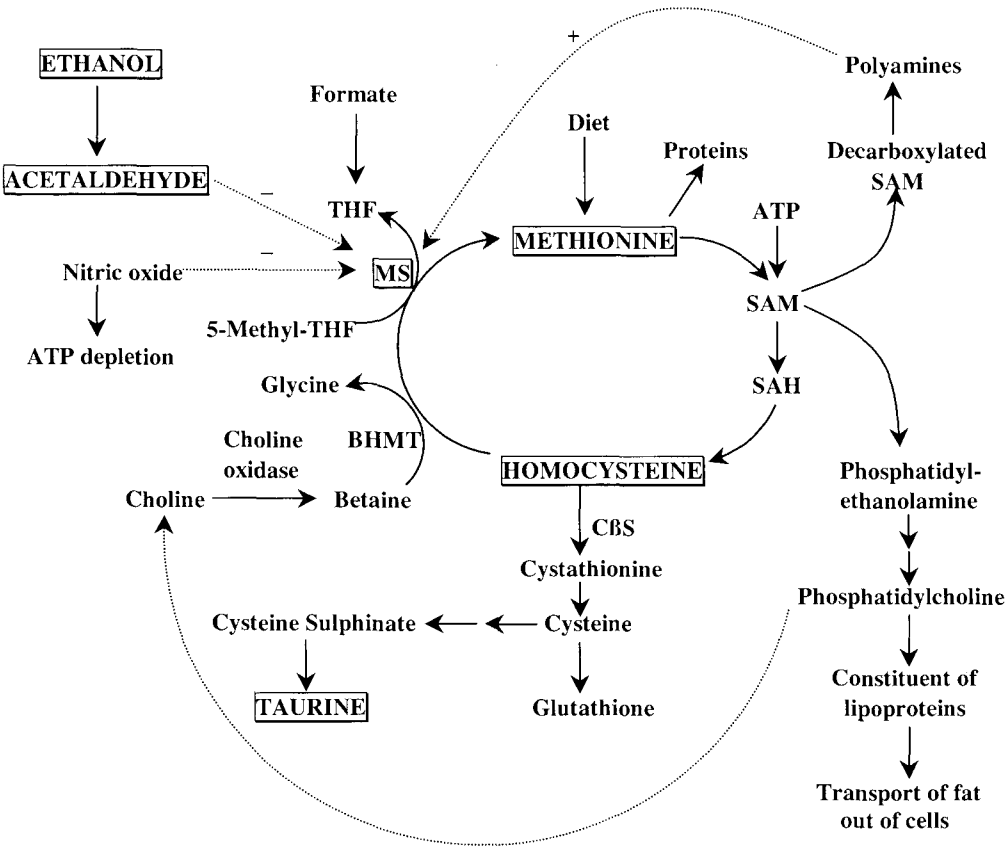

Fig. 2. The methionine cycle and transsulphuration pathway. *ATP* adenosine triphosphate; *THF* tetrahydrofolate; *SAM* S-adenosylmethionine; *SAH* S-adenosylhomocysteine; *MS* methionine synthase; *BHMT* betaine homocysteine methyltransferase; *CβS* cystathionine β-synthase; + stimulatory pathway; − inhibitory pathway. Dotted lines represent modifying reactions

choline and it may be expected that choline reserves will be depleted by this enzyme. Phosphatidylcholine is required for export of lipid from the liver and so induction of BHMT may, in part explain the fatty liver found in alcoholics. Certain compounds have been shown to have protective properties against ethanol-induced changes. For instance S-adenosyl methionine (SAM), a downstream product of methionine synthase activity, is useful for the treatment of early aspects of alcohol-induced liver injury (steatosis) (Lieber et al., 1990) and polyunsaturated lecithin (phosphatidylcholine) for the prevention of fibrosis and cirrhosis (Lieber et al., 1994).

Taurine

Taurine (2-aminoethane sulphonic acid) is a compound which has been shown to protect against hepatotoxicity (Huxtable, 1992). This β-amino acid occurs in high concentrations in many mammalian tissues (mM). It is found predominantly in excitable tissues such as the heart, skeletal muscle and nervous system (Huxtable, 1992). In addition, high concentrations of taurine are also found in the liver (2–10 mM) where it is synthesized from the sulphur-containing amino acid, cysteine. Taurine is not incorporated into proteins but is conjugated to form bile salts including taurocholic acid (Vessey, 1978). Taurine concentrations in the liver are variable, probably due to changes in bile flow and diet (Fukaya et al., 1996). Excess taurine is excreted into the urine. Taurine has been shown to act as a protective agent both *in vivo* and *in vitro* (Timbrell et al., 1995). For example, investigations *in vivo* have shown that taurine, when administered after carbon tetrachloride, suppressed lipid peroxidation (Nakashima et al., 1983) and there is also evidence *in vivo* (Waterfield et al., 1993a) that low liver taurine levels correlate with a greater susceptibility to carbon tetrachloride-induced hepatic injury.

The aim of the following study was to show whether the co-administration of taurine with alcohol to rats would alter or reduce the pathological and biochemical lesions induced by alcohol (for example hepatic steatosis and lipid peroxidation) after intake of amounts of ethanol equivalent to that consumed by man. The role of modulating CYP2E1 activity and methionine synthase activity in the toxicity was also investigated. The method employed the liquid diet technique defined by Lieber and DeCarli (1989).

Materials and methods

Chemicals

The following compounds were supplied by Sigma Chemical Company (Poole, Dorset, UK):- taurine (synthetic), o-phthaldehyde (OPA: HPLC grade), homoserine, (DL)-homocysteine, S-adenosyl L-methionine (iodide salt), ethanol, sodium dihydrogen phosphate, Dowex resins, ATP (disodium salt), GSH, firefly lantern extract (luciferase), DTNB (5, 5′-dithiobis-2-nitrobenzoic acid) for measurement of total non-protein sulphydryls (TNPSH) and glutathione. Chromotropic acid, used in the determination of triglycerides and prepared fresh from 4,5-dihydroxy-2,7-naphthalene disulphuric acid and Zeolite, activated by heating in an oven overnight at 85°C, were also obtained from Sigma

Chemical Company. Water was of ultra high quality (UHQ), prepared using an Elgastat water system. The AG1-X8 resin (200–400 mesh chloride form) was purchased from Biorad and SBD-F (ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate) was obtained from Fluka. Acetaldehyde was from Aldrich Chemical Company and [^{14}C] MTHF (methyltetrahydrofolate, barium salt) was obtained from Amersham (50 μCi/ml).

Animals

Female Sprague Dawley rats (125–150 g) were obtained from Charles River (UK) and acclimatized for at least 7 days after delivery. Animals were housed in communal cages, fed a rat and mouse maintenance cube diet (691 diet, Quest Nutrition Ltd., Wingham, Kent, UK) and water *ad libitum*. During experiments, animals were housed in individual metabolism cages designed to separate and collect faeces and urine (Techmate Ltd., Milton Keynes, UK) and given powdered diet (691 diet, Quest Nutrition Ltd., Wingham, Kent, UK) and water *ad libitum* prior to introduction of the liquid diet. Lighting was controlled to give a regular 12 h light-dark cycle (8am on-8pm off); room temperature was maintained at 21 ± 2°C. Urine samples (24 h) were collected over ice and diluted to 25 ml with UHQ water, centrifuged (2,000 rpm, 10 min, 4°C) to remove hair and food debris and stored (−80°C) in aliquots for later analysis. The body weight and general condition of the animals were monitored twice weekly and liquid diet intake was determined daily.

Liquid diet technique of ethanol administration

Due to the tendency of animals given alcohol to reduce their solid food consumption, animals were given a liquid diet. Chronic ethanol feeding was achieved by incorporation of ethanol in a nutritionally adequate totally liquid diet obtained from Dyets Inc. (Pennsylvania, USA). The liquid diet provided 1 kcal/ml of which 35% of total calories were derived from fat, 47% from carbohydrates and 18% from protein. Ethanol-treated animals were given diet where maltose dextrin was isocalorically replaced by ethanol. The alcohol provided 36% of the calories (Lieber and DeCarli 1989). Animals were started on the diet at a body weight of 125–150 g and ethanol was introduced progressively with 30 g/L of the liquid diet for 2 days, 40 g/L for the subsequent 2 days followed by the final formula containing 50 g/L (Lieber and DeCarli 1989).

Preparation of diet

As vitamin A degrades when mixed in with other dry ingredients, vitamins and minerals were incorporated into the diet at the time of preparation of the liquid diet. The liquid diet was prepared in cold water using a kitchen-type blender. The diet was kept refrigerated, in the dark and used within one week of preparation.

Pair-feeding

The alcohol-fed animals were allowed liquid diet consumption *ad libitum* and their daily intake was monitored. The control animals were then given the same amount of liquid control diet during the following 24 h feeding period. This pair-feeding process was repeated every 24 h. The technique of daily pair-feeding was adopted to assure a strict caloric intake in both ethanol-treated animals and their individual pair-fed controls (Lieber and DeCarli, 1989).

Study design

Rats (n = 6) were treated with alcohol which was administered in the liquid diet for 28 days. Pair-fed control rats (n = 6) were also provided with the same liquid diet but

without alcohol. A second group of animals (n = 6) received alcohol administered in the liquid diet which also contained 3% taurine. Pair-fed control rats (n = 6) were given the same liquid diet containing 3% taurine but no alcohol. After 28 days of treatment, animals were killed and blood and tissue removed for analysis and microsomes prepared from the liver.

Post-mortem procedure

Animals were exanguinated from the abdominal aorta under anaesthesia (Hypnorm: Hypnovel: water, 1:1:2) and blood samples were collected into microcontainers (Beckton Dickinson) for the separation of serum. After allowing to stand at room temperature for at least 45 mins, the microcontainers were centrifuged (13,000 rpm, 45 secs, MSE minifuge) and stored at −80°C. Serum was analyzed for serum enzymes and biochemical parameters using appropriate kits (Boehringer Mannheim GmbH Diagnostica) with a centrifugal IL Monarch 2000 Instrumentation Laboratory, UK, Ltd. The liver was removed, weighed and approximately 200 mg taken from the right lobe and immediately homogenized in trichloroacetic acid (TCA, 10%, 4 ml, 4°C), frozen in liquid nitrogen and stored at −80°C for subsequent analysis of ATP. Approximately 200 mg liver was also taken from the right lobe and immediately homogenized in sulphosalicylic acid (0.2 M, 2 ml, 4°C), frozen in liquid nitrogen and stored at −80°C for subsequent analysis of taurine, total non-protein sulphydryls (TNPSH) and oxidized glutathione (GSSG).

Biochemical determinations

Taurine

A high performance liquid chromatographic method with fluorimetric detection was used for the determination of taurine in urine, serum and liver tissues essentially by the method of Waterfield (1994). Taurine was derivatized with o-phthaldehyde/2-mercaptoethanol prior to injection onto a C_{18} column. Isocratic elution of the adduct was carried out using NaH_2PO_4 (0.05 M, pH 5.4) in methanol and water (43:57 v/v). Homoserine was used as an internal standard to facilitate the standardization and quantitation of samples. Analysis was completed in 6 min with homoserine and taurine eluting after 3 and 4 min, respectively.

Triglycerides

Hepatic content of triglyceride was determined by a modified method of Butler et al. (1962). Briefly, phospholipids were separated from triglycerides by adsorption on a synthetic Zeolite. The triglycerides were then extracted into chloroform, hydrolysed and measured as esterified glycerol with non-esterified samples used as individual blanks.

Lipid peroxidation

Lipid peroxidation, measured as malondialdehyde production in liver samples, was determined by the method of Sawicki et al. (1963) employing malondialdehyde as standard.

ATP

ATP content of liver samples was determined by luciferase-linked bioluminescence in TCA extracts of liver samples using a firefly lantern extract (Jenner and Timbrel, 1994).

Total non-protein sulphydryls (TNPSH)

Liver TNPSH were measured by the method of Ellman (1959) as a measure of reduced liver glutathione, which constitutes most (>95%) of the liver TNPSH (De Master and Redfern, 1987).

GSSG

Hepatic GSSG was determined by the method of Griffith (1980) using 2-vinylpyridine for derivatization of glutathione.

Microsomal analysis

Microsomes were prepared from livers, essentially as described by Lake (1987). Total cytochrome P-450 content of liver samples was determined by the method of Omura and Sato (1964). 4-Nitrophenol hydroxylase activity was determined by the modified method of Prough et al. (1978). P-Nitrophenol is a substrate for the ethanol-inducible CYP2E1. The method relies on the formation of p-nitrocatechol, which can be detected spectrophotometrically after total ionization under alkaline conditions. The protein content of microsomes was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Homocysteine and cysteine

A high performance liquid chromatographic method with fluorimetric detection was used for determination of total homocysteine and cysteine, (oxidized and reduced) in urine and serum by the method of Fortin and Genest (1995). Homocysteine and cysteine were reduced by 10% tri-n-butylphosphine in dimethylformamide then derivatized with SBD-F (ammonium-7-fluorobenzo-2-oxa-1, 3-diazole-4-sulfonate) at 60°C for 1 hour (stable for 1 week at 4°C), prior to injection onto a C_{18} column. Isocratic elution of the adduct was carried out using sodium acetate (0.1 M), acetic acid (0.1 M) and 2% methanol, pH 4.0. N-acetylcysteine was used as an internal standard to facilitate the standardization and quantitation of samples. Analysis was completed in 14 min with cysteine, homocysteine and N-acetylcysteine eluting after 2.5, 3.5 and 6 min, respectively.

Methionine synthase

Methionine synthase was measured in the liver cytosol essentially as described by Nicolaou et al. (1997). Assay mixtures (total volume 300 µl) contained 50 mM potassium phosphate buffer pH 7.2, 400 µM (DL)-homocysteine, 35 µM SAM, 236 µM MTHF (2658 DPM/nmol), 60 µM hydroxycobalamin, 25 mM DTT (DL-dithiothreitol) and the enzyme source. Incubations were performed in light protected stoppered serum vials under nitrogen. Reaction mixtures were preincubated for 5 mins (37°C), prior to the initiation of the reaction by the addition of homocysteine through a syringe. Incubations (37°C) were performed for 45 mins. The enzyme reaction was terminated by the addition of 400 µl ice cold water and solutions immediately passed through a 0.5 × 5.0 cm column of Bio-Rad AG1-X8 resin. [^{14}C]Methionine was eluted with 2 ml of water, collected and quantitated by scintillation spectrometry. Protein concentrations were determined with the Bio-Rad protein assay based on the method of Bradford (1976) with bovine serum albumin as standard.

Urinary protein

Urinary protein was measured by the Coomassie Plus Protein assay kit supplied by Pierce and Warriner (Chester, UK).

Acetaldehyde

Acetaldehyde in the liver and serum was determined by the method of McCloskey and Mahaney (1981).

Histology

Tissues were fixed in 10.5% (v/v) phosphate-buffered formalin (pH 7.2). Frozen liver sections from fixed tissues (10 μm) were cut and stained for lipid with Oil red O in triethylphosphate with Mayer's haematoxylin as counter stain.

Statistical analysis

Statistical evaluation of data was performed by Duncan's multiple range test to make comparisons between groups. Values quoted are means ± SEM of 6 animals. The level of significance was set at 0.05 or less.

Results

Liquid diet consumption, ethanol intake, urine volumes and urinary protein

There was a gradual decrease in liquid diet consumption as alcohol was progressively increased into the liquid diet (Fig. 3a) after which time liquid diet intake remained relatively constant throughout the treatment period. A high ethanol intake of 13 g/kg (2 g/rat/day) by the alcohol treated animals and 12 g/kg (1.8 g/rat/day) by alcohol + taurine treated animals (Fig. 3b) was achieved. Although the alcohol intake in animals given alcohol alone was significantly higher on two days than those receiving alcohol + taurine, the overall intake was for the most part very similar. After administration of the liquid diet, the urine volume of all animals was increased. However, the urinary volumes did not change during the 28 days of ethanol administration (data not shown). There was no effect of alcohol on urinary protein excretion, although animals given taurine excreted significantly more protein (Fig. 3c).

Taurine in the diet, urine, liver and serum

Analysis of taurine in various diets revealed that taurine was present in the powdered diet that was provided during the predose period (4 μmol/g). However, no taurine was detectable in the purified control and alcohol liquid diet. Thus, the incorporation of 3% taurine in the liquid diet significantly raised taurine levels in the diet. During 28 days of alcohol treatment, there was no effect of alcohol (alone) on urinary taurine excretion (Fig. 4a). However, animals consuming the taurine diet excreted significantly more compared to animals on the taurine-free diet (Fig. 4a). There was no effect of alcohol on liver and serum taurine levels (Fig. 4b). However, in the taurine treated animals, there were significantly greater levels of liver taurine (3 fold) and serum taurine (2 fold) levels compared to control animals. Interestingly, both liver taurine

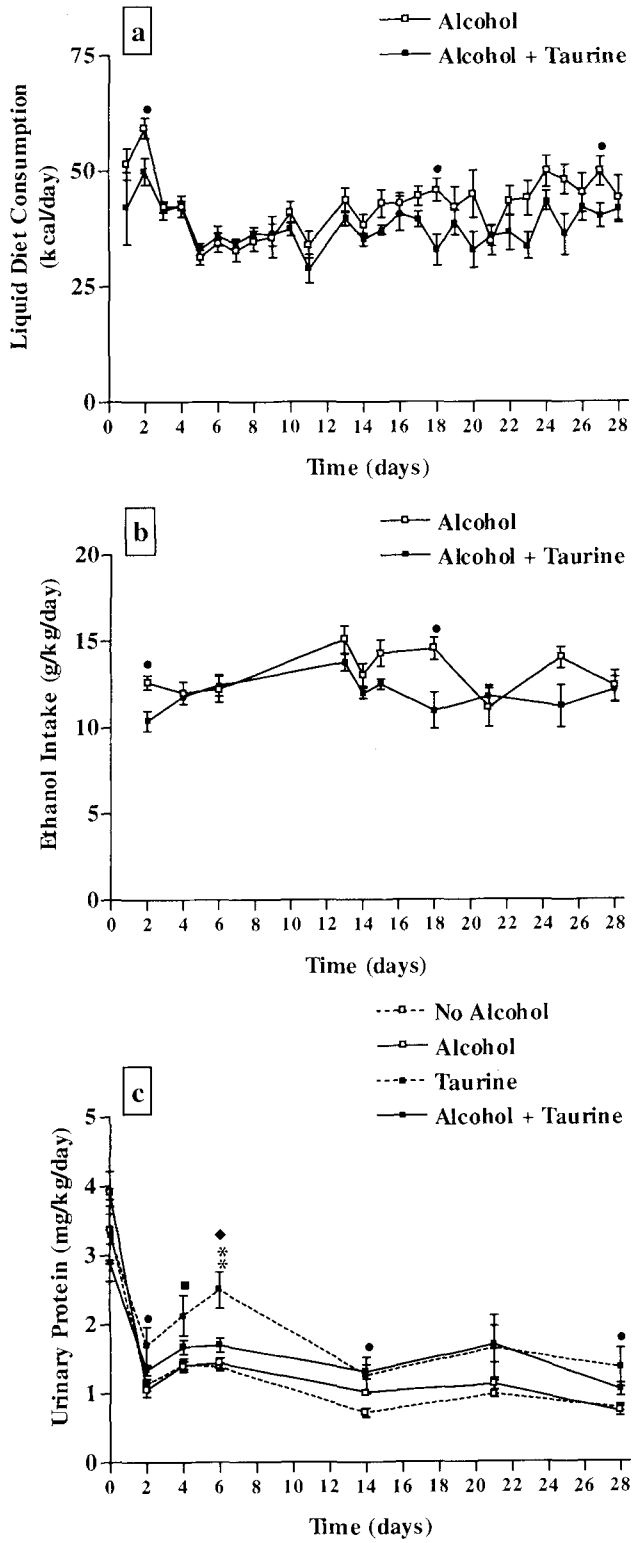

Fig. 3. Liquid diet consumption (**a**), ethanol intake (**b**) and urinary protein (**c**) of animals during 28 days of ethanol administration. Results are expressed as means ± SEM of 6 animals. Asterisks indicate values significantly different between treated and pair-fed controls (**$p < 0.01$). Symbols indicate values significantly different between the no alcohol treated and taurine treated group and between the alcohol treated and alcohol + taurine treated group ($^{\bullet}p < 0.05$, $^{\blacksquare}p < 0.01$, $^{\blacklozenge}p < 0.001$)

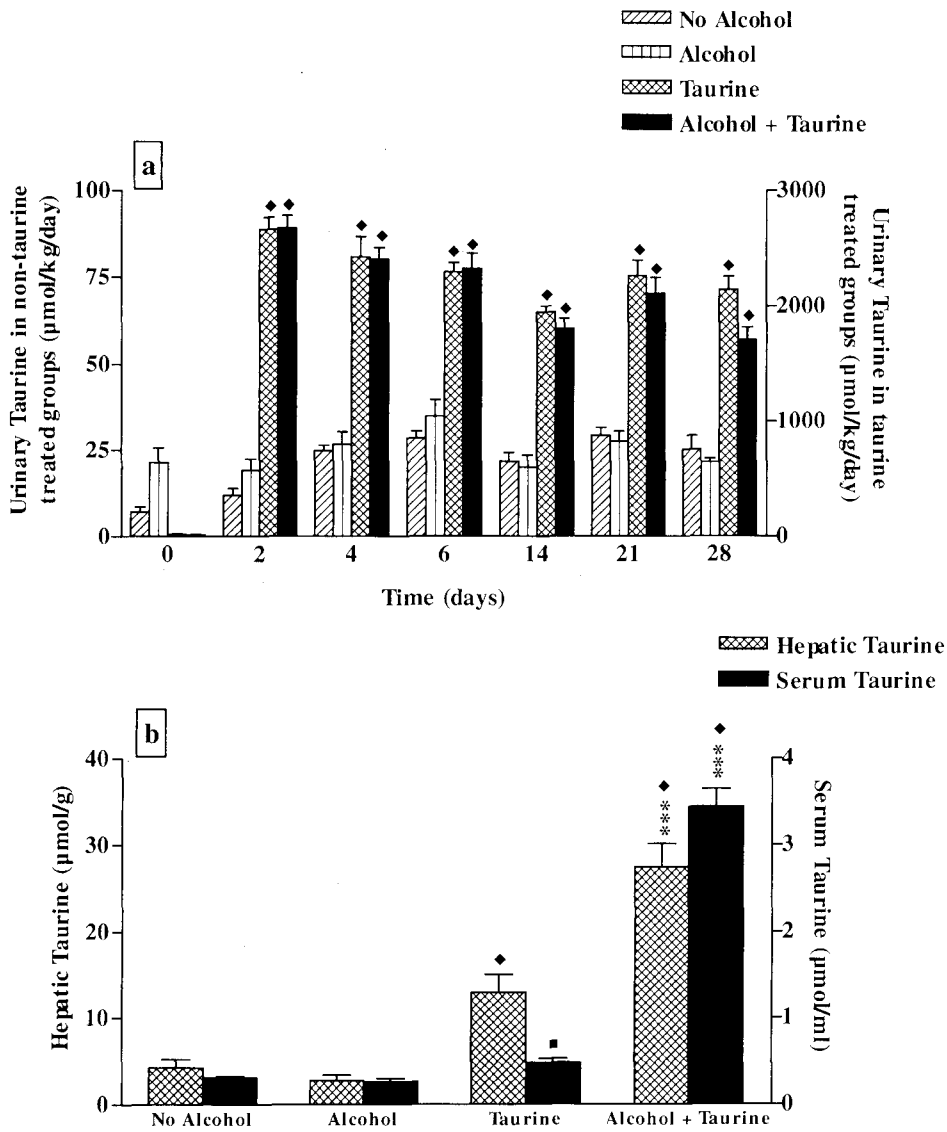

Fig. 4. Levels of urinary taurine (**a**) and hepatic and serum levels of taurine (**b**) in rats after 28 days of ethanol administration. Results are expressed as means ± SEM of 6 animals. Symbols indicate values significantly different between the treated and between the pair-fed controls (■$p < 0.01$, ◆$p < 0.001$). Asterisks indicate values significantly different between alcohol + taurine treated and pair-fed controls (***$p < 0.001$). N. B. use of two different scales

(2 fold) and serum taurine (7 fold) levels were markedly raised in the alcohol + taurine treated animals compared to taurine treated animals alone.

Body weight and post-mortem

Figure 5a shows the gain in body weight in all animals during the 28 days of liquid diet consumption. There was no significant difference in body weights

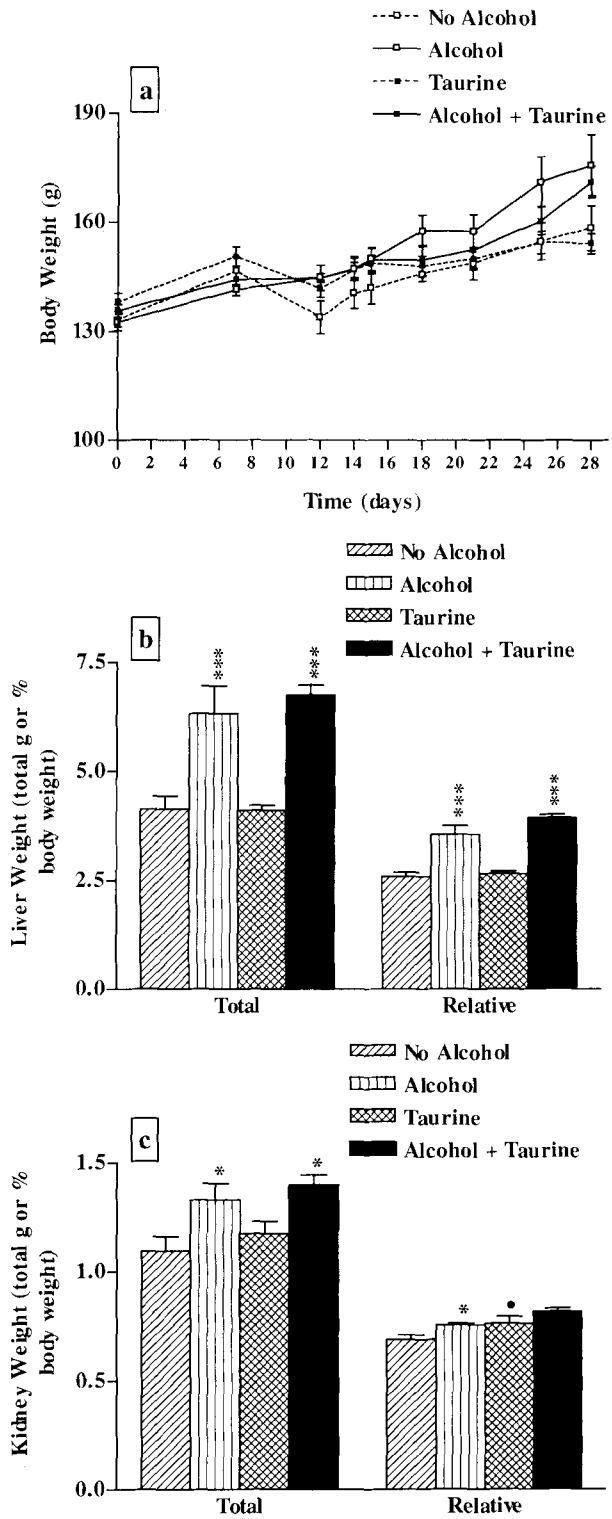

Fig. 5. The effect of ethanol on body weight (**a**), total and relative liver weights (**b**) and total and relative kidney weights (**c**). Results are expressed as means ± SEM of 6 animals. Asterisks indicate values significantly different between treated and pair-fed controls (*$p < 0.05$, ***$p < 0.001$). Symbol indicates values significantly different between the no alcohol treated group and taurine treated group (•$p < 0.05$)

of animals between groups during the treatment period. Both total and relative liver weights (Fig. 5b) and total and relative kidney weights (Fig. 5c) were significantly raised in the alcohol and alcohol + taurine treated animals compared to the pair-fed controls. There was no significant difference in total and relative liver weights between the alcohol and alcohol + taurine treated animals.

Serum analysis

Serum alkaline phosphatase (ALP, a marker of bile duct damage) and serum bile acids (indicating early cholestasis) were significantly raised by alcohol and alcohol + taurine treatment (Fig. 6). Serum cholesterol was also significantly raised by alcohol treatment and alcohol + taurine treatment. Alanine aminotransferase (ALT), a marker of hepatic injury was raised with alcohol and alcohol + taurine treatment (Table 1). Serum urea was significantly raised in the taurine treated and alcohol + taurine treated animals compared to the control and alcohol treated groups, respectively. However, alcohol and alcohol + taurine treatment significantly reduced total bilirubin, but serum albumin was reduced with alcohol + taurine treatment only. There was no effect on serum aspartate aminotransferase (AST).

Histological analysis, liver and serum triglyceride, and lipid peroxidation

Histological examination of liver tissue showed that animals treated with alcohol (Fig. 7c) had developed a marked steatosis compared to pair-fed controls (Fig. 7a). The fat accumulation in animals given alcohol +

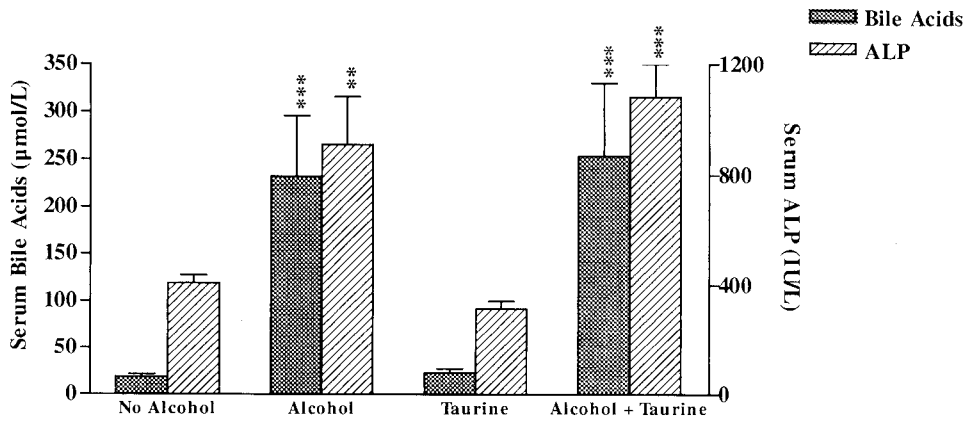

Fig. 6. Levels of serum bile acid and alkaline phosphatase (*ALP*) in rats after 28 days of alcohol administration. Results are means ± SEM of six animals. Asterisks indicate values significantly different between treated and pair-fed controls (**$p < 0.01$ and ***$p < 0.001$)

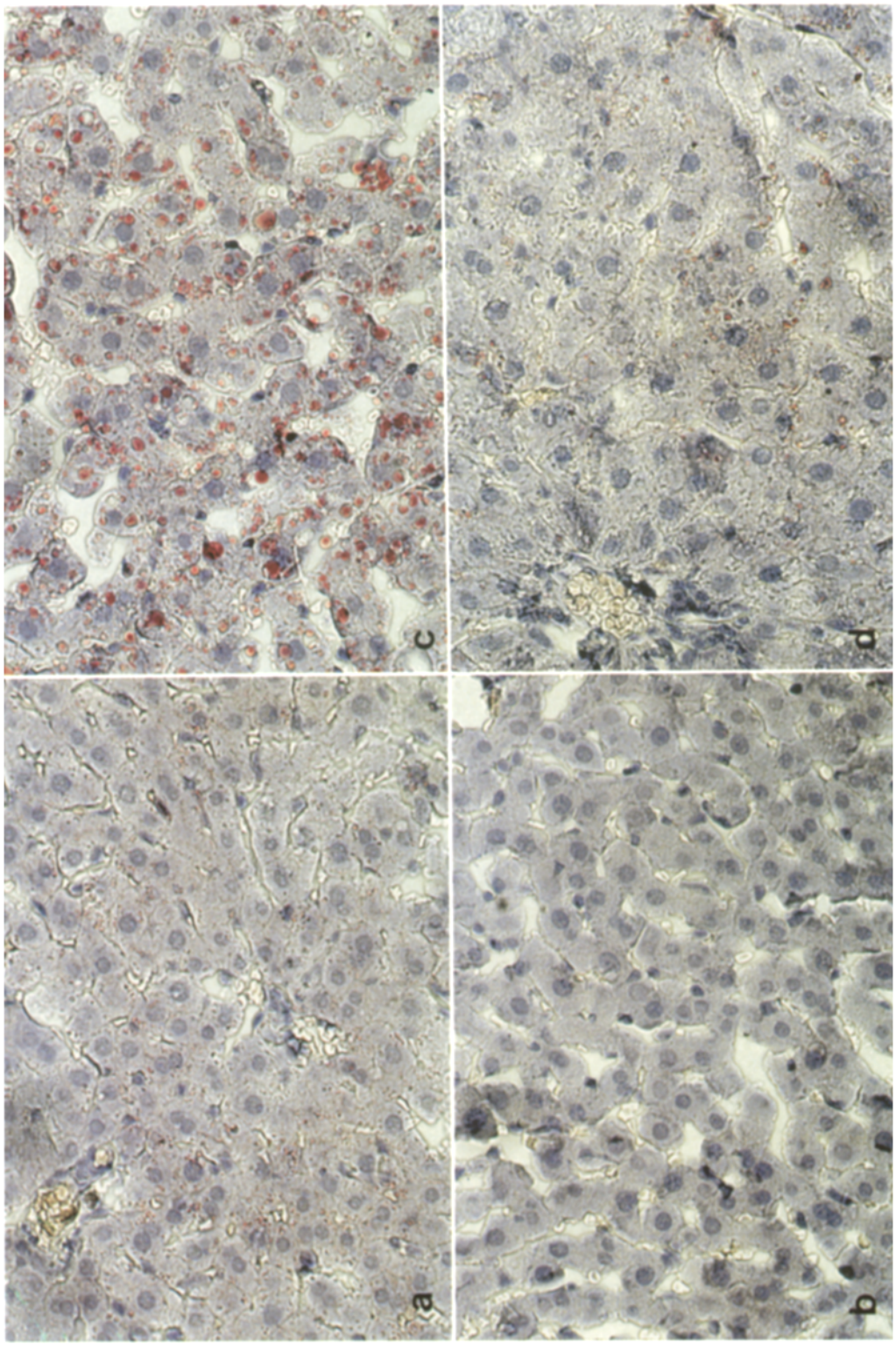

Fig. 7. Frozen sections (X40) taken from (**a**) control, (**b**) taurine alone, (**c**) alcohol and (**d**) alcohol + taurine treated animals. Frozen sections have been stained with Oil red O and counterstained with Mayer's haematoxylin. Lipid droplets are stained red

Table 1. The effect of ethanol and taurine on the serum chemistry after 28 days of ethanol administration

Treatment	No Alcohol	Alcohol	Taurine	Alcohol + Taurine
ALT IU/L	31.13 ± 2.91	60.05 ± 7.26 ***	19.23 ± 0.73	70.32 ± 3.66 ***
AST IU/L	83.00 ± 3.27	86.88 ± 12.59	69.47 ± 6.65	84.98 ± 7.82
Alb g/L	32.88 ± 0.71	31.35 ± 0.46	33.53 ± 0.55	30.83 ± 0.57 **
T-BIL μmol/L	2.80 ± 0.21	2.03 ± 0.38 *	2.75 ± 0.13	2.03 ± 0.16 *
Urea mmol/L	4.19 ± 0.30	4.62 ± 0.14	5.21 ± 0.32 •	5.92 ± 0.38 ■
Creat μmol/L	56.83 ± 1.72	47.83 ± 2.77 **	57.67 ± 1.41	47.67 ± 1.15 **
Trig mmol/L	0.30 ± 0.02	0.91 ± 0.16 **	0.30 ± 0.02	1.06 ± 0.16 ***
Chol mmol/L	1.87 ± 0.15	2.79 ± 0.17 ***	1.87 ± 0.08	2.72 ± 0.20 **

Results are expressed as means ± SEM of 6 animals. Asterisks indicate values significantly different between treated and pair-fed controls groups (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Symbols indicate values significantly different between no alcohol treated group and taurine treated group and between alcohol and alcohol + taurine treated groups (•$p < 0.05$, ■$p < 0.01$). All other values are not significantly different. *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *Alb* albumin; *T-BIL* total bilirubin; *Creat* creatinine; *Trig* triglyceride; *Chol* cholesterol.

taurine (Fig. 7d) was greater than control animals and taurine treated animals (Fig. 7b), but less than in animals treated with alcohol alone. No method of scoring fat accumulation was used as triglycerides were also measured biochemically. It appeared that the lobular distribution of hepatic fat may have been altered in the alcohol + taurine treated animals, with fat being relocalized from the mid zonal region to the area around centrilobular veins. Alcohol significantly raised hepatic triglyceride (Fig. 8) whereas alcohol + taurine treatment resulted in a significant reduction in the triglyceride accumulation compared to alcohol treatment alone (approximately 20%). Serum triglyceride levels were raised 3 fold by alcohol (Table 1) but raised significantly more by alcohol treatment combined with taurine (4 fold), although the difference between alcohol and alcohol + taurine treated animals was not significant. Alcohol treatment caused a significant 2 fold increase in lipid peroxidation, which was absent in the alcohol + taurine treated animals in which malondialdehyde levels were similar to control values (Fig. 8). Thus, there was a highly significant difference in malondialdehyde levels between the alcohol and alcohol + taurine treated animals.

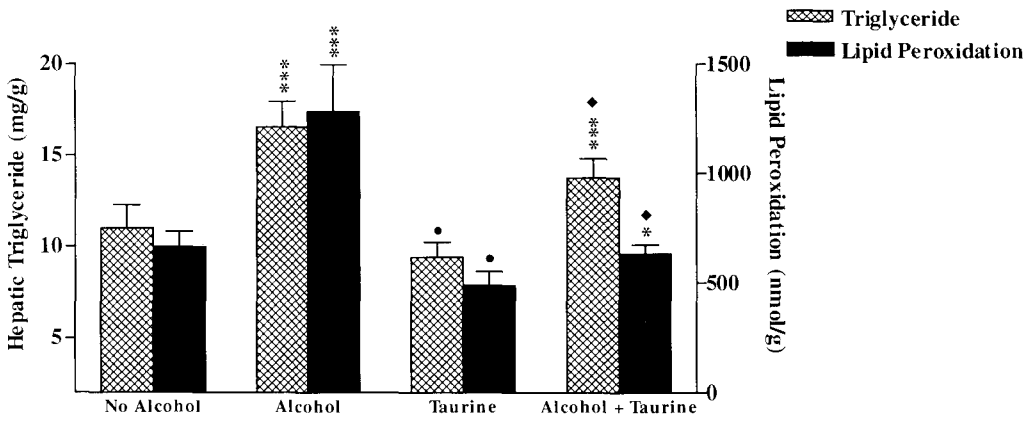

Fig. 8. Lipid peroxidation and accumulation of hepatic triglycerides in rats after 28 days of ethanol administration. Results are expressed as means ± SEM of 6 animals. Asterisks indicate values significantly different between treated and pair-fed controls (*$p < 0.05$, ***$p < 0.001$). Symbols indicate values significantly different between the no alcohol treated group and taurine treated group (•$p < 0.05$) and between alcohol treated and alcohol + taurine treated groups (◆$p < 0.001$)

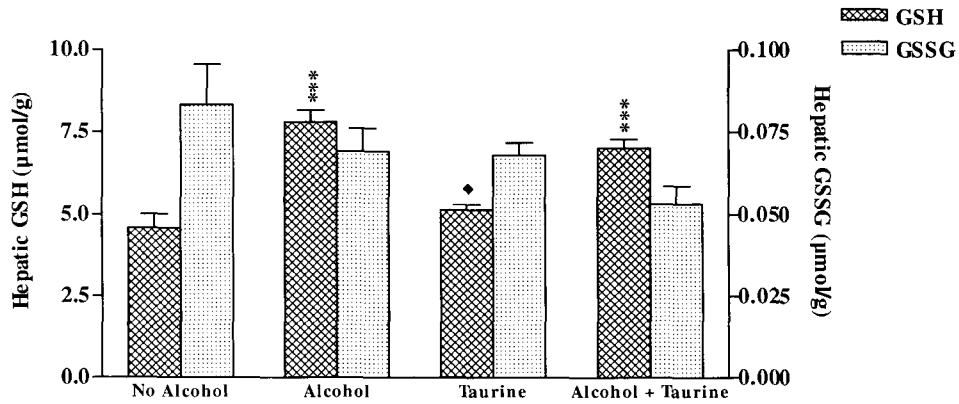

Fig. 9. Changes in hepatic levels of GSH and GSSG. Results are expressed as means ± SEM of 6 animals. Asterisks indicate values significantly different between treated and pair-fed controls (***$p < 0.001$). Symbol indicates values significantly different between the no alcohol treated group and taurine treated group (◆$p < 0.001$)

Hepatic ATP, GSH and GSSG, and microsomal analysis

No difference was detected in the levels of ATP (data not shown) or GSSG between groups after 28 days of alcohol administration (Fig. 9). However, alcohol and alcohol + taurine treatment significantly raised levels of GSH compared to pair-fed controls (Fig. 9). Also, animals given taurine alone had slightly but significantly higher levels of GSH compared to control animals. A 2 fold induction of 4-nitrophenol hydroxylase activity was observed with alcohol treatment (Fig. 10). However, in the alcohol + taurine treated animals, CYP2E1 activity was barely detectable, the levels being 18 fold lower than pair-fed controls and 30 fold lower than alcohol alone treated animals.

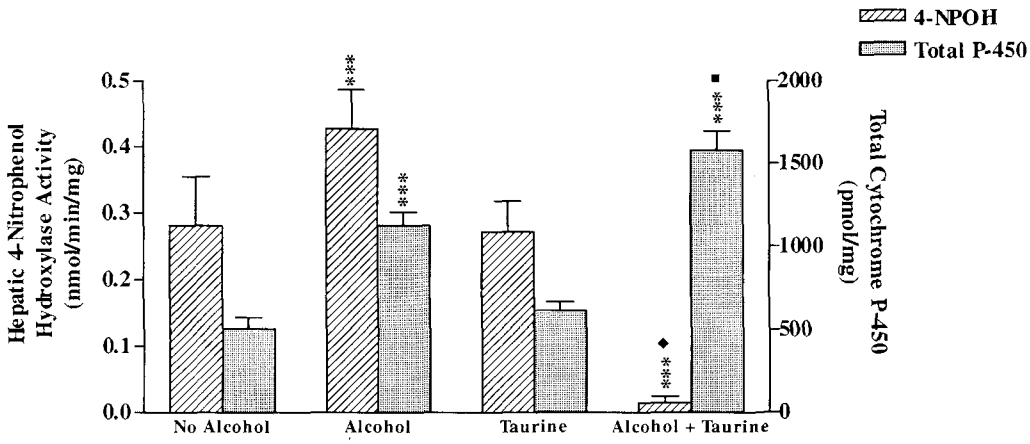

Fig. 10. The effect of ethanol on hepatic 4-nitrophenol hydroxylase activity and total cytochrome P-450 levels. Abbreviations:- 4-NPOH (4-nitrophenol hydroxylase activity). Results are expressed as means ± SEM of 6 animals. Asterisks indicate values significantly different between treated and pair-fed controls (***$p < 0.001$). Symbols indicate values significantly different between the alcohol treated and alcohol + taurine treated group (■$p < 0.01$, ◆$p < 0.001$)

Total cytochrome P-450 was significantly raised by alcohol treatment (2 fold) and more so with alcohol + taurine treatment (3 fold, Fig. 10). There was a statistically significant difference in total cytochrome P-450 levels between alcohol and alcohol + taurine treated animals, the alcohol alone treated animals being lower.

Serum and urinary homocysteine and cysteine and hepatic methionine synthase

From day 2 of treatment (30g/L alcohol), urinary homocysteine levels were raised significantly in the alcohol and alcohol + taurine treated animals compared to the pair-fed controls (Fig. 11a). The higher levels of urinary homocysteine were maintained in these animals throughout 28 days of alcohol treatment. However, alcohol did not affect urinary cysteine levels (Fig. 11b). Although there was no effect of alcohol on levels of serum homocysteine, alcohol and alcohol + taurine treatment significantly reduced levels of serum cysteine (Fig. 11c). Animals treated with alcohol and alcohol + taurine had significantly inhibited hepatic methionine synthase activities compared to pair-fed controls (Fig. 11d). There was no statistical difference in methionine synthase activities between alcohol and alcohol + taurine treated animals.

Discussion

This study aimed to evaluate the protective properties of taurine against adverse alcohol-induced biochemical changes. The liquid diet containing

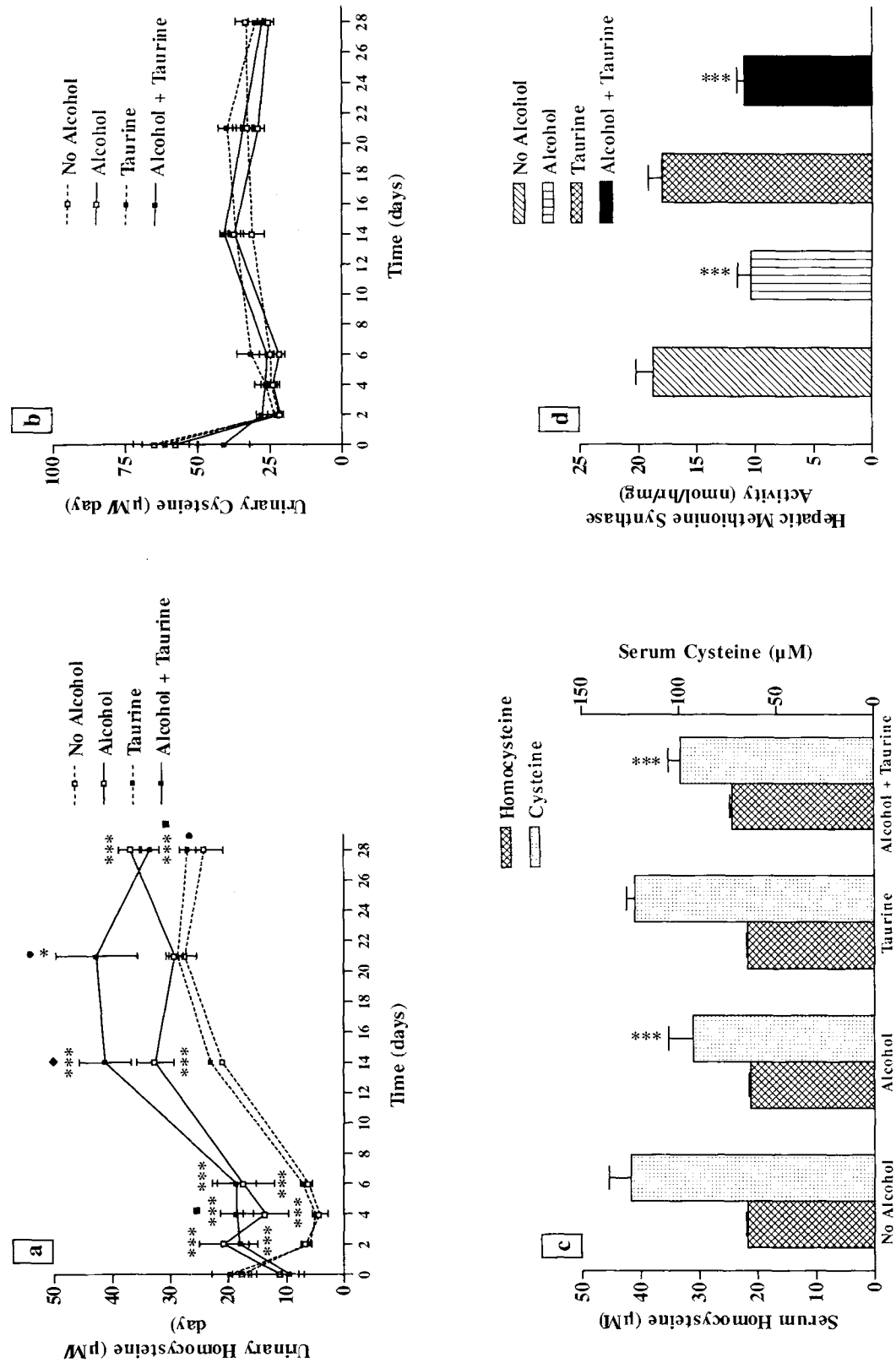

Fig. 11. The effect of alcohol on levels of urinary homocysteine (**a**), urinary cysteine (**b**), serum homocysteine and cysteine (**c**) and hepatic methionine synthase activities (**d**) in rats. Asterisks indicate values significantly different between treated and pair-fed controls (*$p < 0.05$, ***$p < 0.001$). Symbols indicate values significantly different between the no alcohol treated and taurine treated group and between the alcohol treated and alcohol + taurine treated group ($\bullet p < 0.05$, $\blacksquare p < 0.01$, $\blacklozenge p < 0.001$)

alcohol resulted in the accumulation of liver triglycerides and thus provided a model for alcoholic fatty liver as has been previously reported (Lieber and DeCarli, 1989).

The isocaloric pair-feeding resulted in a similar weight gain in all animals. Although animals receiving alcohol appeared to gain more weight than animals receiving alcohol-free diets, the difference was not significant.

The intake of alcohol in the present study was equivalent to approximately 1 kg/day in man and falls within the range of 12–18 g/kg that should be achieved by the liquid diet technique according to Lieber and DeCarli (1989).

The raised serum ALT and ALP in animals treated with alcohol and alcohol + taurine indicated slight parenchymal and bile duct damage, respectively by alcohol. There was an indication of cholestasis as shown by the raised levels of serum bile acids in alcohol (Vendemiale and Lieber 1984) and alcohol + taurine treated animals but total bilirubin was not raised. Taurine has been previously shown to increase bile flow (Fukaya et al., 1996), but this study suggested that this effect may have been blocked in the presence of alcohol. The increase in total and relative liver weights is most likely to be due to fat accumulation.

The raised serum urea in the taurine and alcohol + taurine treated animals indicated that taurine may have increased protein degradation, reduced protein synthesis or affected kidney function. Also, the increased total and relative kidney weights added further evidence of an effect of alcohol alone on the kidneys (Tsuboi et al., 1997). However, urinary protein remained below starting levels suggesting that there was no overt kidney damage and that protein synthesis may have been reduced.

The most common disturbance of lipid metabolism produced by ethanol ingestion is fatty liver. This is characterized by accumulation of triglycerides in the hepatocytes as cytoplasmic lipid droplets and develops progressively over the first month of ethanol administration in the rat (Lieber and Savolainen 1984). The interaction of ethanol with lipid metabolism is complex. When ethanol is present, it becomes a preferred fuel for the liver and displaces fat as a source of energy, which favours fat accumulation. Also there is an altered redox state as a result of ethanol oxidation (Fig. 1), which promotes lipogenesis (Lieber and Savolainen 1984). The triglycerides may accumulate in the liver because of decreased fatty acid oxidation, enhanced fatty acid and triglyceride synthesis, or a combination of these mechanisms. The origin of the fatty acids accumulating in the liver, however, remains the subject of much debate (Trimble et al., 1993). It has been shown that both in man (Lieber and DeCarli, 1989 and Schapiro et al., 1965) and in rats (Horning et al., 1963), large doses of alcohol produce an increase in circulating blood lipids, as was observed in this study.

The high serum triglyceride levels in the alcohol + taurine treated animals suggests that triglycerides were either secreted out of the liver (Yan et al., 1993) or that extrahepatic sources were being mobilized. However, in the alcohol + taurine treated animals, the increased concentration of plasma triglyceride could be a consequence of increased hepatic release of

lipoproteins in these animals (or increased mobilization from adipose tissue). This could have contributed to reduced hepatic triglyceride accumulation in the alcohol + taurine treated animals. The decrease in plasma triglyceride concentration in alcohol treated animals, suggest, indirectly, that decreased hepatic release of lipoproteins plays a primary role in the pathogenesis of the fatty liver produced by chronic alcohol intake. Thus, both alcohol and alcohol + taurine treatment could result in the same amount of increased triglyceride synthesis in the liver, but that taurine limits the accumulation of these triglycerides.

Ethanol metabolism is also associated with the generation of reactive oxygen species, which are capable of initiating or enhancing peroxidation of polyunsaturated fatty acids, the associated membrane damage being a key feature of alcoholic liver injury (Shaw et al., 1988). The increased capacity to produce reactive oxygen species by microsomes or liposomes enriched in CYP2E1, was shown by Ekstrom and Ingelman-Sundberg (1989) to increase the rate of microsomal lipid peroxidation. Lipid peroxidation has also been linked to the increased production of reactive oxygen intermediates by Lieber (1997b) and Reinke et al. (1987) who have demonstrated increased lipid peroxidation in other models of chronic ethanol feeding. Lipid peroxidation not only results from the increased oxygen free radical production by the induced CYP2E1, but also from the enhanced generation of acetaldehyde which causes lipid peroxidation in isolated perfused livers (Müller and Sies, 1982).

Lieber (1997b) reported that ethanol increased lipid peroxidation in the liver could be prevented by antioxidants such as polyenylphosphatidylcholine. Taurine has previously been demonstrated to have protective properties against lipid peroxidation caused by other compounds, such as carbon tetrachloride (Nakashima et al., 1983). In the present study, alcohol was shown to cause lipid peroxidation (measured as malondialdehyde). However, this was completely prevented by the co-administration of taurine.

Lipid peroxidation can also be promoted when GSH is depleted. Binding of acetaldehyde with cysteine and/ or GSH may contribute to GSH depression in the liver (Shaw et al., 1981). Because GSH is able to scavenge toxic free radicals, the depletion of GSH may contribute to lipid peroxidation. However, in the study reported here, there was an alcohol-induced increase in GSH. Fernández-Checa et al. (1993) have shown that GSH depletion precedes steatosis and lipid peroxidation, and have suggested that the depletion of GSH could be a contributing factor in the development of alcoholic liver disease. It is possible that GSH depletion occurred earlier in this study and that by 28 days GSH levels were raised as a result of a) rebound synthesis in GSH, b) conversion of homocysteine (which was raised) to GSH, or c) mild cholestasis (Seabra and Timbrell 1997). Hepatic levels of GSSG, a marker of oxidative stress, were not significantly changed. There was no apparent effect of alcohol on hepatic levels of ATP although ATP levels may have been affected at an earlier time point. Many hepatotoxic compounds which cause hepatic steatosis, such as hydrazine, carbon tetrachloride and cyclohexamide have been shown to raise urinary taurine levels (Waterfield et al., 1993b,

1996). However, chronic alcohol administration did not change urinary taurine levels. Thus, urinary taurine was not a useful marker for alcohol-induced liver steatosis. These findings suggest that the mechanism of fatty liver production may be an important factor in determining urinary taurine levels, and may not be the same in all cases.

Levels of both liver and serum taurine were raised by taurine treatment as expected but this was markedly greater in animals treated with alcohol as well as taurine, but not after alcohol treatment alone. Excess taurine is normally excreted into the urine. However, there was no greater increase in urinary taurine in rats treated with taurine alone compared with those treated with alcohol + taurine. Thus, the greater body burden of taurine in rats given taurine with alcohol did not result in increased excretion of taurine. This could possibly be indicative of kidney dysfunction as all taurine treated animals were excreting high levels of taurine in the urine. Furthermore, both total and relative kidney weights were raised in the alcohol and alcohol + taurine treated animals.

Alcohol may have had an effect on bile flow resulting in the accumulation of taurine in the liver and serum (Seabra and Timbrell, 1997). Liver and serum taurine were raised by approximately the same proportion as urinary taurine was decreased in the alcohol + taurine treated group (compared to taurine alone). Thus, alcohol may reduce the elimination of excess taurine.

Homocysteine is a sulphydryl-containing amino acid that is formed by the demethylation of methionine and is normally metabolized to cysteine or re-methylated to methionine (Fig. 2). Homocysteine may be considered to be a toxic by-product of normal methionine metabolism. The initial reduction in urinary homocysteine in the control and taurine treated groups can be explained by the higher amounts of methionine present in the powdered diet (4.7 g/L), compared to the liquid diet (0.3 g/L). Taurine did not protect against the alcohol-induced increase in homocysteine and the inhibition of methionine synthase. Thus, taurine may not protect against the cardiovascular effects of alcohol (Trimble et al., 1993). The methionine cycle results in the synthesis of up to 30% of phosphatidylcholine which is used to synthesize lipoproteins used in the transportation of triglycerides out of cells. Thus, a reduction in methionine synthesis offers another explanation for the alcohol-induced fatty liver.

The protection of alcohol-induced liver dysfunction, by taurine may have been related to the dramatic reduction of CYP2E1 activity. The alcohol-inducible CYP2E1 was induced 2 fold following alcohol treatment. Taurine completely prevented this induction and reduced values 18 fold lower than controls. This is consistent with Watanabe et al. (1985) who observed the lowering of liver acetaldehyde by taurine in acute studies with alcohol. CYP2E1 is one pathway responsible for the conversion of alcohol to acetaldehyde, which produces many of the alcohol related adverse effects i.e. glutathione depletion, free-radical mediated toxicity, lipid peroxidation and hepatic collagen synthesis. However, acetaldehyde in the liver and serum was not detectable in this study by the method used, probably as a result of its

volatility and its capacity to form protein adducts. It is known that the activity of CYP2E1 depletes the cells of NADPH (Harrison and Burt, 1993) which results indirectly in increased synthesis and accumulation of triglyceride. Thus, an attractive hypothesis to explain the protective properties of taurine co-administration against alcohol-induced fatty liver is associated with the inhibition of CYP2E1. In the present study, the reduced activity of CYP2E1 in the alcohol + taurine treated animals was probably due to the raised levels of serum bile acids. Raised serum ALP and bile acids indicate that slight cholestasis had occurred. In the alcohol + taurine treated animals, the high level of taurine would be preferentially conjugated (rather than glycine) with bile acids such as cholic acid to form taurocholic acid. Taurocholic acid has been reported to be a potent inhibitor of microsomal enzymes (Chen and Farell, 1996) as are chenodeoxycholic acid and deoxycholic acid. Chen et al. (1995) have also shown that CYP2E1 activity is down regulated after bile duct ligation (which results in the accumulation of bile acids). Evidence in favour of this is well illustrated by Kawata et al. (1987), who have shown that the selective reduction of cytochrome P-450 plays a major role in the impairment of microsomal drug oxidation during intrahepatic cholestasis.

Thus, in the present study, the protective effects of taurine against steatosis and lipid peroxidation could be attributed to inhibition of CYP2E1. This would not only prevent the depletion of NADPH by CYP2E1 (reducing steatosis), but also decrease the formation of the toxic metabolite acetaldehyde (reducing lipid peroxidation). In a similar way, Morimoto et al. (1993) have shown diallyl sulphide to be effective in reducing the levels of microsomal CYP2E1 and lipid peroxidation. Although CYP2E1 was reduced in the present study with alcohol + taurine treatment, the total cytochrome P-450 content in the animals was actually greater in this group than animals given alcohol alone. The increase in total cytochrome P-450 could be due to induction of other cytochromes P-450, the likely targets being CYP2B1 (Albano et al., 1991) and/ or CYP4A1 (Lieber, 1997a). It is known that other cytochrome P-450's also participate in the oxidation of ethanol.

This study demonstrated the female rat to be a good model for alcohol-induced fatty liver in a period of 28 days. The findings are the first to demonstrate that taurine co-administration with alcohol can protect against ethanol-induced fatty liver and lipid peroxidation. The protective effects of taurine were attributed to CYP2E1 inactivation. In view of the fact that humans have a limited capacity to biosynthesize taurine, the use of taurine as a dietary supplement with alcohol has the potential to become a promising therapeutic agent in the treatment of alcoholic liver disease.

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