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The protective effect of taurine pretreatment on carbon tetrachloride-induced hepatic damage – A light and electron microscopic study*

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Summary. The results regarding taurine pretreatment on CCl_4-induced hepatic injury are controversial. To assess the therapeutic efficacy of taurine on rat liver injury, hepatic malondialdehyde, glutathione, and hydroxyproline levels together with morphologic alterations in the liver following CCl_4 administration were investigated. The rats were divided into three groups. Taurine-treated animals received 15 ml/kg/day of a 5% taurine solution by a gastric tube for 5 days before administering CCl_4 (2 ml/kg, intraperitoneally, in a single dose). CCl_4-treated rats received the same amount of saline solution. Control animals received no treatment. The increase of hepatic malondialdehyde formation in the CCl_4-treated group was partially prevented by taurine pretreatment, but taurine had no significant effect on the glutathione and hydroxyproline content in the CCl_4-treated rats. Taurine pretreatment induced a marked beneficial effect regarding the prevention of hepatocellular necrosis and atrophy as demonstrated morphologically. In conclusion, these results suggest that taurine pretreatment might not significantly change the biochemical parameters, but prevents the morphologic damage caused by CCl_4 in the early stages.

Keywords: Taurine – Liver – Carbon tetrachloride – Malondialdehyde – Glutathione – Hydroxyproline – Amino acids – Light and electron microscopy

Introduction

Taurine (2-aminoethanesulfonic acid) is an unusual amino acid found in a wide variety of animal species. It is considered to play several important

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physiological functions in each organ. Recently, evidence has accumulated supporting the hypothesis that taurine protects cellular membranes against toxic compounds, including haloalkanes, bile acids, xenobiotics, and oxidants (Huxtable, 1992; Timbrell et al., 1995; Seabra et al., 1997). Carbon tetrachloride (CCl_4) is a toxic agent acting via a reactive free radical metabolite produced in the smooth endoplasmic reticulum which causes lipid peroxidation (Poli et al., 1981; Recnagel et al., 1989). Breakdown of lipids, particularly unsaturated phospholipids in the intracellular and the plasma membranes increases membrane permeability. The beneficial effects of taurine against oxidant induced tissue injury have been attributed to its ability to stabilize biomembranes (Huxtable, 1992; You et al., 1998) and scavenge reactive oxygen derivatives including hypochlorous acid (Wright et al., 1986; Marquez et al., 1994; Marcinkiewicz et al., 2000).

It has been shown that the administration of taurine provides a protective effect in both in vitro and in vivo settings in the liver. Nakashima et al. (1982) found that taurine administration after exposure CCl_4 protected the liver against lipid peroxidation, as measured by malondialdehyde (MDA) production. In addition, preincubation of the liver microsomes in vitro with very high concentrations of taurine (70 mM) reduced the lipid peroxidation caused by CCl_4. However, the same authors reported that the pretreatment with taurine (3% aqueous solution, p.o. for 3 days) actually facilitated lipid peroxidation (as shown by increased MDA levels) and significantly enhanced the hepatocellular damage (as evidenced by marked necrosis of hepatocytes) in the rat liver after CCl_4 administration (Nakashima et al., 1983).

Conversely, depletion of liver taurine in rats increased their susceptibility to the hepatotoxicity of CCl_4 (Waterfield et al., 1993a; Timbrell et al., 1995). Recently, Zhou et al. (1996) demonstrated that taurine administration in drinking water to rats inhibited hepatocellular degeneration, necrosis, and DNA damage induced by CCl_4.

Consequently, there is no agreement about the role of taurine pretreatment on the CCl_4-induced hepatotoxicity. The present study was therefore carried out to investigate the effect of taurine pretreatment on the CCl_4-induced liver injury by determining the morphological and biochemical parameters.

Materials and methods

This study was performed on male Wistar rats weighing 150–200 g. Rats were divided into three groups. In the control group, animals received no treatment. In the taurine-treated group, taurine (5% aqueous solution, 15 ml/kg/day) was administered using a gastric tube for 5 days before CCl_4 administration. In the CCl_4-treated group, rats received the same amount of saline solution before CCl_4 dosing. CCl_4 was administered intraperitoneally in a single dose (2 ml/kg) on day 6. The rats were sacrificed at 24 hours after CCl_4-treatment by an overdose of pentobarbital sodium anaesthesia. The livers were excised; rinsed in saline; and then dried. The livers were then divided for biochemical and morphologic analysis. The specimens for biochemical analysis were frozen immediately in liquid nitrogen.

Liver MDA levels were determined by the method of Uchiyama and Mihara (1978). The breakdown product 1,1,3,3-tetraethoxypropane was used as standard and the results were expressed as nmol MDA/g tissue.

Total liver nonprotein sulfhydryl groups (TNPSH) were assayed by the method of Ellman (Elmann, 1959). This was used as a measure of reduced liver glutathione, which constitutes most (>95%) of the liver TNSPH. Results were expressed as μmol GSH/g tissue.

Liver hydroxyproline levels were measured by the method of Woessner (Woessner, 1961). The hydroxyproline values were determined by using the L-hydroxyproline standard curve and given as μg hydroxyproline/mg tissue.

Liver tissue samples were obtained for morphologic examination. For light microscopy, tissues were fixed in 10% buffered formalin and embedded in paraffin. The sections were stained with both haematoxylin and eosin (HE). For electron microscopy, tissues were fixed with gluteraldehyde and osmium tetroxide. The thin sections were stained with uranyl acetate and lead citrate.

Statistical analysis

The data are shown as mean ± SD. Differences among groups were analyzed by using the one way ANOVA and Mann-Whitney U tests. P value of <0.05 was considered as significant.

Results

Liver MDA, GSH and hydroxyproline levels of all groups are shown in Table 1.

MDA formation in the liver was significantly increased in CCl_4 and taurine administered rats compared with controls ($p < 0.05$). The levels of MDA were lower (but not significantly) in the taurine-treated group compared with CCl_4-treated group ($p > 0.05$).

Hepatic GSH and hydroxyproline concentrations did not show any change in the CCl_4-treated and taurine-treated groups compared with the control group ($p > 0.05$).

The morphological changes in the CCl_4 group included marked necrosis, congestion and atrophy of hepatocytes in the perivenular area (Fig. 1). Mitochondria and endoplasmic reticulum cisternae were irregular. Nucleus was ovoid and nuclear chromatin appearance was also irregular (Fig. 2). Taurine pretreatment prevented these changes. In the taurine-treated group, the

Table 1. The effect of taurine on hepatic malondialdehyde, glutathione and hydroxyproline levels in CCl_4-intoxicated rat liver

Groups	n	MDA mean ± SD	GSH mean ± SD	HP mean ± SD
Control	6	57 ± 7	8.4 ± 0.7	0.19 ± 0.04
CCl_4-treated	6	77 ± 11.2*	7.4 ± 1.4	0.14 ± 0.06
Taurine-treated	6	71.8 ± 6.4*	7.1 ± 0.9	0.20 ± 0.09

*$p < 0.05$, significantly different from the control. *MDA*, malondialdehyde (nmol/g tissue); *GSH*, glutathione (μmol/g tissue); *HP*, hydroxyproline (μg/mg tissue); *CCl_4*, carbontetrachloride was administered 2 ml/kg, i.p. as a single dose. Taurine (5% aqueous solution) was administered 15 ml/kg/day using a gastric tube for 5 days before CCl_4 administration.

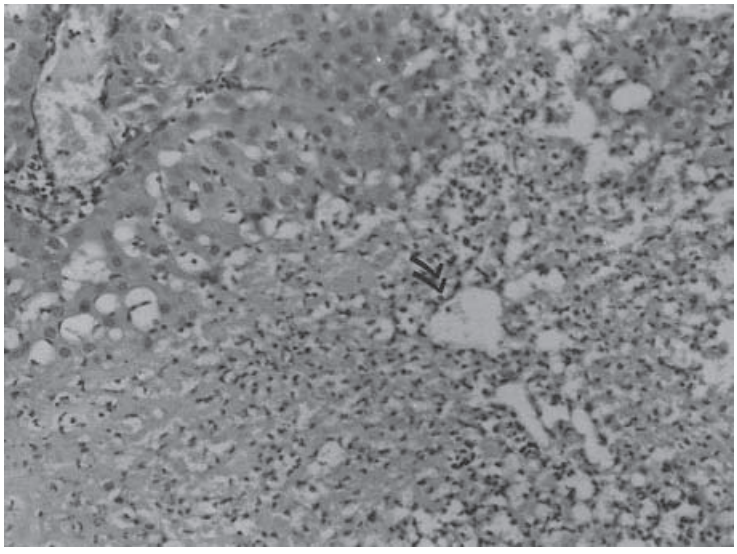

Fig. 1. A light microscopic view in CCl_4-treated group. Necrosis, congestion and hepatocellular atrophy in the perivenular area (arrows), HE × 200

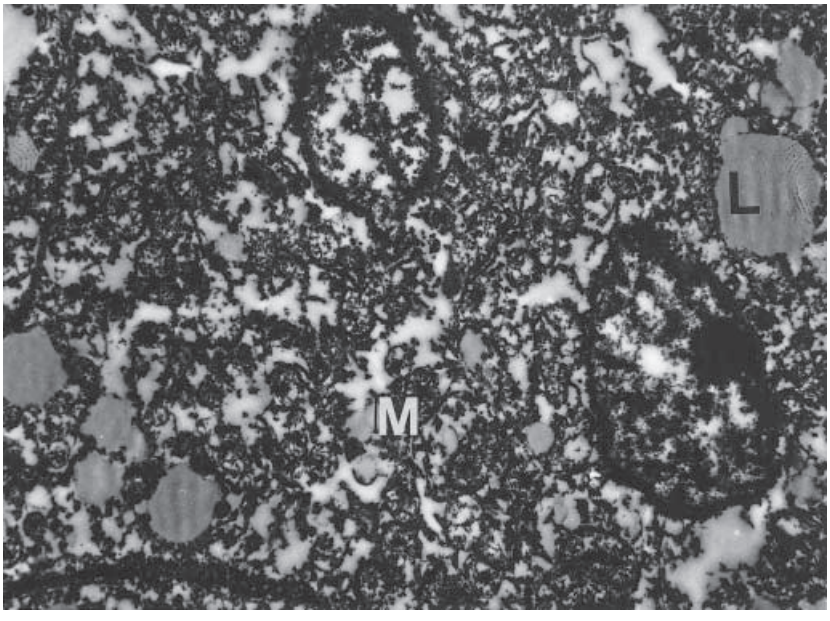

Fig. 2. An electron microscopic view in CCl_4-treated group. Degenerated cytoplasm and organelles. Mitochondrion (*M*), lipid droplets (*L*), uranyl acetate, lead citrate, ×3000

cytoplasm of the perivenuler hepatocytes were large and pale, and the nuclei of these cells had some activity findings such as nuclear enlargement and hyperchromasia (Fig. 3). There were lucent lipid droplets in the taurine-treated group, and nuclei, mitochondria and other organelles were more regular than in the CCl_4 group (Fig. 4).

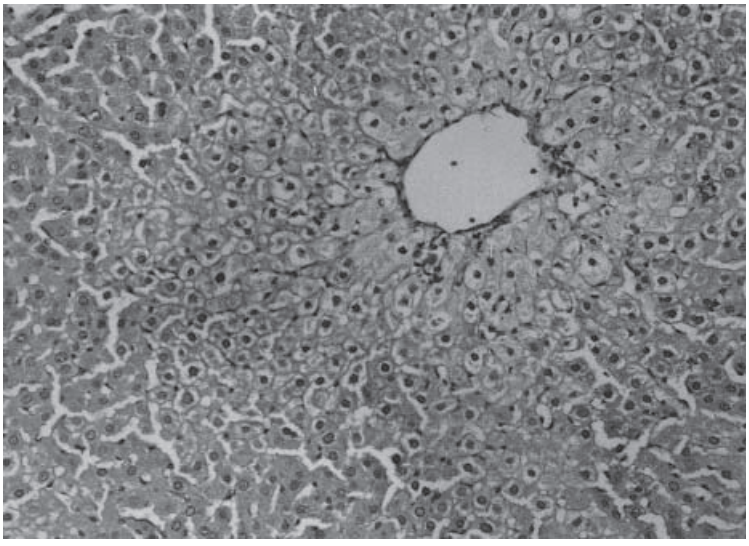

Fig. 3. Hepatocytes having larger, paler cytoplasm located in the perivenular region compared to the adjacent hepatocytes, taurine-treated group, HE × 200

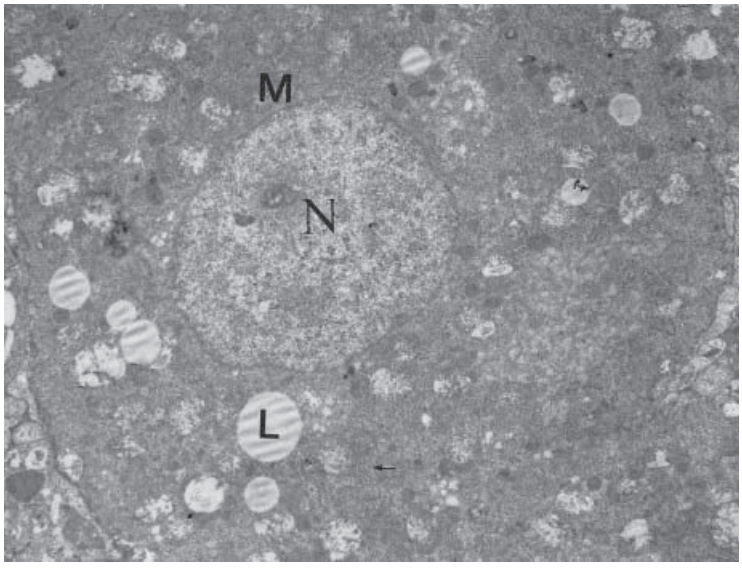

Fig. 4. An electron microscopic view in taurine-treated group, mitochondrion (*M*), nucleus (*N*), lipid droplets (*L*), uranyl acetate, lead citrate, ×3000

Fig. 5 and Fig. 6 show light and electron microscopic views of control liver tissues, respectively.

Discussion

Assessment of malondialdehyde (MDA) has been extensively used during the past 3 to 4 decades as a marker of lipid peroxidation (Jentzsch et al., 1996). It

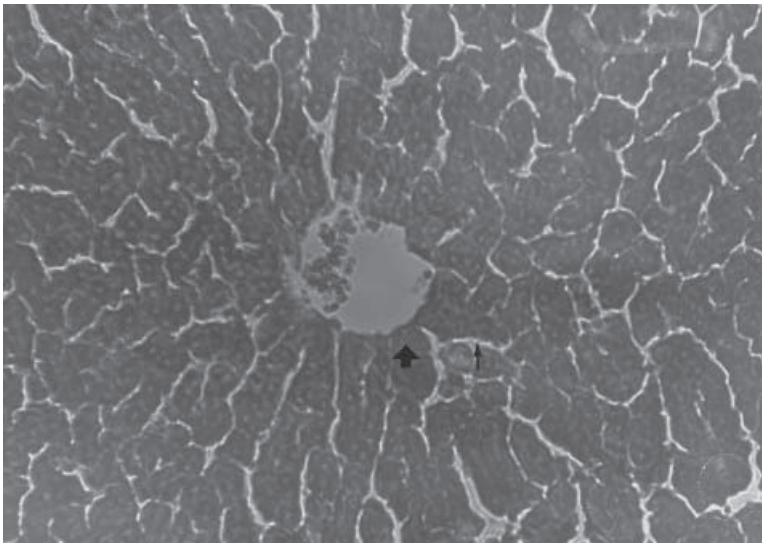

Fig. 5. A light microscopic view in control group. Hepatocyte (thick arrow), sinusoids (thin arrow), toluidin blue ×400

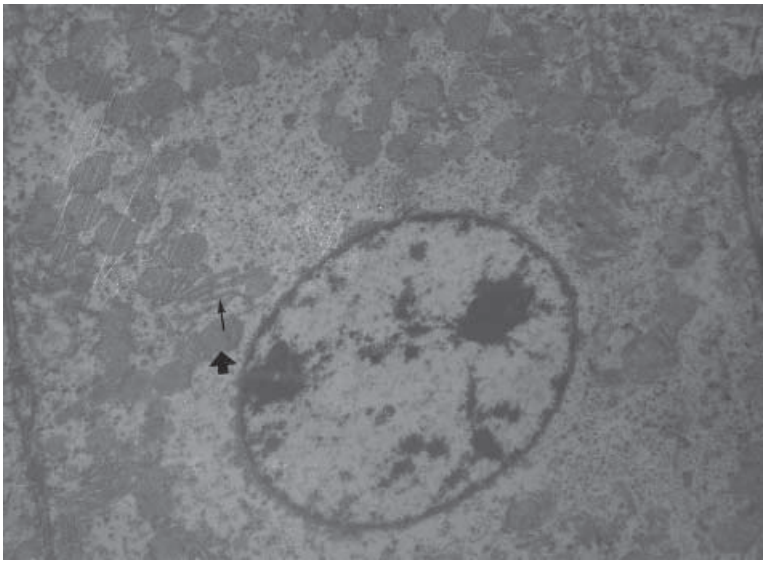

Fig. 6. An electron microscopic view in control group. Mitochondrion (thick arrow), rough endoplasmic reticulum cisternae (thin arrow), uranyl acetate, lead citrate, ×3000

has been known that taurine decreases MDA levels in the liver (Lim et al., 1998; Hwang et al., 1998).

In the present study, the effects of CCl_4 and taurine on lipid peroxidation in the liver were determined by measurement of MDA production. MDA levels were found to be significantly increased in the CCl_4-treated and taurine-treated rats compared with controls ($p < 0.05$), but this increase was lower in the taurine-treated group. These results indicate that pretreatment with

taurine limits the CCl_4-induced lipid peroxidation. Our findings are not consistent with Nagashima's results (1983).

Waterfield et al. (1993b) suggested that if the tissue taurine level is below threshold, susceptibility to CCl_4 will be increased. In that study, a highly significant correlation was found between the liver damage in rats due to CCl_4 and the predicted liver taurine concentration. Holecek et al. (1999) showed that plasma taurine concentrations at 48 hours after administration of various doses of CCl_4 decreased to below the control values. Nakashima et al. (1982) reported that hepatic taurine content decreased significantly 24 hour after CCl_4 administration, and oral administration of taurine to CCl_4-intoxicated rats was able to protect these rats from hepatic taurine depletion. In our study, pretreatment with taurine may have prevented the decrease of hepatic taurine content.

Thiol groups play a complex role in biological systems and GSH in particular, is an important constituent of cellular protective mechanisms against a number of noxious stimuli including oxygen derived free radicals. GSH is among the most widely cited water soluble forms of biological antioxidants (Basaga, 1990). Nakashima et al. (1983) reported that the contents of watersoluble antioxidants in the liver were not altered after CCl_4 administration, and taurine did not have any effect on the content of these antioxidants in the CCl_4-intoxicated liver. On the contrary, Wu et al. (1999) established that the cytoprotective ability of taurine in CCl_4-induced hepatotoxicity is related to polyamines which may prevent GSH and protein SH depletion. In our study, GSH contents in the CCl_4-treated and taurine-treated groups were not significantly different when compared with the control group. These results are compatible with Nakashima's study.

Penttila et al. (1990) suggested that GSH accumulates faster in the periportal (PP) hepatocytes compared to perivenous (PV) hepatoytes, because of the higher biosynthesis rate of the GSH in PP cells. The limited replenishment capacity of GSH potentiates the cellular injury triggered by toxic chemicals. Penttila and colleagues suggested that the slow GSH synthesis in PV cells relates to their large capacity to catabolize intracellular cysteine to taurine. In the present study, perivenular hepatocytes showed more necrosis and atropy compared with peripheral hepatocytes in the CCl_4-treated group. Our findings suggest that the exogenous taurine inhibited this catabolism and prevented the GSH depletion and perivenular cell damage.

Hydroxyproline is an abundant amino acid found in collagen, and in our study, the levels of hydroxyproline were measured to predict hepatic collagen metabolism. The hydroxyproline levels in CCl_4-treated and taurine-treated groups did not show any changes. In our study, the rats were killed after 24 hour of CCl_4 treatment and an explanation migth be that the exposure time of the liver to CCl_4 was not sufficient to alter collagen metabolism. The general method of CCl_4 administration in rats requires at least biweekly administration of the hepatotoxin and the chronicity of liver damage is certainly an important aspect of liver fibrosis (Seyer et al., 1992). Chen et al. (1999) showed that there are significant elevations of hepatic collagen I, III, IV, laminin and hyaluronic acid levels in rats after 12 weeks of CCl_4 treatment. In

addition, they observed that taurine prevented increases in type I and III procollagen mRNA expression as well as the accumulation of collagens, laminin and hyaluronic acid in the liver. They suggest that taurine has a protective effect in CCl_4-induced hepatic fibrosis. Zhou et al. (1996) have also observed that abnormal expressions of hepatic extracellular matrix in the light microscopic examination of the rat liver sections become observable five weeks after CCl_4 administration. However, in this study, taurine (1,5% solution, p.o.), not only inhibited hepatocellular degeneration, necrosis and DNA damage, but also inhibited the lesions in the extracellular matrix induced by CCl_4.

It has been reported that taurine or taurine conjugated bile acids enhances cell proliferation (Kendler, 1989, Panella et al., 1995; Barone et al., 1996; Alpini et al., 1999). In the present study, histopathologic findings demonstrated that taurine prevented hepatocellular necrosis and atrophy as well as stimulating nuclear activity in the hepatocyte (Fig. 3). This may be related to the cell proliferative effect of taurine. In a previous study, we observed that taurine administration enhanced proliferation of the epidermis and accelerated the healing of incisional skin wounds in mice (Dinçer et al., 1996).

It is generally considered that hepatic lipid peroxidation is an important factor in inducing hepatocellular necrosis following CCl_4 administration. One of the suggested roles of lipid peroxidation is the control of cell proliferation. Products of the peroxidation of lipids, such as aldehydes and peroxides, possess a cytostatic activity (Tirmenstein et al., 1988). In our study, taurine was probably able to mitigate CCl_4-induced lipid peroxidation and hepatocellular necrosis by stimulating cell proliferation.

In conclusion, although taurine pretreatment might not significantly change the biochemical parameters, taurine does prevent the morphologic damage caused by CCl_4 in the early stages.

References

Alpini G, Glaser SS, Ueno Y, Rodgers R, Phinizy JL, Francis H, Baiocchi L, Holcomb LA, Caligiuri A, LeSage GD (1999) Bile acid feeding induces cholangiocyte proliferation and secretion: evidence for bile acid-regulated ductal secretion. Gastroenterology 116/1: 179–186

Basaga HS (1990) Biochemical aspects of free radicals. Biochem Cell Biol 68: 989–998

Barone M, Francavilla A, Polimeno L, Ierardi E, Romanelli D, Berloco P, Di Leo A, Panella C (1996) Modulation of rat hepatocyte proliferation by bile salts: in vitro and in vivo studies. Hepatology 23/5: 1159–1166

Chen Y, Li S, Zhang X (1999) Taurine inhibits deposition of extracellular matrix in experimental liver fibrosis in rats. Chung Hua Kan Tsang Ping Tsa Chih 7/3: 165–167

Dinçer S, Babül A, Erdoğan D, Özoğul C, Dinçer SL (1996) Effect of taurine on wound healing. Amino Acids 10: 59–71

Ellman G (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82: 70–77

Holecek M, Skalska H, Mraz J (1999) Plasma amino acid levels after carbon tetrachloride induced acute liver damage. A dose-response and time-response study in rats. Amino Acids 16: 1–11

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Hwang DF, Wang LC, Cheng HM (1998) Effect of taurine on toxicity of copper in rats. Food Chem Toxicol 36/3: 239–244

Jentzsch AM, Bachmann H, Fürst P, Biesalski HK (1996) Improved analysis of malondialdehyde in human body fluids. Free Radic Biol Med 20: 251–256

Kendler BS (1989) Taurine: an overview of its role in preventive medicine. Prev Med 18/1: 79–100

Lim E, Park S, Kim H (1998) Effect of taurine supplementation on the lipid peroxide formation and the activities of glutathione-related enzymes in the liver and islet of type I and II diabetic model mice. Adv Exp Med Biol 442: 99–103

Marcinkiewicz J, Chain B, Nowak B, Grabowska A, Bryniarski K, Baran J (2000) Antimicrobial and cytotoxic activity of hypochlorous acid: interactions with taurine and nitrite. Inflammation Research 49/6: 280–289

Marquez LA, Dunford HB (1994) Chlorination of taurine by myeloperoxidase. J Biol Chem 269: 7950–7956

Nakashima T, Taniko T, Kuriyama K (1982) Therapeutic effect of taurine administration on carbon tetrachloride-induced hepatic injury. Jap J Pharm 32: 583–589

Nakashima T, Takino T, Kuriyama K (1983) Pretreatment with taurine facilitates hepatic lipid peroxide formation associated with carbon tetrachloride intoxication. Japan J Pharmacol 33: 515–523

Panella C, Ierardi E, De Marco MF, Barone M, Guglielmi FW, Polimeno L, Francavilla A (1995) Does tauroursodeoxycholic acid (TUDCA) treatment increase hepatocyte proliferation in patients with chronic liver disease? Ital J Gastroenterol 27/5: 256–258

Penttila KE (1990) Role of cysteine and taurine in regulating glutathione synthesis by periportal and perivenous hepatoytes. Biochem J 269: 659–664

Poli G, Cheesman K, Slater TF, Dianzani MU (1981) The role of lipid peroxidation in CCl_4-induced damage to liver microsomal enzymes: comparative studies in vitro using microsomes and isolated liver cells. Chem Biol Interact 37: 13–24

Recknagel NO, Glende Jr EA, Dolak JA, Waller RL (1989) Mechanism of carbontetrachloride toxicity. Pharmacol Ther 43: 749–790

Seabra V, Timbrell JA (1997) Modulation of taurine levels in the rat liver alters methylene dianiline hepatotoxicity. Toxicology 122/3: 193–204

Seyer JM, Raghow R (1992) Hepatic fibrosis. In: Cohen IK, Diegelmann RF, Lindblad WJ (eds) Wound healing. Biochemical & clinical aspects. W.B. Saunders Company, Philadelphia, pp 416–432

Timbrell JA, Seabra V, Waterfield CJ (1995) The in vivo and in vitro protective properties of taurine. Gen Pharmac 26: 453–462

Tirmenstein MA, Reed DJ (1988) Characterization of glutathione-dependent inhibition of lipid peroxidation of isolated rat liver nuclei. Arch Biochem Biophys 261: 1–11

Uchiama M, Mihara M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86: 271–278

Waterfield CJ, Turton JA, Scales MDC, Timbrell JA (1993a) Reduction of liver taurine in rats by β-alanine treatment increases carbontetrachloride toxicity. Toxicology 77: 7–20

Waterfield CJ, Mesquita M, Parnham P, Timbrell JA (1993b) Taurine protects against the cytotoxicity of hydrazine, 1,4-naphthoquinone and carbon tetrachloride in isolated rat hepatocytes. Biochem Pharmac 46: 589–595

Woessner F (1961) The determination of hydroxyproline in tissue and protein samples containing small proportion of this imino acid. Arch Biochem Biophys 93: 440–447

Wright CE, Tallan HH, Lin YY, Gaull GE (1986) Taurine: biological update. Ann Rev Biochem 55: 427–453

Wu C, Kennedy DO, Yano Y, Otani S, Matsui-Yuasa I (1999) Thiols and polyamines in the cytoprotective effect of taurine on carbon tetrachloride-induced hepatotoxicity. J Biochem Mol Toxicol 13: 71–77

You JS, Chang KJ (1998) Taurine protects the liver against lipid peroxidation and membrane disintegration during rat hepatocarcinogenesis. Adv Exp Med Biol 442: 105–112

Zhou JW, Zhou JL, Zhong XM, Chen L, Yang XF (1996) Abnormal expressions of hepatocellular proteins and extracellular matrix in CCl_4-induced liver injury in rats. Chinese Medical J 109/5: 366–371

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