Food Chemistry 119 (2010) 62–68

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Protective effects of taurine against hepatic abnormality in NZB/W F1 mice fed a hypercholesterolemic diet

Tsai-Ching Hsu [a,1], Yi-Chen Chen [b,1], Chun-Chou Tsai [a], Jen-Huang Wu [c], Sin-Lun Li [c], Bor-Show Tzang [c,d,e,*]

[a] Institute of Immunology, Chung Shan Medical University, Taichung, Taiwan, ROC
[b] School of Nutrition, Chung Shan Medical University, Taichung, Taiwan, ROC
[c] Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung, Taiwan, ROC
[d] Department of Biochemistry, School of Medicine, Chung Shan Medical University, Taichung, Taiwan, ROC
[e] Clinical Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan, ROC

ARTICLE INFO

Article history:
Received 6 February 2009
Received in revised form 15 May 2009
Accepted 27 May 2009

Keywords:
Hypercholesterolemic diet
Systemic lupus erythematosus (SLE)
Taurine
Hepatic abnormality
NZB/W F1

ABSTRACT

A hypercholesterolemic diet has been associated with the hepatic abnormalities and the pathological processes of systemic lupus erythematosus (SLE). To investigate the effects of taurine on the hepatic abnormality in SLE, NZB/W F1 mice were used as an animal model by receiving control, cholesterol, or cholesterol + taurine diets, respectively. Reductions ($P < 0.05$) of liver-to-body weight ratio, lipid deposit, mean arterial pressure, serum total cholesterol (TC), triacylglycerol (TAG), AST, ALT and hepatic CRP levels were detected in the cholesterol + taurine group as compared to those of the cholesterol group. In addition, stress-related molecules in livers, including HSP70, HSP90, MMP9 and iNOS, were also lower ($P < 0.05$) in the cholesterol + taurine group compared to the cholesterol group. These findings demonstrated the protective effects of taurine on the hepatic abnormality in NZB/W F1 mice fed a hypercholesterolemic diet and may suggest taurine as a dietary supplementation for SLE patients.

1. Introduction

A high-cholesterol dietary habit is known to play an important role in the induction of chronic diseases such as hypercholesterolemia and hepatic abnormalities (Kleemann et al., 2007). For instance, the progression of non-alcoholic fatty liver disease was demonstrated in obese rats by feeding a high-fat/cholesterol diet (Carmiel-Haggai, Cederbaum, & Nieto, 2005; Pan, Song, Xu, & Gan, 2006). Another study also indicated that increased fat deposits, insulin resistance, and liver oxidative stress are associated with hepatic disorders, and aggravate the metabolic syndrome (Xu et al., 2003). Recently, an altered lipid profile characterised by increased triacylglycerol (TAG) and decreased high-density lipoprotein cholesterol (HDL-C) concentrations were reported in patients with systemic lupus erythematosus (SLE) (Chung et al., 2007) which also implied connections between hepatic abnormality and SLE (Abraham, Begum, & Isenberg, 2004).

SLE is an autoimmune disorder with an unknown etiology (Hahn, 1993) that impacts various organs including liver (Mukai et al., 2000; Hsu et al., 2008; Tzang et al., 2008). Indeed, increased hepatic diseases are reported in SLE patients and recognised as important consequences of SLE (Abraham et al., 2004; Hahn, 1993). Additionally, hepatic abnormality has been linked to the pathogenesis of SLE (Abraham et al., 2004; Hahn, 1993; Lu, Li, Hsieh, Wu, & Yu, 2006) with the involvement of various inflammatory related molecules such as C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Herlong, 1994; Zein, Ganuza, & Kushner, 1979), heat shock proteins (HSPs) (Dhillon et al., 1993), iNOS (Belmont et al., 1997), and matrix metalloproteinases (MMPs) (Faber-Elmann, Sthoeger, Tcherniack, Dayan, & Mozes, 2002). However, association between inflammatory-factors and hepatic abnormality in SLE is still transient or non-existent.

Taurine is the major intracellular free β-amino acid in most mammalian tissues and known as a conditionally essential nutrient, which can be synthesised from methionine and cysteine and obtained largely from the diet, predominantly through eggs, meat and seafood (Birdsall, 1998; Boucknooghe, Remacle, & Reusens, 2006) such as oyster and squid (Jacobsen & Smith, 1968; Sakaguchi & Murata, 1989). Many studies have indicated that taurine plays various important physiological roles in osmoregulation, bile acid conjugation, central nervous system modulation, cell proliferation, viability, and prevention of oxidant-induced injury (Cañas & Valenzuela, 1989; Chesney, 1985). Taurine was also reported to reduce the serum lipid and lipid oxidation (Hagar, 2004), and blood pressure in a spontaneously hypertensive rat model (Racasan

* Corresponding author. Tel.: +886 4 23248168; fax: +886 4 23248195.
E-mail address: bstzang@csmu.edu.tw (B.-S. Tzang).
[1] Hsu T.C. and Chen Y.C. contributed equally as co-first authors in this paper.

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.05.088

et al., 2004). Additionally, the protective effects of taurine against cyclosporine-A-induced oxidative stress and hepatotoxicity have been documented previously (Hagar, El Etter, & Arafa, 2006). Moreover, our research team also demonstrated reduced hepatic apoptosis in NZB/W F1 mice with taurine supplementation (Hsu, Chiang, et al., 2008).

Although many studies have indicated the beneficial effects of taurine in a variety of disorders, none or little research has been carried out about the protective effect of taurine against hepatic abnormality in SLE with a high-cholesterol dietary habit. In the current study, we investigated the effects of taurine on aggravated hepatic abnormality in NZB/W F1 mice induced by feeding a hypercholesterolemic diet and may suggest the therapeutic potential of taurine in SLE with hepatic abnormality.

2. Materials and methods

2.1. Mice and diets

Female NZB/W F1 is a well-described lupus-prone mice strain that has been widely used as an SLE animal model (Liang, Gardner, Griswold, Bugelski, & Song, 2006), which reveals the symptoms of SLE spontaneously from the age of 12 weeks (Russell & Denman, 1969). Female NZB/W F1 were purchased from the animal centre of National Taiwan University, Taiwan and housed in an animal room at 22 + 2 °C with a 12/12 h light–dark cycle under supervision of the Institutional Animal Care and Use Committee at Chung Shan Medical University. Disease activity of mice was determined by monitoring the uric protein biweekly with Albustix test strips (Bayer Diagnostics, Hong Kong) from the age of 12 weeks as described previously (Hsu, Chiang, et al., 2008; Hsu et al., 2008; Tzang et al., 2008). Chow diet and cholesterol were purchased from TestDiet® Division, PMI® Nutrition International/Purina Mills LLC, Richmond, IN, USA and ICN Biomedicals Inc., Irvine, CA, USA, respectively. Taurine was purchased from Sigma (Sigma, St. Louis, MO, USA) and the formulation of the experimental diets is shown in Table 1. Thirty-six female NZB/W F1 mice of 112-day-old were divided into three groups and were given diets on control, cholesterol, and cholesterol/taurine for 12 weeks, respectively. Mice were sacrificed by CO_2 asphyxiation. Liver and blood of mice were obtained after CO_2 sacrifice and stored at −80 °C for further analyses.

2.2. Physiological values

Body weight of mice was recorded individually every week. Heart rate and blood pressures of mice were measured by a non-invasive measurement (Matoba et al., 2001) every week. First, mice were held in a small and dark-coloured plastic holder. After about 10 min of equilibration, heart rate and mean arterial pressure were monitored in conscious mice by the tail-cuff method with a BP Monitor MK-2000A (Muromachi Co. Ltd., Tokyo, Japan) consecutively at least three times per mouse. The mean arterial pressure is defined when the amplitude of the pulse wave is the greatest.

Table 1
Compositions of experimental diets (g/kg).

	Experimental groups		
Ingredients	Control	Cholesterol	Cholesterol + taurine
5001[a]	1000	990	980
Cholesterol	0	10	10
Taurine	0	0	10

[a] 5001 indicates chow diet, Rodent 5001 (PMI® Nutrition International/Purina Mills LLC., USA).

2.3. Haematoxylin-Eosin and Sudan III staining

Liver tissues obtained as described above were embedded into OCT compound (Tissue-Tek, Miles Inc., Elkhart, IN, USA) and snap frozen in liquid nitrogen. For Sudan III staining, the frozen sections were sectioned at 5 μm and socked in 50% ethanol before immersed in the dark in the Sudan III solution in 70% ethanol for 20 min. The sections were then washed with 50% ethanol and immersed in Hematoxyl solution for 3 min as the negative staining. Photomicrographs were obtained using Zeiss Axiophot microscopes.

2.4. Detection of AST, ALT, total cholesterol (TC), triacylglycerol (TAG), and CRP levels in serum

The mice were sacrificed as described above and the heart blood was collected by direct puncture with a sterilised 1 mL syringe. The serum values of AST, ALT, TC, and TAG were detected by a nephelometric system according to the manufacturer's instruction (Cobas Mira, Roche, Swiss). Additionally, the serum CRP level was also determined by Western blot.

2.5. Tissue extraction and Western blot

Liver tissue extracts were obtained by homogenisation in a PBS buffer (pH 7.0, 0.14 M NaCl, 3 mM KCl, 1.4 mM KH_2PO_4, 14 mM K_2HPO_4) at a ratio of 100 mg tissue/0.5 ml PBS for 5 min. The homogenates were placed on ice for 10 min and then centrifuged at 12,000*g* for 30 min. The supernatant was collected and stored at −80 °C for further analyses. For Western blots, protein samples were denatured for 5 min in boiling water with sample buffer (pH 6.8, 0.0625 M Tris–HCl, containing 2.3% SDS, 5% 2-mercaptoethanol, and 10% glycerol). Samples applied to the gel were run at 100–150 V for 1.5 h and electrophoretically transferred to nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ, USA). The membrane was then soaked in PBS with 5% non-fat dry milk for 30 min at room temperature to saturate irrelevant protein binding sites. Antibodies against CRP, HSP70, HSP90, MMP9, iNOS and β-actin (Upstates, Charlottesville, VA, USA) were diluted in PBS with 2.5% BSA and incubated for 1.5 h with gentle agitation at room temperature. The membranes were washed twice with PBS-Tween for 1 h and secondary antibody conjugated with horseradish peroxidase (HRP) was added. Pierce's Supersignal West Dura HRP Detection Kit (Pierce Biotechnology Inc., Rockford, IL) was used to detect antigen–antibody complexes. The blots were also scanned and quantified by densitometry (Appraise, Beckman-Coulter, Brea, CA, USA).

2.6. Statistical analysis

All the statistical analyses were performed by using Student's *t*-test (SPSS 10.0 software, SPSS Inc, Chicago, IL). Three independent experiments (4 mice/treatment/experiment) were repeated. A significant difference was defined at 0.05 probability level.

3. Results and discussion

3.1. Physiological changes of NZB/W F1 mice

To investigate the association between hypercholesterolemic diets and taurine on hepatic abnormality in SLE, NZB/W F1 mice were used as an animal model and randomly divided into three groups that were fed with different diets for 12 weeks as described in Section 2. The formulations of diets are presented in Table 1. The increased ($P < 0.05$) ratio of liver-to-body weight was observed in

Table 2
The ratios of liver-to-body weight of NZB/W F1 mice.

Groups	Liver weight/body weight	*P* value
Control	0.0490 ± 0.0025	–
Cholesterol	0.0552 ± 0.0023[a]	0.0026
Cholesterol + taurine	0.0483 ± 0.0038[b]	0.0015

[a] Cholesterol group vs. control group.
[b] Cholesterol + taurine group vs. cholesterol group.

the cholesterol group compared to the control group, which was normalised ($P < 0.05$) by supplementing taurine into the hypercholesterolemic diet (Table 2). No ($P > 0.05$) differences on the average body weight and average heartbeats were recorded between cholesterol and cholesterol + taurine groups (Fig. 1A and B). However, an increased ($P < 0.05$) relative mean arterial pressure of the cholesterol group to the control group was observed when compared to that of cholesterol + taurine group to the control group

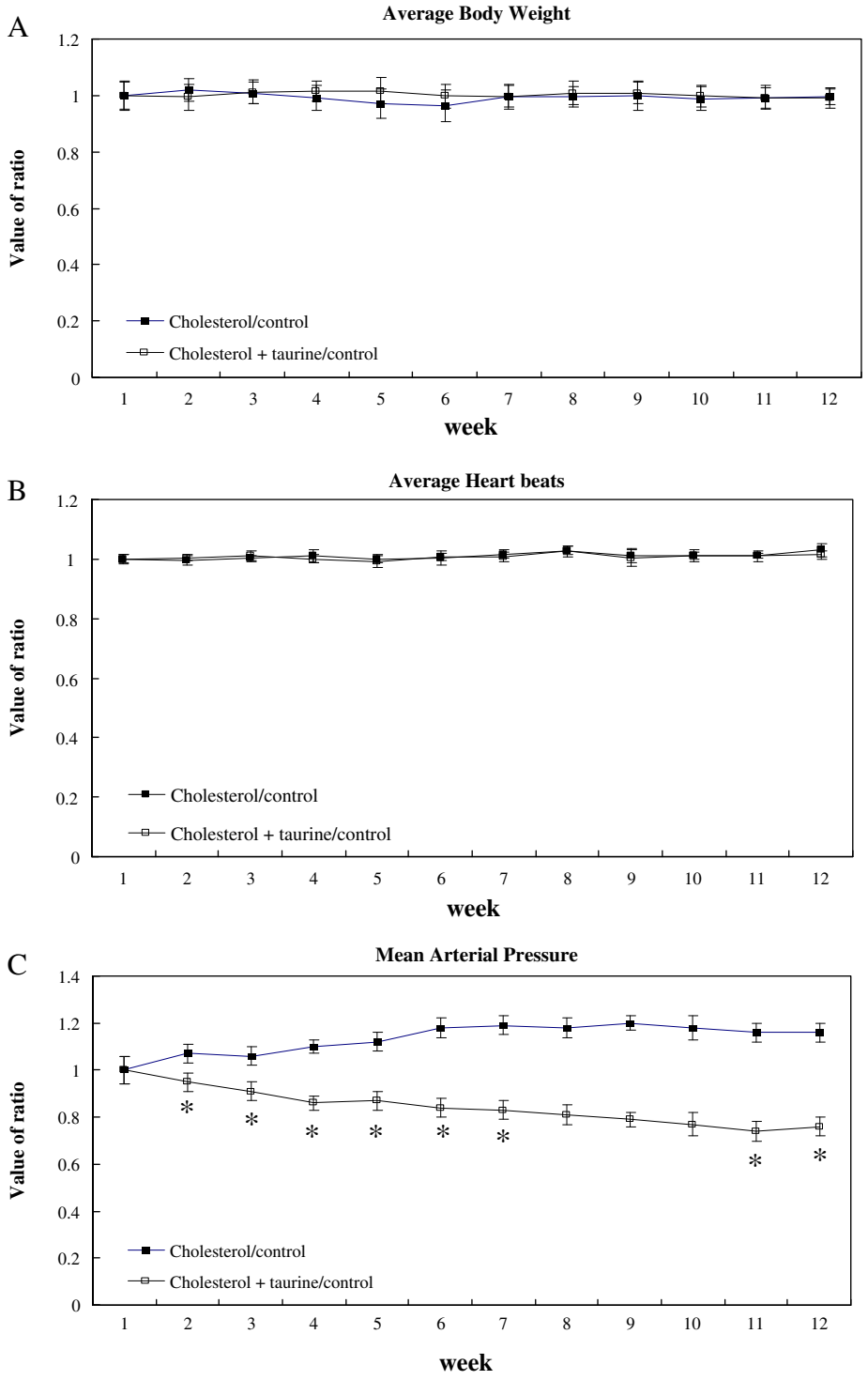

Fig. 1. Average body weight, heartbeats, and mean arterial pressure of NZB/W F1 mice. The ratios of (A) body weight, (B) heartbeats, and (C) mean arterial pressure in cholesterol and cholesterol + taurine groups were relative to the values of the control group. * indicates a significant difference.

from second week (Fig. 1C). To confirm the effect of taurine on lipid deposit in livers of NZB/W F1 mice, liver cross section was performed and analysed by Sudan III staining (Fig. 2). An apparent lipid deposit in livers was observed in the cholesterol group, whereas little lipid deposit was observed in the cholesterol + taurine group (Fig. 2A). Although an increase ($P < 0.05$) of total serum cholesterol (TC) was detected in mice fed with hypercholesterolemic diets (cholesterol and cholesterol + taurine groups), taurine supplementation did not ($P > 0.05$) reduce TC level (Fig. 2B). In the contrast, a decreased ($P < 0.05$) serum triacylglycerol (TAG) was detected in cholesterol/taurine group compared to the control or the cholesterol group, respectively (Fig. 2C). Moreover, both serum AST and ALT levels was lower ($P < 0.05$) in cholesterol + taurine group than those in the control and the cholesterol groups (Fig. 2D and E).

Taurine, the 2-aminoethane sulphuric acid, is known as a major intracellular free β-amino acid and has the function of protecting various disorders. In a clinical application, taurine has been demonstrated to have beneficial effects in platelet aggregation, nephropathy, cardiomyopathy, neuropathy, and fetal-development (Szymański & Winiarska, 2008). Besides, taurine was reported to reduce serum lipid and lipid oxidation (Hagar, 2004), as well as blood pressures in a spontaneously hypertensive rat model (Racasan et al., 2004). Additionally, an amelioration of taurine on non-alcoholic fatty livers was discussed via a decrease of ratios of glycine/taurine-conjugated bile acids, recovery of GPT values, an inhibition of lipid peroxidation, improvement of lipid and glucose metabolism, decreases of synthesis of TNF-α and TGF-β1, and increase of adiponectin biosynthesis (Chen, Chen, Shi, Lin, & Xie, 2006; Obinata, Maruyama, Hayashi, Watanabe, & Nittono,

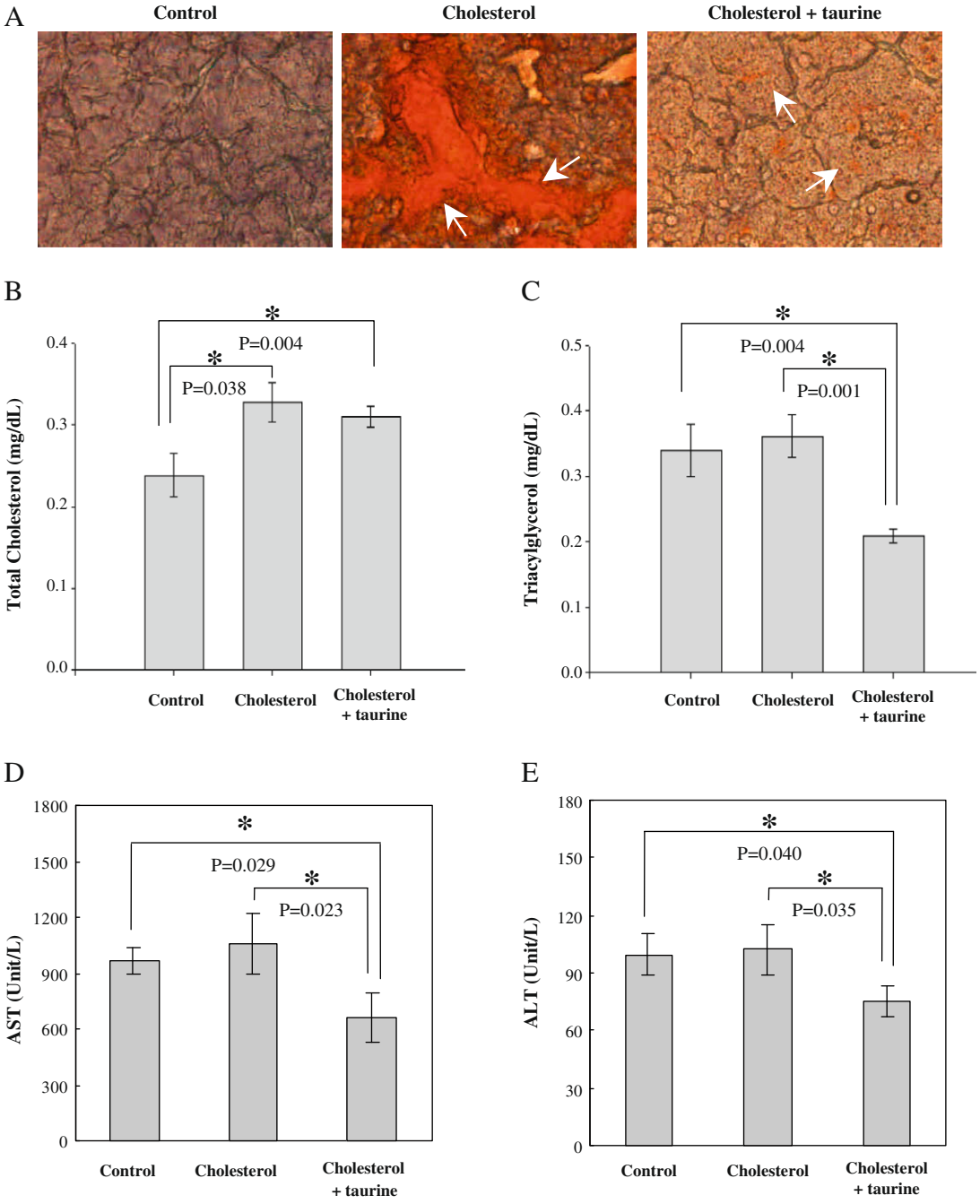

Fig. 2. (A) Sudan III stains, and (B) serum total cholesterol, (C) triacylglycerol, (D) AST and (E) ALT levels of NZB/W F1 mice. * indicates a significant difference ($P < 0.05$).

1996). Altogether, these investigations are similar to and do support the findings in the current study and could provide a clue that taurine supplementation has potential beneficial effect in alleviating hepatic abnormalities in SLE.

3.2. Effect of taurine on expression of stress- and inflammatory-related proteins in NZB/W F1 mice

To further investigate the effect of taurine on inflammatory and stress responses in NZB/W F1 mice fed a hypercholesterolemic diet, Western blots were performed to detect the expressions of CRP, HSP70, HSP90, MMP-9 and iNOS proteins. Fig. 3 reveals the expressions of CRP levels in serum and liver lysate of NZB/W F1 mice. An increased ($P < 0.05$) serum CRP level was detected in mice fed on a hypercholesterolemic diet without taurine supplementation as compared to that of the control group. In the contrast, taurine supplementation reduced ($P < 0.05$) serum CRP levels of mice fed a hypercholesterolemic diet (Fig. 3A). Similar results were shown in liver lysate where CRP level was decreased ($P < 0.05$) in the cholesterol + taurine group (Fig. 3B) than that of the cholesterol group. Additionally, decreased ($P < 0.05$) HSP70 and HSP90 levels in liver lysate were detected in the cholesterol + taurine group as compared to those in the cholesterol group (Fig. 4A and B). Fig. 4C and D reveal the expressions of MMP9 and iNOS levels. MMP and iNOS levels in livers were increased ($P < 0.05$) in the cholesterol group as compared to that of the control group, while MMP and iNOS levels were decreased ($P < 0.05$) by supplementing taurine into the hypercholesterolemic diet. Besides, it is interesting that CRP and MMP-9 levels in livers of the cholesterol + taurine group were lower than those of the control group (Figs. 3B and 4C).

A high-cholesterol dietary habit has been recognised as an important risk to cause hepatic abnormality that is associated with

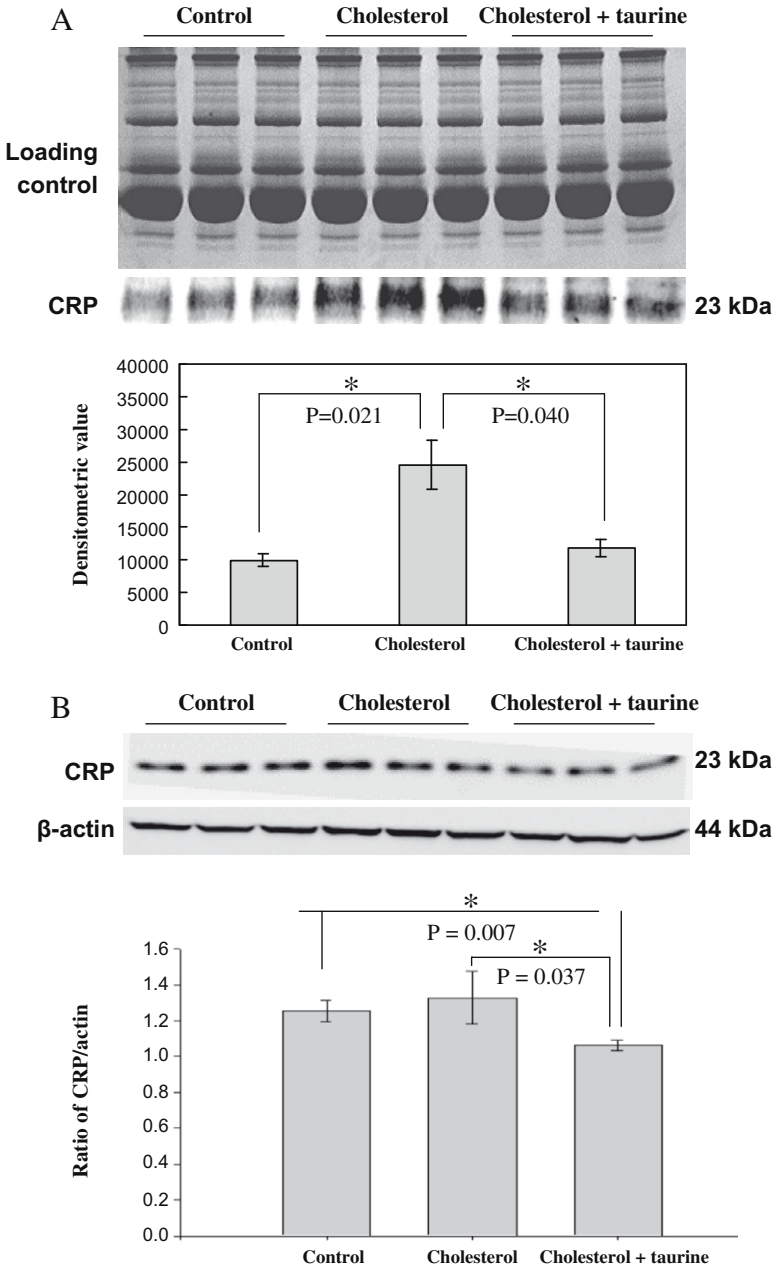

Fig. 3. Ratios of CRP levels in (A) serum and (B) liver samples of NZB/W F1 mice. The value of CRP levels was normalised to the value of β-actin. * indicates a significant difference ($P < 0.05$).

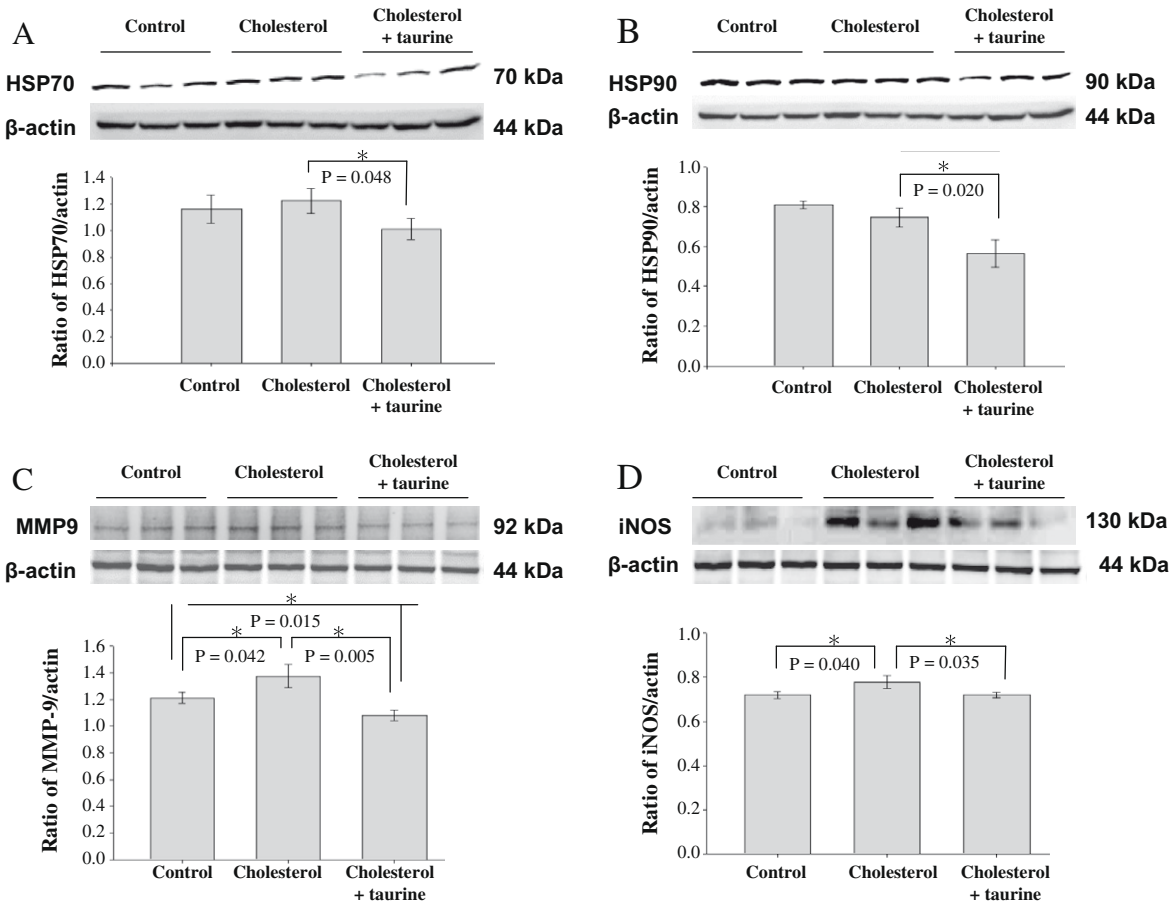

Fig. 4. Ratios of (A) HSP70, (B) HSP90, (C) MMP9 and (D) iNOS protein levels in livers of NZB/W F1 mice. The values of HSP70, HSP90, MMP9 and iNOS protein levels were normalised to the value of β-actin. * indicates a significant difference ($P < 0.05$).

SLE. Indeed, increasing hepatic abnormality has been reported in SLE patients and considered to play a crucial diagnosis in SLE pathogenesis (Abraham et al., 2004; Hahn, 1993; Lu et al., 2006). A recent study reported that increased serum triacylglycerol (TAG) and decreased HDL cholesterol concentrations were characterised in SLE patients (Chung et al., 2007). Moreover, increased stress- and inflammatory-proteins related to liver damages, i.e., AST, ALT, CRP, HSPs, iNOS and MMPs were detected in serum from SLE patients (Belmont et al., 1997; Dhillon et al., 1993; Faber-Elmann et al., 2002; Herlong, 1994; Zein et al., 1979), which stimulates the development of SLE pathogenesis. Although taurine is known to be beneficial on lipid metabolism and hepatic abnormality, little is known about the effects of taurine on SLE (Abraham et al., 2004; Chung et al., 2007). The present study demonstrated that taurine supplementation indeed reduces the mean arterial pressure and liver size, consistent with results of previous research (Yokogoshi & Oda, 2002). Furthermore, a hypercholesterolemic diet elevates stress- and inflammatory-factors, i.e., TC, TG, AST, ALT, CRP, HSP70, HSP90, MMP9, and iNOS protein in NZB/W F1 mice. Indeed, an alleviation of those stress and inflammatory-factors was observed by supplementating taurine which indicates that taurine has a protective effect against hepatic abnormalities of SLE induced by a high-cholesterol dietary habit.

4. Conclusions

A consensus has been postulated that cholesterol could induce hepatic abnormality and is associated with the pathogenesis of SLE (Abraham et al., 2004; Hahn, 1993; Hsu et al., 2008; Lu et al., 2006; Tzang et al., 2008). Although increasing hepatic abnormality is observed in SLE patients (Abraham et al., 2004) and has been related to the level of SLE pathogenesis (Hahn, 1993), suitable treatment for SLE remains unclear. In the current study, a hypercholesterolemic diet indeed increased the serum TC and hepatic cholesterol deposit in NZB/W F1 mice, thus increasing stress- and inflammatory-factors, i.e., AST, ALT, CRP, HSP70, HSP90, MMP9, and iNOS protein. However, taurine supplementation showed a protective effect against the hepatic abnormality of mice induced by a hypercholesterolemic diet. As we know, damaged hepatic function always couples with SLE patients, even with a normal dietary habit. Inspiringly, those inflammatory-factors related to liver are lower when taurine is supplemented in the diet, even which contains high cholesterol. These novel observations strongly suggest that taurine supplementation may retard on the progressive hepatic abnormality in SLE patients.

Acknowledgements

This study was supported by the grants NSC96-2320-B-040-025-MY3 from the National Science Council, Taiwan, Republic of China.

References

Abraham, S., Begum, S., & Isenberg, D. (2004). Hepatic manifestations of autoimmune rheumatic diseases. *Annals of the Rheumatic Diseases, 63*, 123–129.

Belmont, H. M., Levartovsky, D., Goel, A., Amin, A., Giorno, R., Rediske, J., et al. (1997). Increased nitric oxide production accompanied by the up-regulation of

inducible nitric oxide synthase in vascular endothelium from patients with systemic lupus erythematosus. *Arthritis Rheumatism, 40*, 1810–1816.
Birdsall, T. C. (1998). Therapeutic applications of taurine. *Alternative Medicine Review, 3*, 128–136.
Boucknooghe, T., Remacle, C., & Reusens, B. (2006). Is taurine a functional nutrient? *Current Opinion in Clinical Nutrition and Metabolic Care, 9*, 728–733.
Cañas, P., & Valenzuela, A. (1989). Biologic and physiologic role of taurine and its derivates. *Revista médica de Chile, 117*, 454–459.
Carmiel-Haggai, M., Cederbaum, A. I., & Nieto, N. (2005). A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *The Journal of the Federation of American Societies for Experimental Biology, 19*, 136–138.
Chen, S. W., Chen, Y. X., Shi, J., Lin, Y., & Xie, W. F. (2006). The restorative effect of taurine on experimental nonalcoholic steatohepatitis. *Digestive Diseases and Sciences, 51*, 2225–2234.
Chesney, R. W. (1985). Taurine: Its biological role and clinical implications. *Advances in Pediatrics, 32*, 1–42.
Chung, C. P., Oeser, A., Solus, J., Avalos, I., Gebretsadik, T., Shintani, A., et al. (2007). Inflammatory mechanisms affecting the lipid profile in patients with systemic lupus erythematosus. *Journal of Rheumatology, 34*, 1849–1854.
Dhillon, V. B., McCallum, S., Norton, P., Twomey, B. M., Erkeller-Yuksel, F., Lydyard, P., et al. (1993). Differential heat shock protein overexpression and its clinical relevance in systemic lupus erythematosus. *Annals of the Rheumatic Diseases, 52*, 436–442.
Faber-Elmann, A., Sthoeger, Z., Tcherniack, A., Dayan, M., & Mozes, E. (2002). Activity of matrix metalloproteinase-9 is elevated in sera of patients with systemic lupus erythematosus. *Clinical and Experimental Immunology, 127*, 393–398.
Hagar, H. H., El Etter, E., & Arafa, M. (2006). Taurine attenuates hypertension and renal dysfunction induced by cyclosporine A in rats. *Clinical and Experimental Pharmacology and Physiology, 33*, 189–196.
Hagar, H. H. (2004). The protective effect of taurine against cyclosporine-A induced oxidative stress and hepatotoxicity in rats. *Toxicology Letter, 151*, 335–343.
Hahn, B. H. (1993). An overview of the pathogenesis of systemic lupus erythematosus. In D. J. Wallace & B. H. Hahn (Eds.), *Dubois' lupus erythematosus* (pp. 69–76). Philadelphia: Williams and Wilkins.
Herlong, H. F. (1994). Approach to the patient with abnormal liver enzymes. *Hospital Practice (Off. Edition.), 29*, 32–38.
Hsu, T. C., Chiang, S. Y., Wu, J. H., Tsai, C. C., Huang, C. Y., Chen, Y. C., et al. (2008). Treatment with taurine attenuates hepatic apoptosis in NZB/W F1 mice fed with a high-cholesterol diet. *Journal of Agriculture and Food Chemistry, 56*, 9685–9691.
Hsu, T. C., Huang, C. Y., Chiang, S. Y., Lai, W. X., Tsai, C. H., & Tzang, B. S. (2008). Transglutaminase inhibitor cystamine alleviates the abnormality in liver from NZB/W F1 mice. *European Journal of Pharmacology, 579*, 382–389.
Jacobsen, J. G., & Smith, L. H. (1968). Biochemistry and physiology of taurine and taurine derivatives. *Physiological Reviews, 48*, 424–511.
Kleemann, R., Verschuren, L., van Erk, M. J., Nikolsky, Y., Cnubben, N. H., Verheij, E. R., et al. (2007). Atherosclerosis and liver inflammation induced by increased dietary cholesterol intake: A combined transcriptomics and metabolomics analysis. *Genome Biology, 8*, R200.
Liang, B., Gardner, D. B., Griswold, D. E., Bugelski, P. J., & Song, X. Y. (2006). Anti-interleukin-6 monoclonal antibody inhibits autoimmune responses in a murine model of systemic lupus erythematosus. *Immunology, 119*, 296–305.
Lu, M. C., Li, K. J., Hsieh, S. C., Wu, C. H., & Yu, C. L. (2006). Lupus-related advanced liver involvement as the initial presentation of systemic lupus erythematosus. *Journal of Microbiology, Immunology and Infection, 39*, 471–475.
Matoba, N., Doyama, N., Yamada, Y., Maruyama, N., Utsumi, S., & Yoshikawa, M. (2001). Design and production of genetically modified soybean protein with anti-hypertensive activity by incorporating potent analogue of ovokinin(2–7). *Federation of European Biochemical Societies Letters, 497*, 50–54.
Mukai, M., Bohgaki, T., Notoya, A., Kohno, M., Tateno, M., & Kobayashi, S. (2000). Liver dysfunction due to apoptosis in a patient with systemic lupus erythematosus. *Lupus, 9*, 74–77.
Obinata, K., Maruyama, T., Hayashi, M., Watanabe, T., & Nittono, H. (1996). Effect of taurine on the fatty liver of children with simple obesity. *Advances in Experimental Medicine and Biology, 403*, 607–613.
Pan, M., Song, Y. L., Xu, J. M., & Gan, H. Z. (2006). Melatonin ameliorates nonalcoholic fatty liver induced by high-fat diets in rats. *Journal of Pineal Research, 41*, 79–84.
Racasan, S., Braam, B., van der Giezen, D. M., Goldschmeding, R., Boer, P., Koomans, H. A., et al. (2004). Perinatal L-arginine and antioxidant supplements reduce adult blood pressure in spontaneously hypertensive rats. *Hypertension, 44*, 83–88.
Russell, A. S., & Denman, A. M. (1969). Normal induction and maintenance of tolerance in (NZB × NZW) F1 mice. *Clinical and Experimental Immunology, 5*, 265–272.
Sakaguchi, M., & Murata, M. (1989). Seasonal variations of free amino acids in oyster whole body and adductor muscle. *Nippon Suisan Gakkaishi, 55*, 2037–2041.
Szymański, K., & Winiarska, K. (2008). Taurine and its potential therapeutic application. *Postepy higieny i medycyny doswiadczalnej, 62*, 75–86.
Tzang, B. S., Chiang, S. Y., Lai, W. X., Tsai, C. C., Wu, J. H., & Hsu, T. C. (2008). Treatment with cystamine reduces apoptosis in liver from NZB/W F1 mice. *International Immunopharmacology, 8*, 589–596.
Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., et al. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation, 112*, 1821–1830.
Yokogoshi, H., & Oda, H. (2002). Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet. *Amino Acids, 23*, 433–439.
Zein, N., Ganuza, C., & Kushner, I. (1979). Significance of serum C-reactive protein elevation in patients with systemic lupus erythematosus. *Arthritis Rheumatism, 22*, 7–12.