JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Treatment with Taurine Attenuates Hepatic Apoptosis in NZB/W F1 Mice Fed with a High-Cholesterol Diet

TSAI-CHING HSU,[†] SZU-YI CHIANG,[‡] JEN-HUANG WU,[§] CHUN-CHOU TSAI,[†] CHIH-YANG HUANG,[||,⊥,#] YI-CHEN CHEN,[#,▽] AND BOR-SHOW TZANG*[,§,#]

Institute of Immunology, Institute of Biochemistry and Biotechnology, and School of Nutrition, Chung Shan Medical University, Taichung, Department of Health, Executive Yuan, Hua-Lien Hospital, Hua-Lien, Institute of Basic Medical Science and Graduate Institute of Chinese Medical Science, China Medical University, Taichung, and Department of Health and Nutrition Biotechnology, Asia University, Taichung, Taiwan, Republic of China

Cholesterol-rich diets are known to cause hepatic apoptosis, which has been associated with the pathogenesis of systemic lupus erythematosus (SLE). However, the mechanisms and treatments for hepatic apoptosis in SLE are poorly understood. To clarify the effects of taurine on hepatic apoptosis in SLE, NZB/W F1 mice received control, cholesterol, and cholesterol/taurine diets. Significant reductions of caspase-3 activity, TUNEL-positive cells, and Fas- and mitochondrial- dependent apoptosis were detected in liver from the cholesterol/taurine group as compared to the cholesterol group. Moreover, significant increases of phosphorylated AKT, NF-κB (p65), and ERK1/2 proteins were detected in liver from the cholesterol/taurine group as compared to the cholesterol group. In contrast, a significant reduction of phosphorylated p38 protein was observed in the cholesterol/taurine group. These experimental results demonstrated positive effects of taurine against hepatic apoptosis in NZB/W F1 mice fed a high-cholesterol diet and suggested the therapeutic potential of taurine in SLE.

KEYWORDS: Apoptosis; cholesterol; liver; systemic lupus erythematosus (SLE); taurine

INTRODUCTION

Diets with high amounts of lipids are a known risk factor for hepatic abnormalities including hypercholesterolemia, steatohepatitis, inflammation, and apoptosis (*1*−*5*) Recently, a rabbit model of steatohepatitis using a cholesterol-rich, high-fat diet has revealed increased oxidative stress, inflammation, and fibrosis in the liver (*6*). Additionally, hepatic abnormality has been associated with the pathogenesis of autoimmune disease, including systemic lupus erythematosus (SLE) (*7, 8*). Increased triglycerides (TGs) and decreased high-density lipoprotein (HDL) cholesterol concentrations have also been reported in patients with SLE, which has been postulated to the condition and pathogenesis of SLE (*9, 10*). Indeed, these findings suggest the effect of cholesterol on aggravated conditions in SLE.

SLE is known as an autoimmune disease with unknown etiology (*11*). Impaired clearance of apoptotic cells is recognized to play a crucial role in the pathogenesis of SLE by accumulation of the apoptotic cells in tissues (*12*) as well as apoptosis induced by liver dysfunction. Recently, various studies have indicated the increased population of SLE patients with hepatic abnormality (*9, 13, 14*). Notably, our recent study also indicated that both Fas- and mitochondria-dependent apoptosis were increased in the livers of NZB/W F1 mice (*15*). Moreover, growing evidence has demonstrated that increased hepatic dysfunction, apoptosis, and fibrosis in patients with SLE are associated with the condition of disease (*7, 8, 16, 17*). However, the treatment for SLE with hepatic abnormality or apoptosis remains obscure.

On the basis of our knowledge, dieting may be the most natural way to treat physiological disorders. Meanwhile, taurine, a conditionally essential amino acid, is known to regulate the immune response and to protect biological systems against various injuries (*18*−*20*). Moreover, taurine has been shown to possess antioxidant properties and to regulate the proinflammatory cytokines (*21*−*24*). In a rat model of hepatoxicity, taurine attenuates hypertension and renal dysfunction induced by cyclosporine A (*25*) as well as the protective effect of taurine in another nephrotoxicity rat model induced by cisplatin (*26*). Notably, taurine has also been reported to reduce adult blood

* To whom correspondence should be addressed. Tel: +886-4-23248168. Fax: +886-4-23248195. E-mail: bstzang@csmu.edu.tw.
[†] Institute of Immunology, Chung Shan Medical University.
[‡] Hua-Lien Hospital.
[§] Institute of Biochemistry and Biotechnology, Chung Shan Medical University.
[||] China Medical University.
[⊥] Asia University.
[#] These authors contributed equally.
[▽] School of Nutrition, Chung Shan Medical University.

10.1021/jf8020255 CCC: $40.75
Published on Web 09/25/2008

pressure in a spontaneously hypertensive rat model (*27*). Additionally, taurine is known to reduce ischemia-induced apoptosis in cardiac cells (*28*) and methotrexate-induced leukocyte death (*29*). Although these studies indeed demonstrate the beneficial effects of taurine in biological protection, little is known about the effect of taurine in SLE. In the current study, we demonstrate the beneficial effects of taurine in NZB/W F1 mice fed a cholesterol-rich diet by reducing the hepatic apoptosis and suggest the therapeutic potential of taurine in SLE.

MATERIALS AND METHODS

Mice and Diets. Female NZB/W F1 mice, a well-known and popular utilized lupus-prone mice strain, were purchased from the animal center, 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. The disease conditions of the mice were determined by monitoring the proteinuria biweekly with Albustix test strips from the age of 12 weeks as described previously (*30*), and they were scored according to the manufacturer's scoring system (Bayer Diagnostics, Hong Kong). Chow diet, soybean oil, and cholesterol were purchased (TestDiet Division, PMI Nutrition International/Purina Mills LLC, Richmond, IN). Taurine was purchased from Sigma (Sigma, St. Louis, MO), and the ingredients of the experimental diets were prepared as follows. The control diet was composed of 93% Rodent 5001 chow diet and 7% soybean oil. The cholesterol diet was composed of 92% Rodent 5001 chow diet, 7% soybean oil, and 1% cholesterol. The cholesterol/taurine diet was composed of 91% Rodent 5001 chow diet, 7% soybean oil, 1% cholesterol, and 1% taurine. Thirty female NZB/W F1 mice that were 112 days old were divided into three groups (10 mice/group) and were given control, cholesterol, and cholesterol/taurine diets for 12 weeks, respectively. Mice were sacrificed at the age of 196 days old by CO_2 asphyxiation and rinsed in 70% ethanol solution. Liver and heart blood samples of the mice were obtained after CO_2 sacrifice and stored at −80 °C until use.

TUNEL Assay. Liver tissues obtained as described above were embedded into OCT compound (Tissue-Tek, Miles Inc., Elkhart, IN) and snap frozen in liquid nitrogen. The frozen tissue blocks were sectioned at 5 μm and fixed in 4% paraformaldehyde (Sigma) in 0.1 M PBS, pH 7.4, for 20 min at room temerpature. After they were washed for 30 min with 0.1 M phosphate-buffered saline (PBS), the tissue sections were incubated with 3% H_2O_2 in methanol for 10 min at room temperature. The TUNEL reaction mixture was freshly prepared according to the manufacturer's instructions (Roche Applied Science, Inc., United States), and a total volume of 100 μL of terminal deoxytransferase reaction mixture was incubated with the tissue sections for 1 h at room temperature in the dark. The tissue sections were then rinsed with 0.1 M PBS containing DAPI and observed with a fluorescence microscope. The number and percentage of TUNEL-positive cells were counted and determined by counting 1×10^3 hepatic cells from five random selected fields. All measurements were performed by at least three independent animals in a blinded manner.

Caspase-3 Activity Assay. A caspase-3 enzyme-linked immunosorbent assay kit (BD Pharmingen, San Diego, CA) was used for in vitro determination of caspase-3 enzymatic activity in 20 μg of liver lysates derived from NZB/W F1 mice given PBS or cystamine according to the manufacturer's instructions.

Western Blot. The liver samples from the 18 mice of each group were analyzed for immunoblotting, and similar results were observed in the 18 mice of the same group. The loading sample for each lane of Western blot was a pool of four randomly selected mice of the same group. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE), using 12.5% acrylamide gel, was performed as previously described (*31*). Protein samples were denatured for 5 min in boiling water with sample buffer (0.0625 M Tris-HCl buffer, pH 6.8, 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 a nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ). The membrane was then soaked in PBS with 5% nonfat dry milk for 30 min at room temperature to saturate irrelevant protein binding sites. Antibodies against Fas, caspase-9, caspase-8, Bad, Bax, cytochrome *c*, Apaf-1, BCL-2, AKT-p, Erk1/2-p, c-JUN-p, p-38, p38-p, NF-κB (p65-p), and actin (Upstates, Charlottesville, VA; Santa Cruz Biotechnology, Santa Cruz, CA) were diluted in PBS with 2.5% bovine serum albumin 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).

Statistical Analysis. All of the statistical analyses were performed using SPSS 10.0 software (SPSS Inc., Chicago, IL). Three independent experiments were repeated. Statistical analyses were performed using the analysis of variance plus posterior multiple comparison test to test the difference. $P < 0.05$ was considered statistically significant.

RESULTS

Taurine Attenuates Hepatic Apoptosis in Liver from NZB/W F1 Mice. To clarify the effect of cholesterol and taurine on hepatic apoptosis in SLE, liver samples from NZB/W F1 mice were detected with TUNEL and caspase-3 activity assays. A significantly higher caspases-3 activity was detected in the livers of mice from all experimental groups at the age of 196 days as compared to those at the age of 112 days (**Figure 1A**). Moreover, significant caspase-3 activity was revealed in the livers of mice from the cholesterol group as compared to those from the control group (**Figure 1A**). However, a significant reduction of caspase-3 activity was detected in the livers of mice from the cholesterol/taurine group as compared to the cholesterol group (**Figure 1A**). **Figure 1B** illustrates the fluorescence results of nicked DNA labeled by FITC-labeled terminal deoxytransferase (TdT) in the livers of mice from the control, cholesterol, and cholesterol/taurine group, respectively (**Figure 1B**). Notably, apparent nicked DNA was observed in the livers of mice from the cholesterol group as compared to those from the control group, whereas a significant reduction of nicked DNA was observed in the livers of mice from the cholesterol/taurine group as compared to those from the cholesterol group. The percentage of TUNEL-positive hepatic cells is also shown (**Figure 1C**).

Taurine Attenuates the Hepatic Apoptosis via Both Fas- and Mitochondria-Dependent Apoptotic Pathways. Because Fas engagement is crucial on the induction of hepatic cell apoptosis (*32, 33*), here, we investigate the effects of taurine on Fas-dependent apoptotic proteins in the livers of NZB/W F1 mice. The expressions of Fas and caspase-8 proteins were examined by Western blot (**Figure 2**). A significant increase of Fas protein was observed in the livers of mice from the cholesterol group as compared to the control group, whereas a significant reduction of Fas was detected in the livers of mice from the cholesterol/taurine group as compared to the cholesterol group (**Figure 2A**). Additionally, the expression of caspase-8, a downstream molecule of Fas protein, and its cleaved forms were also investigated. **Figure 2B** shows the Western blot results of procaspase-8 and its two cleaved forms with molecular masses at 40 and 23 kDa, respectively. A significant increase of procaspase-8 was detected in mice from the cholesterol/taurine group as compared to the control or the cholesterol group, whereas a significant reduction of both cleaved caspase-8 was observed in the cholesterol/taurine group as compared to the control or the cholesterol group, respectively (**Figure 2B**). To further investigate the effect of taurine on mitochondrial-dependent apoptosis in the livers from NZB/W F1 mice, Western

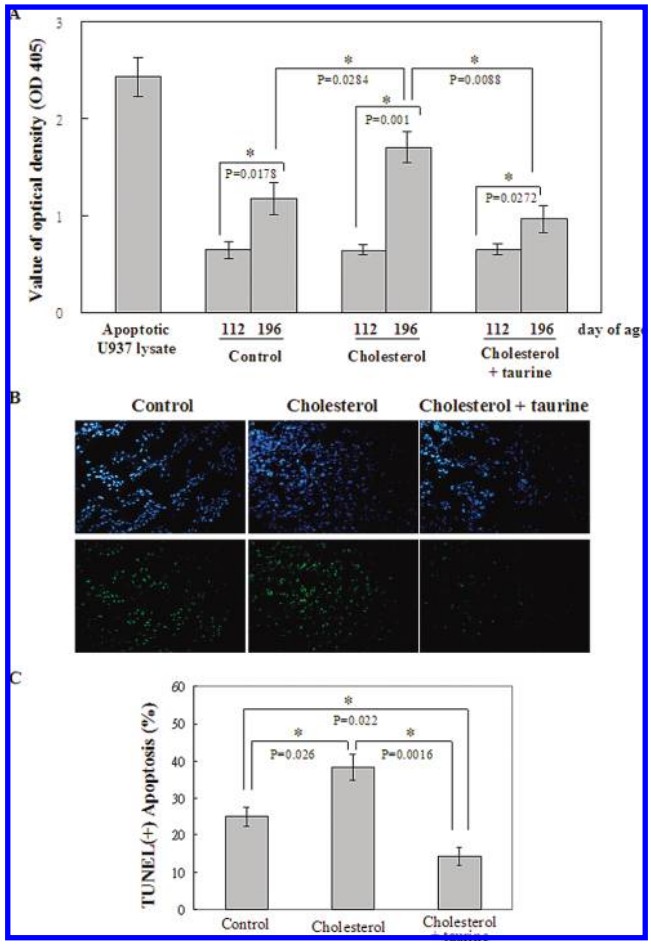

Figure 1. Detection of apoptosis. (**A**) The activity of caspase-3 was measured in 20 μg of liver lysates of NZB/W F1 mice from the control, cholesterol, and cholesterol/taurine groups, respectively. The UV-induced apoptotic U937 lysate that comes along with the kit was used as a positive control. (**B**) TUNEL assay of liver sections of NZB/W F1 mice from the control, cholesterol, and cholesterol/taurine groups. FITC-labeled terminal deoxy-transferase was bound to the nicked end of DNA as the arrows indicate. (**C**) The percentage of TUNEL-positive cells in liver sections of NZB/W F1 mice from the control, cholesterol, and cholesterol/taurine groups. Three independent experiments were performed, and * indicates the significant difference.

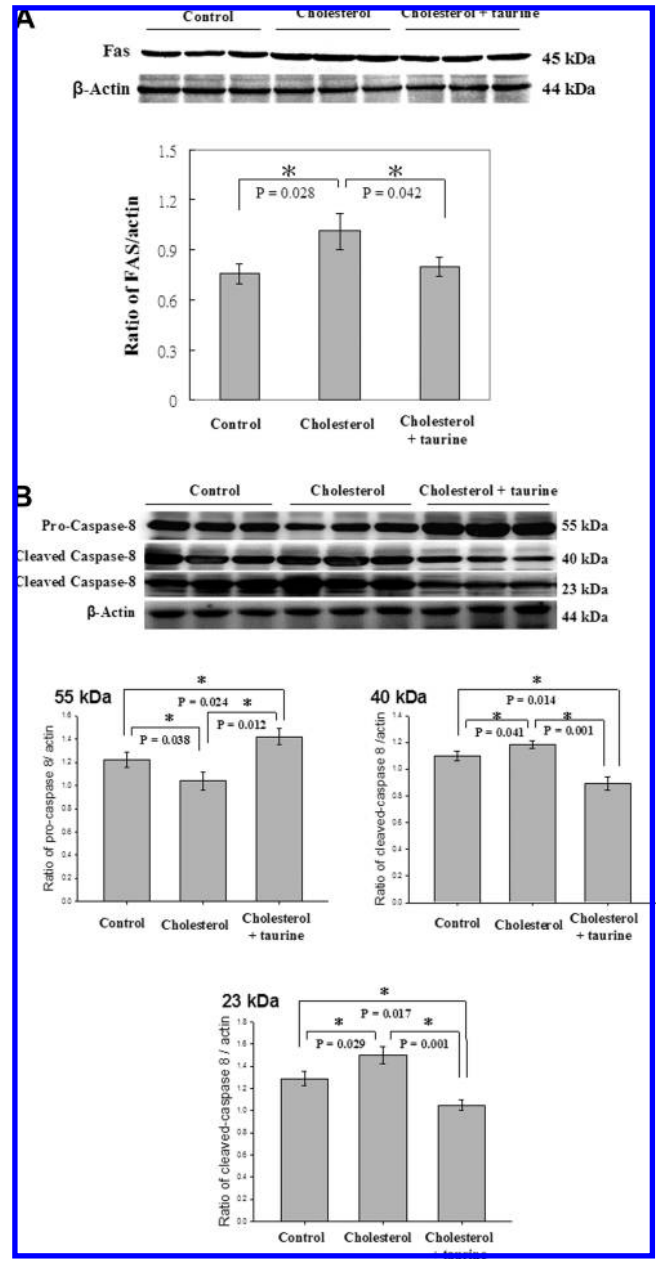

Figure 2. Expression of Fas and caspase-8. Liver lysates obtained from the control, cholesterol, and cholesterol/taurine groups were probed with antibody against (**A**) Fas or (**B**) caspase-8, respectively. Densitometric analysis is shown in the lower panel. Similar results were obtained in three independent experiments, and * indicates the significant difference.

blots were performed to examine the molecules involving the mitochondria-dependent apoptotic pathway. A significant increase of activate caspase-9 was detected in mice from the cholesterol group, whereas a significant reduction of active caspase-9 was observed in the livers of mice from the cholesterol/taurine group as compared to those from the control and the cholesterol group, respectively (**Figure 3A**). Additionally, Apaf-1, the upstream molecule of caspase-9, was significantly increased in the livers from the mice of the cholesterol group as compared to those from the control group. In contrast, a significant reduction of Apaf-1 expression was detected in the livers from the mice of the cholesterol/taurine group as compared to those from the cholesterol group (**Figure 3B**). Moreover, a significant increase of Bax and Bad proteins was detected in the livers of mice from the cholesterol group as compared to those from the control group, whereas a significant reduction of both Bax and Bad proteins was observed in the livers of mice from the cholesterol/taurine group as compared to those from the cholesterol group (**Figure 4**). Similar results were also observed in the expression of cytochrome *c* protein that was increased significantly in the livers of mice from the cholesterol group as compared to those from the control group (**Figure 5A**). In contrast, a significant reduction of cytochrome *c* protein was detected in the livers of mice from the cholesterol/taurine group (**Figure 5A**). Because Bcl-2 is known to play crucial roles in inhibiting cytochrome *c* expression (*34*), herein, we further examined the expression of Bcl-2 protein. Notably, the expression of Bcl-2 protein was significantly decreased in the livers of mice from the cholesterol group as compared to those from the control group, whereas a significant increase of Bcl-2 protein was observed in the livers of mice from the cholesterol/taurine group as compared to those from the cholesterol group (**Figure 5B**).

Signaling Pathway Involved in the Taurine-Reduced Hepatic Apoptosis in NZB/W F1 Mice. To clarify the possible signaling pathway involved in the effect of taurine on the livers

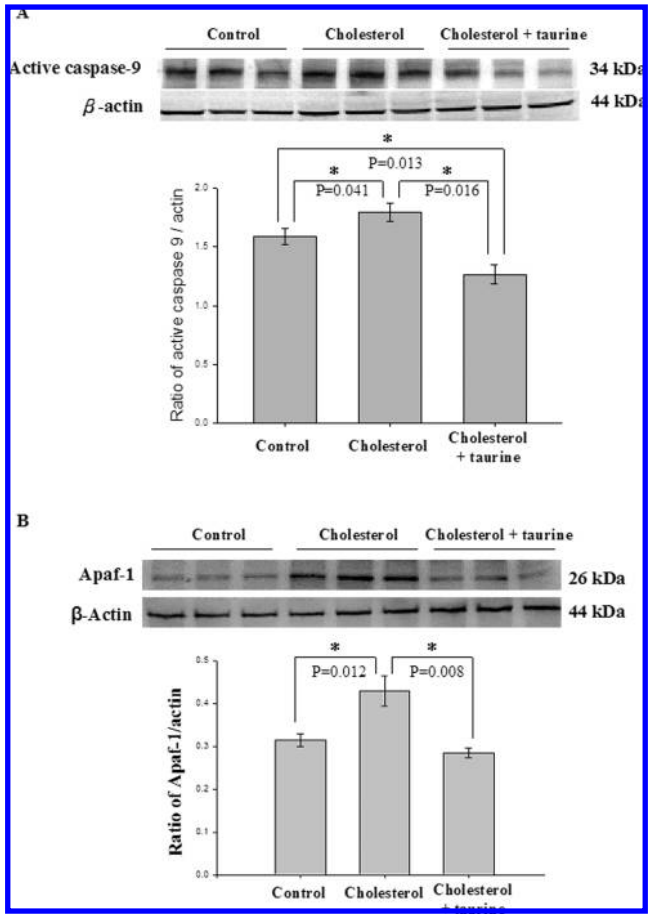

Figure 3. Expression of caspase-9 and Apaf-1. Liver lysates obtained from the control, cholesterol, and cholesterol/taurine groups were probed with antibody against (**A**) caspase-9 or (**B**) Apaf-1, respectively. Densitometric analysis is shown in the lower panel. Similar results were obtained in three independent experiments, and * indicates the significant difference.

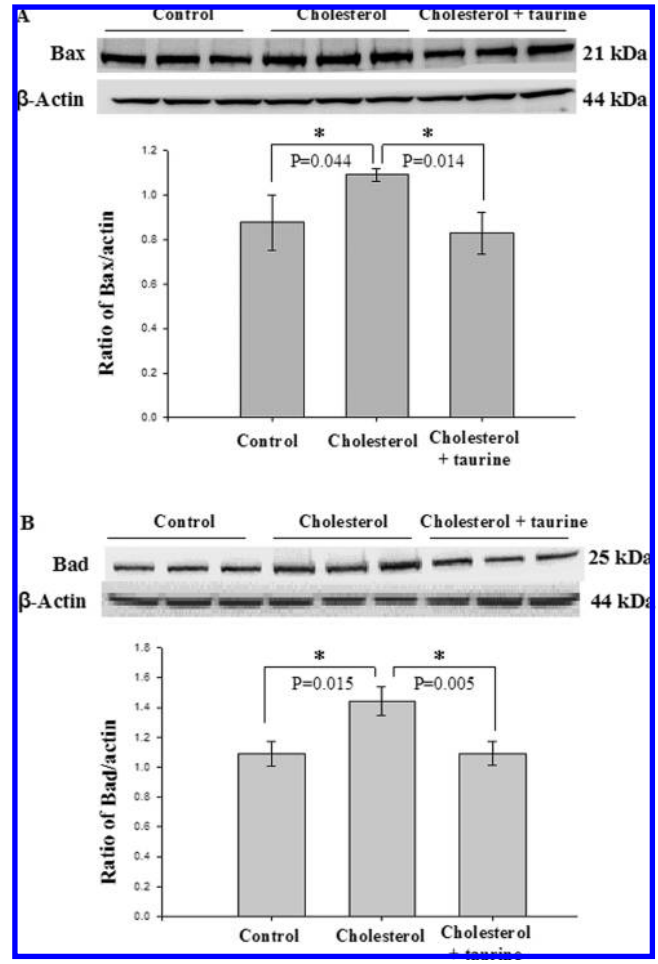

Figure 4. Expression of Bax and Bad. Liver lysates obtained from the control, cholesterol, and cholesterol/taurine groups were probed with antibody against (**A**) Bax or (**B**) Bad, respectively. Densitometric analysis is shown in the lower panel. Similar results were obtained in three independent experiments, and * indicates the significant difference.

from NZB/W F1 mice that were fed with a high-cholesterol diet, various signaling molecules, including AKT-p, Erk1/2-p, p38-p, and NF-κB (p65-p), were examined. The expression of both phosphorylated AKT and NF-κB (p65) protein was significantly increased in the livers of mice from the cholesterol/taurine group as compared to those from the cholesterol group (**Figure 6A**). The quantified results are shown in the lower panel. Similar results were observed that phosphorylated ERK-1/2 increased significantly in the livers of mice from the cholesterol/taurine group as compared to those from the cholesterol group (**Figure 6B**). Additionally, the expression of p38 protein was also examined, and no variation of total p38 was detected among all experimental groups (**Figure 7**). However, the expression of phosphorylated p38 protein was significantly increased in the livers of mice from the cholesterol group as compared to those from the control group (**Figure 7**). Notably, a significant decrease of phosphorylated P38 protein was detected in the livers of mice from the cholesterol/taurine as compared to those from the cholesterol group (**Figure 7**). The quantified results are shown in the lower panel (**Figure 7**).

DISCUSSION

High-cholesterol diets are known to cause various disorders including hepatic apoptosis, which has been recognized to play crucial roles in the pathogenesis of SLE. However, the mechanisms and treatments for hepatic apoptosis in SLE have been poorly understood. For the first time, this study revealed the aggravated condition of disease in NZB/W F1 mice fed a high-cholesterol diet by increased hepatic apoptosis. However, the treatment with taurine significantly reduced both Fas- and mitochondrial-dependent apoptosis in the liver from NZB/W F1 mice that were fed a high-cholesterol diet.

Liver dysfunction is known to induce apoptosis in patients with SLE and is recognized as an important factor for self-antigen exposure (*7, 35*). Indeed, hepatic abnormality is more common in SLE than is usually thought (*9, 13, 14*) and is postulated to the pathogenesis of SLE (*12*). Notably, increased abnormal lipid metabolism has been reported in patients with SLE and is considered as a consequence of hepatic abnormality (*9, 10*). Another study of lupus-prone mice, Ldl-r (−/−) strain, and a high-cholesterol diet could enhance arterial apoptosis and inflammation and accelerate atherosclerosis (*36*). These studies did postulate a connection among high-cholesterol diets, hepatic abnormality, and pathogenesis of SLE. In this study, beneficial effects of taurine are revealed in NZB/W F1 mice. A significant increase of Fas- and mitochondrial-dependent apoptosis was observed in the livers of NZB/W F1 mice from the cholesterol group. In contrast, a significant reduction of caspase-3 activity, TUNEL-positive cells, Fas, cleaved caspase-8, caspase-9, Bad, Bax, cytochrome *c*, and Apaf-1 was observed in the livers of NZB/W F1 mice from the cholesterol/taurine group. These experimental results suggest multiple

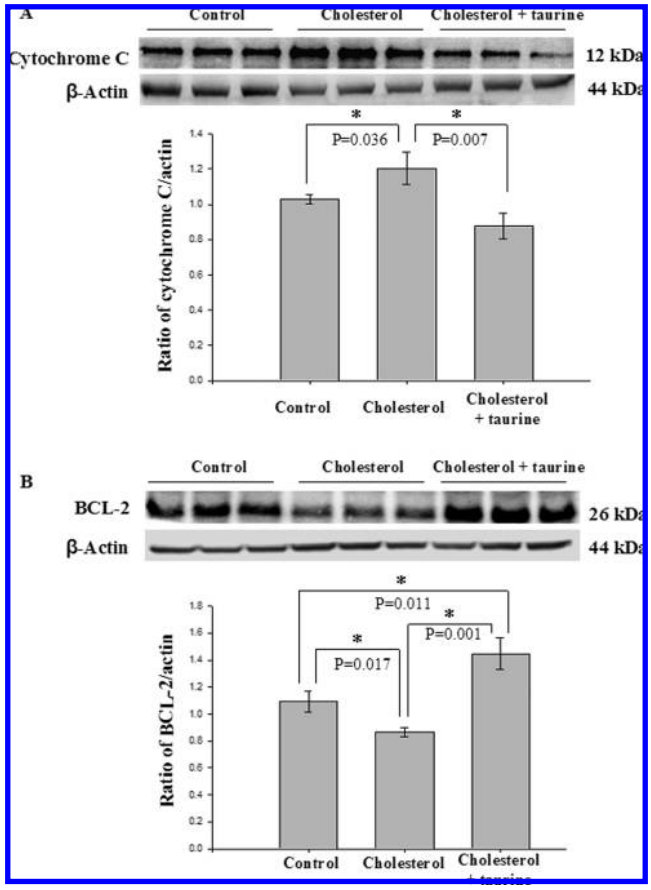

Figure 5. Expression of cytochrome *c* and Bcl-2. Liver lysates obtained from the control, cholesterol, and cholesterol/taurine groups were probed with antibody against (**A**) cytochrome *c* or (**B**) Bcl-2, respectively. Densitometric analysis is shown in the lower panel. Similar results were obtained in three independent experiments, and * indicates the significant difference.

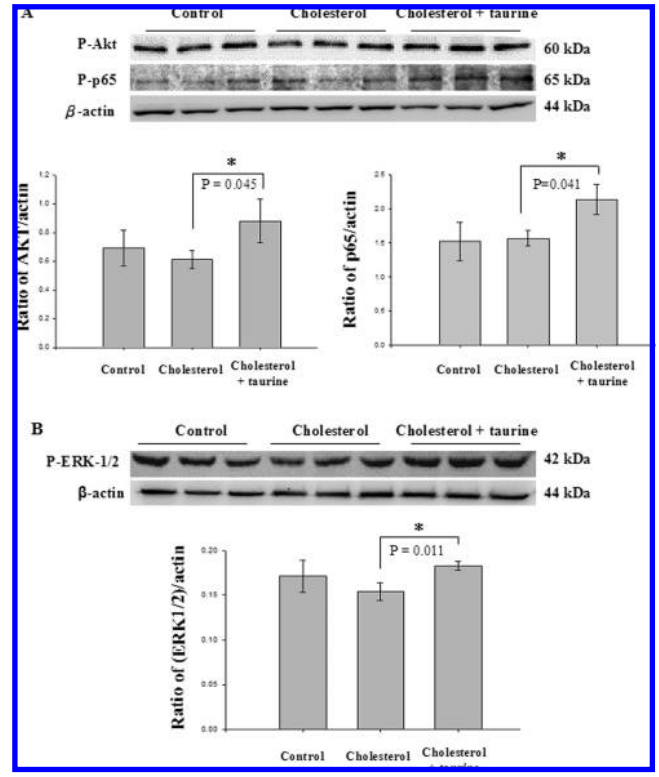

Figure 6. Expression of phosphoryalted Akt and ERK1/2. Liver lysates obtained from the control, cholesterol, and cholesterol/taurine groups were probed with antibody against phosphorylated (**A**) Akt or (**B**) ERK 1/2, respectively. Densitometric analysis is shown in the lower panel. Similar results were obtained in three independent experiments, and * indicates the significant difference.

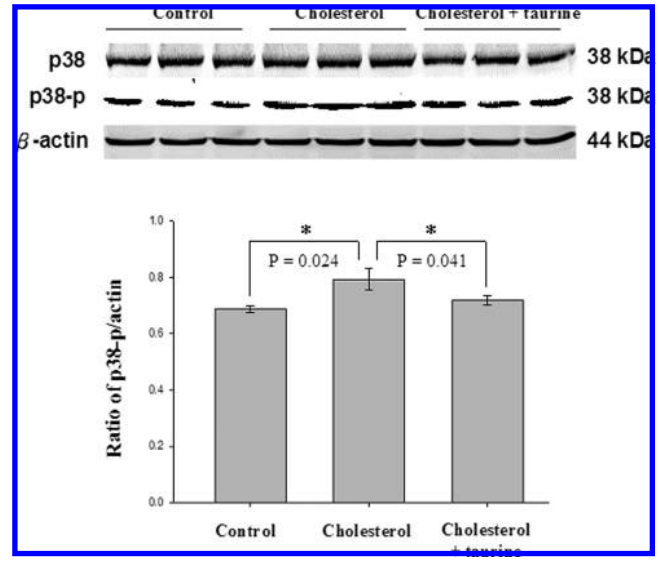

Figure 7. Expression of phosphorylated p38. Liver lysates obtained from the control, cholesterol, and cholesterol/taurine groups were probed with antibody against total p38 and phosphorylated p38. Densitometric analysis is shown in the lower panel. Similar results were obtained in three independent experiments, and * indicates the significant difference.

antiapoptosis effects of taurine in the livers from NZB/W F1 mice by inhibiting both Fas- and mitochondrial-dependent apoptotic pathways.

Taurine is known as the 2-aminoethanesulfonic acid and is important in bile salt synthesis, which is involved in a number of crucial physiological processes including modulation of calcium flux and neuronal excitability, osmoregulation, detoxification, and membrane stabilization (*37*). Various studies have demonstrated the protective effect of taurine against free radical-mediated damage in biological systems, including heart, liver, and kidney (*26, 38*), as well as the protective effect in the inhibition of hepatic apoptosis induced by bile acid in rats (*39, 40*). Recently, taurine and its derivatives have been indicated to promote the cell survival by activation of the ERK 1/2 and Akt/PKB signaling pathway, including the downstream effector of NF-kB (*41*−*44*). Additionally, taurine has been demonstrated to attenuate the phosphorylation of p38 protein (*45*) that is known as a mediator of apoptotic cell death (*46*). Notably, a similar effect of taurine in alleviating hepatic apoptosis was observed in this study. The phosphorylation of Akt, NF-kB, and ERK1/2 proteins increases significantly in the livers of mice from the cholesterol/taurine group as compared to those from the cholesterol group, whereas the significant decrease of phosphorylated-p38 protein in the cholesterol/taurine group as compared to the cholesterol group. These results indicated the involvement of the MAPK survival signaling pathway and p38-mediated apoptotic pathway in taurine-induced antihepatic apoptosis in NZB/W F1 mice. However, the physiological activities of taurine and their precise mechanisms are still unclear and merit further investigation.

Taken together, this study first demonstrated the beneficial effect of taurine by attenuating both Fas- and mitochondrial-dependent apoptosis in the livers from NZB/W F1 mice that were fed with a high-cholesterol diet. Besides, increased phosphorylation of AKT, ERK1/2, and NF-kB proteins and

decreased phosphorylation of p38 protein contributed to the taurine-induced antiapoptosis in the livers of NZB/W F1 mice that were fed a high-cholesterol diet. Indeed, these experimental results strongly suggest therapeutic potential of taurine supplements in SLE.

LITERATURE CITED

(1) Xu, H.; Barnes, G. T.; Yang, Q.; Tan, G.; Yang, D.; Chou, C. J.; Sole, J.; Nichols, A.; Ross, J. S.; Tartaglia, L. A.; Chen, H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* **2003**, *112*, 1821–1830.

(2) Kim, S.; Sohn, I.; Ahn, J. I.; Lee, K. H.; Lee, Y. S.; Lee, Y. S. Hepatic gene expression profiles in a long-term high-fat diet-induced obesity mouse model. *Gene* **2004**, *340*, 99–109.

(3) Carmiel-Haggai, M.; Cederbaum, A. I.; Nieto, N. A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *FASEB J.* **2005**, *19*, 136–138.

(4) Milagro, F. I.; Campión, J.; Martínez, J. A. Weight gain induced by high-fat feeding involves increased liver oxidative stress. *Obesity* **2006**, *14*, 1118–1123.

(5) Kleemann, R.; Verschuren, L.; van Erk, M. J.; Nikolsky, Y. O.; Cnubben, N. H.; Verheij, E. R.; Smilde, A. K.; Hendriks, H. F.; Zadelaar, S.; Smith, G. J.; Kaznacheev, V.; Nikolskaya, T.; Melnikov, A.; Hurt-Camejo, E.; van der Greef, J.; van Ommen, B.; Kooistra, T. Atherosclerosis and liver inflammation induced by increased dietary cholesterol intake: a combined transcriptomics and metabolomics analysis. *Genome Biol.* **2007**, *8*, R200.

(6) Otogawa, K.; Kinoshita, K.; Fujii, H.; Sakabe, M.; Shiga, R.; Nakatani, K.; Ikeda, K.; Nakajima, Y.; Ikura, Y.; Ueda, M.; Arakawa, T.; Hato, F.; Kawada, N. Erythrophagocytosis by liver macrophages (Kupffer cells) promotes oxidative stress, inflammation, and fibrosis in a rabbit model of steatohepatitis: Implications for the pathogenesis of human nonalcoholic steatohepatitis. *Am. J. Pathol.* **2007**, *170*, 967–980.

(7) Mukai, M.; Bohgaki, T.; Notoya, A.; Kohno, M.; Tateno, M.; Kobayashi, S. Liver dysfunction due to apoptosis in a patient with systemic lupus erythematosus. *Lupus* **2000**, *9*, 74–77.

(8) Hsu, T. C.; Huang, C. Y.; Chiang, S. Y.; Lai, W. X.; Tsai, C. H.; Tzang, B. S. Transglutaminase inhibitor cystamine alleviates the abnormality in liver from NZB/W F1 mice. *Eur. J. Pharmacol.* **2008**, *579*, 382–389.

(9) Abraham, S.; Begum, S.; Isenberg, D. Hepatic manifestations of autoimmune rheumatic diseases. *Ann. Rheum. Dis.* **2004**, *63*, 123–129.

(10) Chung, C. P.; Oeser, A.; Solus, J.; Avalos, I.; Gebretsadik, T.; Shintani, A.; Linton, M. F.; Fazio, S.; Stein, C. M. Inflammatory mechanisms affecting the lipid profile in patients with systemic lupus erythematosus. *J. Rheumatol.* **2007**, *34*, 1849–1854.

(11) Hahn, B. H. An overview of the pathogenesis of systemic lupus erythematosus. In *Dubois' Lupus Erythematosus*; Wallace, D. J., Hahn, B. H., Eds.; Williams and Wilkins: Philadelphia, 1993; pp 69−76.

(12) Gaipl, U. S.; Kuhn, A.; Sheriff, A.; Munoz, L. E.; Franz, S.; Voll, R. E.; Kalden, J. R.; Herrmann, M. Clearance of apoptotic cells in human SLE. *Curr. Dir. Autoimmun.* **2006**, *9*, 173–187.

(13) Mackay, I. R.; Taft, L. I.; Cwoling, D. C. Lupoid hepatitis and the hepatic lesions of systemic lupus erythematosus. *Lancet* **1959**, *1*, 65–69.

(14) Runyon, B. A.; LaBrecque, D. R.; Anuras, S. The spectrum of liver disease in systemic lupus erythematosus report of 33 histologically-proved cases and review of the literature. *Am. J. Med.* **1980**, *69*, 187–194.

(15) Tzang, B. S.; Chiang, S. Y.; Lai, W. X.; Tsai, C. C.; Wu, J. H.; Hsu, T. C. Treatment with cystamine reduces apoptosis in liver from NZB/W F1 mice. *Int. Immunopharmacol.* **2008**, *8*, 589–596.

(16) Amasaki, Y.; Kobayashi, S.; Takeda, T.; Ogura, N.; Jodo, S.; Nakabayashi, T.; Tsutsumi, A.; Fujisaku, A.; Koike, T. Up-regulated expression of Fas antigen (CD95) by peripheral naive and memory T cell subsets in patients with systemic lupus erythematosus (SLE): A possible mechanism for lymphopenia. *Clin. Exp. Immunol.* **1995**, *99*, 245–250.

(17) Louzir, B.; Ben Abdelhafidh, N.; Bahri, M.; M'Sadek, F.; Ksontini, I.; Boussema, E.; Othmani, S. Association of systemic lupus erythematosis and myasthenia gravis. A new case report. *Tunis. Med.* **2003**, *81*, 963–966.

(18) Chesney, R. W. Taurine: Its biological role and clinical implications. *Adv. Pediatr.* **1985**, *32*, 1–42.

(19) Huxtable, R. J. Physiological actions of taurine. *Physiol. Rev.* **1992**, *72*, 101–163.

(20) Redmond, H. P.; Wang, J. H.; Bouchier-Hayes, D. Taurine attenuates nitric oxide- and reactive oxygen intermediate-dependent hepatocyte injury. *Arch. Surg.* **1996**, *131*, 1280–1287.

(21) Kontny, E.; Szczepanska, K.; Kowalczewski, J.; Kurowska, M.; Janicka, I.; Marcinkiewicz, J.; Maslinski, W. The mechanism of taurine chloramine inhibition of cytokine (interleukin-6, interleukin-8) production by rheumatoid arthritis fibroblast-like synoviocytes. *Arthritis Rheum.* **2000**, *43*, 2169–2177.

(22) Grimble, R. F. Sulphur amino acids and the metabolic response to cytokines. *Adv. Exp. Med. Biol.* **1994**, *359*, 41–49.

(23) Huxtable, R. J. Taurine past, present, and future. *Adv. Exp. Med. Biol.* **1996**, *403*, 641–650.

(24) Chorazy, M.; Kontny, E.; Marcinkiewicz, J.; Maslinski, W. Taurine chloramine modulates cytokine production by human peripheral blood mononuclear cells. *Amino Acids* **2002**, *23*, 407–413.

(25) Hagar, H. H.; El Etter, E.; Arafa, M. Taurine attenuates hypertension and renal dysfunction induced by cyclosporine A in rats. *Clin. Exp. Pharmacol. Physiol.* **2006**, *33*, 189–196.

(26) Saad, S. Y.; Al-Rikabi, A. C. Protection effects of taurine supplementation against cisplatin-induced nephrotoxicity in rats. *Chemotherapy* **2002**, *48*, 42–48.

(27) Racasan, S.; Braam, B.; van der Giezen, D. M.; Goldschmeding, R.; Boer, P.; Koomans, H. A.; Joles, J. A. Perinatal l-arginine and antioxidant supplements reduce adult blood pressure in spontaneously hypertensive rats. *Hypertension* **2004**, *44*, 83–88.

(28) Takatani, T.; Takahashi, K.; Uozumi, Y.; Shikata, E.; Yamamoto, Y.; Ito, T.; Matsuda, T.; Schaffer, S. W.; Fujio, Y.; Azuma, J. Taurine inhibits apoptosis by preventing formation of the Apaf-1/caspase-9 apoptosome. *Am. J. Physiol. Cell. Physiol.* **2004**, *287*, C949–C953.

(29) Cetiner, M.; Sener, G.; Sehirli, A. O.; Ekşioğlu-Demiralp, E.; Ercan, F.; Sirvanci, S.; Gedik, N.; Akpulat, S.; Tecimer, T.; Yeğen, B. C. Taurine protects against methotrexate-induced toxicity and inhibits leukocyte death. *Toxicol. Appl. Pharmacol.* **2005**, *209*, 39–50.

(30) Karpuj, M. V.; Becher, M. W.; Springer, J. E.; Chabas, D.; Youssef, S.; Pedotti, R.; Mitchell, D.; Steinman, L. Prolonged survival and decreased abnormal movements in transgenic model of Huntington disease, with administration of the transglutaminase inhibitor cystamine. *Nat. Med.* **2002**, *8*, 143–149.

(31) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–684.

(32) Wang, X. Z.; Zhang, S. J.; Chen, Y. X.; Chen, Z. X.; Huang, Y. H.; Zhang, L. J. Effects of platelet-derived growth factor and interleukin-10 on Fas/Fas-ligand and Bcl-2/Bax mRNA expression in rat hepatic stellate cells in vitro. *World J. Gastroenterol.* **2004**, *10*, 2706–2710.

(33) Zhang, L. J.; Zheng, W. D.; Shi, M. N.; Wang, X. Z. Effects of interleukin-10 on activation and apoptosis of hepatic stellate cells in fibrotic rat liver. *World J. Gastroenterol.* **2006**, *12*, 1918–1923.

(34) Shen, Y.; White, E. p53-dependent apoptosis pathways. *Adv. Cancer Res.* **2001**, *82*, 55–84.

(35) Al-Maini, M. H.; Mountz, J. D.; Al-Mohri, H. A.; El-Ageb, E. M.; Al-Riyami, B. M.; Svenson, K. L.; Zhou, T.; Richens, E. R. Serum levels of soluble Fas correlate with indices of organ damage in systemic lupus erythematosus. *Lupus* **2000**, *9*, 132–9.

(36) Gautier, E. L.; Huby, T.; Ouzilleau, B.; Doucet, C.; Saint-Charles, F.; Gremy, G.; Chapman, M. J.; Lesnik, P. Enhanced immune system activation and arterial inflammation accelerates atherosclerosis in lupus-prone mice. *Arterioscler., Thromb., Vasc. Biol.* **2007**, *27*, 1625–1631.

(37) Kendler, B. S. Taurine: An overview of its role in preventive medicine. *Prev. Med.* **1989**, *18*, 79–100.

(38) Hagar, H. H. The protective effect of taurine against cyclosporine-A induced oxidative stress and hepatotoxicity in rats. *Toxicol. Lett.* **2004**, *151*, 335–343.

(39) Graf, D.; Kurz, A. K.; Reinehr, R.; Fischer, R.; Kircheis, G.; Häussinger, D. Prevention of bile acid-induced apoptosis by betaine in rat liver. *Hepatology* **2002**, *36*, 829–839.

(40) Schoemaker, M. H.; Conde de la Rosa, L.; Buist-Homan, M.; Vrenken, T. E.; Havinga, R.; Poelstra, K.; Haisma, H. J.; Jansen, P. L.; Moshage, H. Tauroursodeoxycholic acid protects rat hepatocytes from bile acid-induced apoptosis via activation of survival pathways. *Hepatology* **2004**, *39*, 1563–1573.

(41) Song, G.; Ouyang, G.; Bao, S. The activation of Akt/PKB signaling pathway and cell survival. *J. Cell. Mol. Med.* **2005**, *9*, 59–71.

(42) Dent, P.; Fang, Y.; Gupta, S.; Studer, E.; Mitchell, C.; Spiegel, S.; Hylemon, P. B. Conjugated bile acids promote ERK1/2 and AKT activation via a pertussis toxin-sensitive mechanism in murine and human hepatocytes. *Hepatology* **2005**, *42*, 1291–1299.

(43) Deldicque, L.; Theisen, D.; Bertrand, L.; Hespel, P.; Hue, L.; Francaux, M. Creatine enhances differentiation of myogenic C2C12 cells by activating both p38 and Akt/PKB pathways. *Am. J. Physiol. Cell. Physiol.* **2007**, *293*, C1263–C1271.

(44) Raufman, J. P.; Shant, J.; Guo, C. Y.; Roy, S.; Cheng, K. Deoxycholyltaurine rescues human colon cancer cells from apoptosis by activating EGFR-dependent PI3K/Akt signaling. *J. Cell. Physiol.* **2008**, *215*, 538–549.

(45) Xu, Y. J.; Saini, H. K.; Zhang, M.; Elimban, V.; Dhalla, N. S. MAPK activation and apoptotic alterations in hearts subjected to calcium paradox are attenuated by taurine. *Cardiovasc. Res.* **2006**, *72*, 163–174.

(46) Feuerstein, G. Z.; Young, P. R. Apoptosis in cardiac diseases: Stress- and mitogen-activated signaling pathways. *Cardiovasc. Res.* **2000**, *45*, 560–569.

Received for review July 3, 2008. Revised manuscript received August 25, 2008. Accepted September 3, 2008. This study was supported by Grants NSC96-2320-B-040-025-MY3 from the National Science Council and Department of Health, Taiwan, Republic of China.

JF8020255