

Original Article

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## Alleviative Effects of Taurine on Hepatic Fibrosis and Cox-2 Expression in Lupus-prone Mice

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**Purpose:** There is growing evidence of a possible association between high-cholesterol diet and hepatic abnormality in systemic lupus erythematosus (SLE). To investigate the effects of taurine on the hepatic abnormality in SLE, NZB/W F1 mice were randomly divided into three groups: a control group, cholesterol group, and a cholesterol plus taurine group.

**Methods:** Hematoxylin-Eosin staining and Immunoblots were used to perform the analysis.

**Results:** Mice treated with cholesterol plus taurine had improvements in both hepatic cleft and irregular architecture in the liver, compared to the other two groups. Additionally,  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), the hepatic associated factor, was significantly more decreased in mice treated with cholesterol and taurine than in those of the other two groups.

Moreover, mice treated with cholesterol and taurine had significant greater decrease in Cyclooxygenase-2 (Cox-2) protein in the liver than the other groups.

**Conclusions:** Our findings suggested that taurine is a beneficial dietary supplement for alleviating hepatic fibrosis and inflammation in SLE.

**Keywords:** systemic lupus erythematosus (SLE), taurine, liver fibrosis

### Introduction

SLE is known as an autoimmune disorder of unknown etiology that impacts a variety of

organs, including the liver<sup>[1-2]</sup>. Recent evidence has indicated that impaired clearance of apoptotic cells play a crucial role in the development of SLE by exposure of self-antigens<sup>[3]</sup>, which also induces the liver dysfunction in SLE patients. Indeed, many studies have reported an increase in population of SLE patients with hepatic abnormality<sup>[4-6]</sup>. Moreover, growing evidence has shown that increased hepatic dysfunction, apoptosis, and fibrosis in patients with SLE are associated with disease status<sup>[2, 7-8]</sup>. Notably, our recent studies have also detected significantly higher hepatic apoptosis in NZB/W F1 mice<sup>[2, 9]</sup>. However, the

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precise pathogenesis and possible treatments for the development of hepatic abnormality in SLE are still unclear.

Taurine, a conditionally essential amino acid, plays important roles in a variety of immune responses<sup>[10-11]</sup>. Taurine has been suggested to exhibit antioxidant properties in regulating the expression of various inflammatory associated cytokines<sup>[12-13]</sup>. Besides, taurine has been demonstrated to reduce blood pressure in a hypertensive rat model<sup>[14]</sup> and attenuate hypertension and renal dysfunction in a hepatotoxic rat model<sup>[15-16]</sup>. Additionally, taurine also reduces ischemia-induced apoptosis in cardiac cells<sup>[17]</sup>. Notably, taurine reduced the hepatic apoptosis in livers of NZB/W F1 mice receiving a high-cholesterol diet in our recent study<sup>[2]</sup>.

Although these findings support the potential importance of taurine in biological protection, little is known about the effect of taurine on hepatic fibrosis in SLE. In the current study, we further investigated the beneficial effects of taurine on hepatic fibrosis and Cox-2 expression in NZB/W F1 mice receiving a high-cholesterol diet to evaluate the therapeutic potential of taurine in SLE.

## Materials and Methods

### Mice and liver samples

Female NZB/W F1 mice, a well-known and popularly 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. Chow diet, soybean oil, and cholesterol were purchased (TestDiet Division, PMI Nutrition International/Purina Mills LLC, Richmond, IN, USA). Taurine was purchased from Sigma (Sigma, St. Louis, MO, USA), and the ingredients of the experimental diets were prepared as follows and described in our recent publications<sup>[2, 18]</sup>. Briefly, 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 (16 weeks) old were divided into three groups (10 mice/group) and given control, cholesterol, and cholesterol/taurine diets for 12 weeks, respectively. Mice were sacrificed at the age of 196 days (28-week) old by CO<sub>2</sub> asphyxiation and the mice were rinsed in 70% ethanol solution. Liver samples of the mice were obtained after CO<sub>2</sub> sacrifice and stored at -80 °C until use.

### Hematoxylin-Eosin Staining

The hepatic tissues from NZB/W F1 mice were embedded into OCT compound (Tissue-Tek, Miles Inc.) and snap frozen in liquid nitrogen. The frozen sections were sectioned at 5 μm and soaked in 50% ethanol before immersion in the dark of eosin solution in 70% ethanol for 20 min. The sections were then washed with 50% ethanol and immersed in hematoxylin solution for 3 min as the negative stain. Photomicrographs were obtained using Zeiss Axiophot microscopes.

### Immunoblots

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 belonging to the same group. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12.5% acrylamide gel) was performed as previously described<sup>[2]</sup>. 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 or tissue section was then soaked in PBS with 2% gelatin for 30 min at room temperature to saturate irrelevant protein binding sites. Antibodies against α-SMA, Cox-2 (Upstates, Charlottesville, VA, USA) and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) 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, USA) was used to detect antigen-antibody complexes. The blots were also scanned and quantified by densitometry (Appraise, Beckman-Coulter, Brea, CA, USA).

### Statistical Analysis

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

## Results

### Hepatic Histopathological Changes in NZB/W F1 mice

To investigate the effect of taurine on hepatic architecture in NZB/W F1 mice fed with high-cholesterol, we performed histopathological analysis with hematoxylin and eosin (Fig.1). Markedly disarray of hepatocyte and clefts were observed in liver-sections of NZB/W F1 mice that were fed with this diet compared to those mice

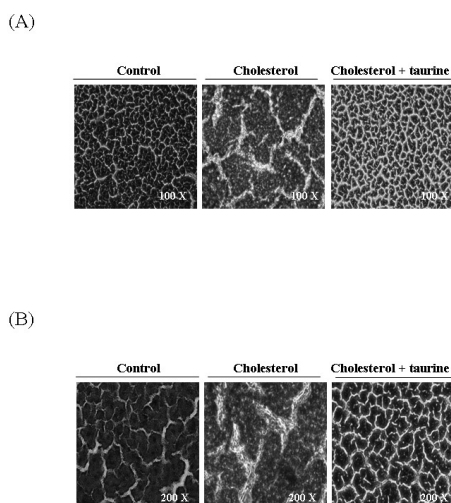


Fig. 1. (A) Histopathological analysis of hepatic tissue sections with hematoxylin and eosin staining and was observed at magnified 100 times and (B) 200 times. Similar results were obtained in three independent experiments

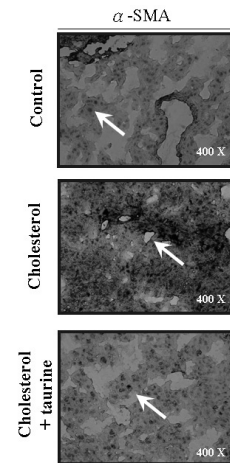


Fig. 2. Presence of  $\alpha$ -SMA. Hepatic tissue sections of NZB/W F1 mice obtained from the control, cholesterol, and cholesterol/taurine groups probed with antibody against  $\alpha$ -SMA and observed at magnified 400 times. Similar results were obtained in three independent experiments.

from control group. Notably, hepatocyte disarray and clefts were observed in liver-sections of rats feeding with cholesterol plus taurine diet compared to those mice from cholesterol group.

### Presence of $\alpha$ -SMA in livers of NZB/W F1 mice

To verify the influence of taurine on hepatic fibrosis, we performed hepatic tissue sections and Immunoblots to detect the presence of  $\alpha$ -SMA protein in livers of NZB/W F1 mice receiving different dietary supplements. A Marked presence of  $\alpha$ -SMA protein was observed in hepatic tissue sections of NZB/W F1 mice that were fed the cholesterol diet compared to those mice fed the control diet (Fig.2). In contrast, there was an obviously reduced presence was detected in livers of NZB/W F1 mice that were fed the cholesterol plus taurine diet compared to those fed the cholesterol diet (Fig. 2). Similar results were observed in liver lysates of NZB/W F1 mice fed the cholesterol plus taurine diet compared to those mice from control or cholesterol group, respectively (Fig. 3A). Quantified results are shown in Figure 3B.

### Expression of Cox-2 protein in livers of NZB/W F1 mice

To examine the influence of taurine on inflammatory associated protein in livers of NZB/W F1 mice fed with high-cholesterol, we measured expression of Cox-2. Figure 4 shows the results of immunoblotting for Cox-2 in livers of NZB/W F1 mice. A significant increase in Cox-2 protein was detected in the hepatic tissue sections of NZB/W F1 mice fed the cholesterol diet compared to those fed the control diet (Fig. 4A). In contrast, a significant reduction in Cox-2 expression was observed in the livers of NZB/W F1 mice fed the cholesterol plus taurine diet compared to those fed the cholesterol diet (Fig. 4A). Quantified results are shown in Figure 4B.

### Discussion

Hepatic abnormality is disorder found worldwide and common in patients with SLE. A variety of studies have indicated an increase in number of SLE patients with hepatic dysfunction, apoptosis, and fibrosis<sup>[2, 7-8]</sup>. Recently, we have reported the beneficial effects of taurine on hepatic abnormality and apoptosis in lupus-prone mice<sup>[2]</sup>, and it has been found to be beneficial to hearts<sup>[18]</sup>. In this study, we further reported the alleviative effects of taurine on hepatic fibrosis and inflammation through its reduction of the expression of  $\alpha$ -SMA and Cox-2.

Alpha-smooth muscle actin ( $\alpha$ -SMA) is associated with fibrosis and commonly known as an early marker of renal scarring progression and response to treatment<sup>[19]</sup>. Recently, renal alpha-smooth muscle actin is considered as a new prognostic factor for lupus nephritis<sup>[20]</sup>. Since taurine plays crucial roles in many immune responses<sup>[10-11]</sup> and has been demonstrated to be beneficial on SLE<sup>[2, 18]</sup>, we investigated the effect of taurine on hepatic fibrosis in NZB/W F1 mice and demonstrated its alleviative effect on fibrosis in SLE reducing the expression of  $\alpha$ -SMA.

Cyclooxygenase type 2(Cox-2), a pro-inflammation factor, plays crucial roles in inflammation, carcinogenesis, hemodynamics, and renal function<sup>[21-22]</sup>. A growing body of evidence

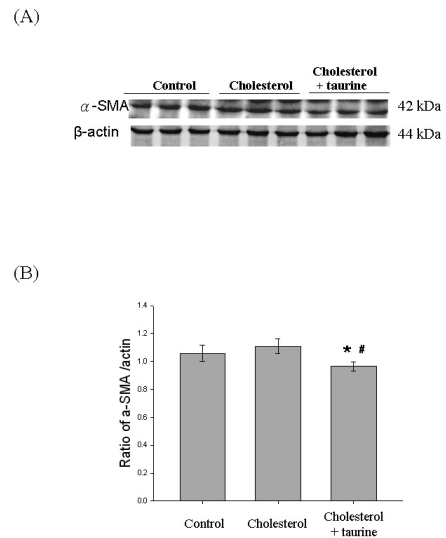


Fig. 3. Expression  $\alpha$ -SMA. Liver lysates of NZB/W F1 mice obtained from the control, cholesterol, and cholesterol/taurine groups were probed with antibody against (A) $\alpha$ -SMA. (B) Bars depict the results of the densitometric analysis relative to  $\beta$ -actin. \* and # indicate significant differences as compared to the control or cholesterol group, respectively.

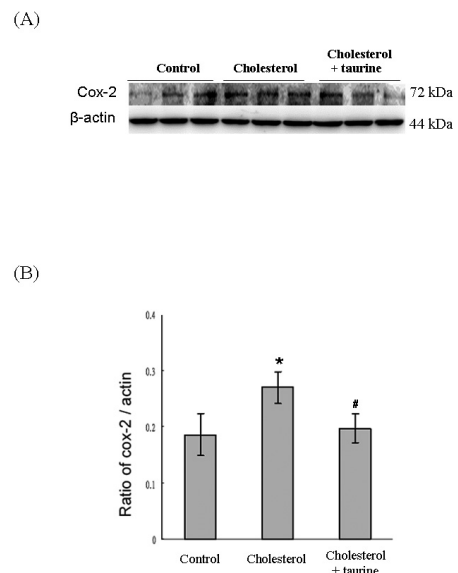


Fig. 4. Expression Cox-2. Liver lysates of NZB/W F1 mice obtained from the control, cholesterol, and cholesterol/taurine groups were probed with antibody against (A) Cox-2. (B) Bars depict the densitometric analysis relative to  $\beta$ -actin. \* and # indicate significant differences as compared to the control or cholesterol group, respectively.



suggests that increased Cox-2 is associated with a variety of liver disorders including inflammation<sup>[23-25]</sup>. Additionally, Cox-2 has been also associated with the pathogenesis of SLE. Cox-2 inhibitors have been found able to suppress the production of pathogenic autoantibodies to DNA by causing autoimmune T-cell apoptosis<sup>[26]</sup>. The results of our study were similar. We found a significant decrease Cox-2 in liver of NZB/W F1 mice fed with cholesterol and taurine compared to those mice from the control and the cholesterol groups.

## Conclusion

Altogether, these findings strongly suggest that taurine has beneficial effects on hepatic fibrosis and inflammation through its reduction of the expression of  $\alpha$ -SMA and Cox-2. It also suggests that taurine has therapeutic potential for the treatment of SLE with hepatic abnormality.

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## 牛磺酸減緩狼瘡小鼠肝纖維化及環氧合酶蛋白質表現

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**目的：**目前已有許多證據指出，高膽固醇食物與紅斑性狼瘡病人中，肝臟異常之關聯性。為瞭解牛磺酸對紅斑性狼瘡肝臟異常的影響，我們將NZB/W F1小鼠隨機分為三組，分別餵食對照食物、膽固醇食物、牛磺酸加上膽固醇食物。

**方法：**我們採用蘇木紫—伊紅染色法以及西方墨點法來進行相關的分析。

**結果：**實驗結果發現餵食膽固醇加上牛磺酸食物的狼瘡小鼠比起餵食膽固醇食物的小鼠，其肝臟細胞呈現較規則排列，且肝臟中纖維化相關蛋白質 $\alpha$ -SMA的表現顯著降低，此外，重要的發炎相關蛋白質及環氧合酶也呈現顯著下降。

**結論：**這些實驗結果證明牛磺酸能減緩狼瘡小鼠肝臟纖維化蛋白質及環氧合酶的表現。

**關鍵詞：**紅斑性狼瘡、牛磺酸、肝臟纖維化

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