European Review for Medical and Pharmacological Sciences 2017; 21: 1868-1875

The protective effects of taurine on experimental autoimmune myocarditis

Y.-F. LU[1], Q.-Y. ZHANG[2], L.-H. WANG[3], X.-Y. LIU[4], S.-X. ZHANG[5,6]

[1]Department of Clinical Laboratory, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong, China
[2]Department of Clinical Laboratory Penglai Traditional Chinese Medicine Hospital Yantai, Shandong, China
[3]Department of Ultrasonography, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong, China
[4]Department of Reproductive Medicine, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong, China
[5]Biochip Laboratory, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong, China Yantai, China
[6]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Education Ministry of China, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China

Abstract. – OBJECTIVE: To investigate the protective effects of taurine on experimental autoimmune myocarditis and its mechanisms. BALB/c mice were immunized with porcine cardiac myosin to induce experimental autoimmune myocarditis (EAM).

MATERIALS AND METHODS: We administered taurine (5 mg/kg, 10 mg/kg or 20 mg/kg) or vehicle to EAM mice daily. On day 21, the severity of myocarditis was evaluated by determination of heart weight/body weight ratio (Hw/Bw), histopathological and echocardiographic examination of heart tissue. The levels of Th1 and Th2 cytokines in serum were measured by ELISA. Moreover, ELISA was also used to determine the levels of MDA, SOD and GSH-Px in heart homogenates.

RESULTS: The mice treated with taurine had significantly decreased Hw/Bw ($p < 0.05$). Treatment with 10 or 20 mg/ kg taurine prevented the LV dysfunction, significantly increased the LVEDs, LVEDd and LVPW. Furthermore, Th1 cytokines (TNF-α, IFN-γ and IL-2) were significantly downregulated, accompanied by Th2 cytokines (IL-4 and IL-10) markedly upregulated after treatment with taurine. Meanwhile, the activities of SOD and GSH-Px in heart tissues significantly increased, while the content of MDA significantly decreased after treatment with taurine.

CONCLUSIONS: The results indicated that taurine has a protective effect against EAM by modulating Th1/Th2 cytokine balance and suppressing of oxidative stress.

Key Words:
Experimental autoimmune myocarditis, Taurine, Th1/Th2 balance, Oxidative stress.

Introduction

Myocarditis, a representative inflammatory heart disease, is the precursor of dilated cardiomyopathy (DCM) and can cause severe left ventricular (LV) dysfunction, arrhythmia or even cardiac sudden death[1,2]. Although the precise mechanisms for the development of myocarditis are not fully understood, there is substantial evidence suggestion that its pathogenesis is related to natural killer cells, viral-specific cytotoxic T cells, and antimyosin antibodies. Experimental autoimmune myocarditis (EAM) induced by inoculation with cardiac myosin in rodents is the most widely animal model, due to the highest similarity between the fulminant form and histopathological features of EAM and human myocarditis[3,4].

EAM in mice is a T-cell mediated disease characterized by infiltration of T cells and macrophages, leading to massive myocardial necrosis which later develops into dilated cardiomyopathy in the chronic phase[5,6]. Compelling evidence suggested that proinflammatory cytokines and chemokines secreted by both macrophages and T cells played an important role in the induction and progression of EAM[7]. Th1 type cytokines (IL-2, TNF-α and IFN-γ) have been detected only in the inflammatory phase. However, the production of Th2 cytokines (IL-6 and IL-10) have been found in the recovery phase. IL-6 and IL-10 produced mainly by antigen-presenting cells has been suggested to play

Corresponding Author: Shouxin Zhang, MD; e-mail: 9712zhangsx@163.com

a key role in the development of EAM[8,9]. Targeted anti-inflammation by regulation of Th1/Th2 balance may be represent possible strategic points for therapeutic intervention of EAM. Despite the prominent inflammatory process is involved in the pathogenesis of EAM, recent researches[10,11] have reported the significant participation of oxidative myocardial injury in the development of heart failure. Taurine, one of the most abundant non-essential amino acids in mammals, has many physiological functions in the nervous, cardiovascular, renal, endocrine, and immune systems [12]. It has been reported exhibiting functions of anti-inflammatory and antioxidant properties in several models of cardiovascular diseases[13,14]. Therefore, the purpose of the present work was to examine the effect of taurine on EAM, focusing on its inhibitory effects on inflammatory and oxidative stress.

Materials and Methods

Animals

Sixty BALB/c mice (male, 8 weeks old) were purchased from the Experimental Animal Center of Suzhou Aiermaite Technology (Co., Ltd. SPF grade, Certificate No. SCXK20150012, Suzhou, Jiangsu, China). All experiments were performed in accordance with the guidelines of Animal Experiments of Yuhuangding Hospital of Yantai, Affiliated Hospital of Qingdao Medical University. Animals were kept in the departmental animal house under controlled conditions of 23±3°C, relative humidity of 40-70% and a 12 h light/dark cycle. They were fed with food pellets and water ad libitum.

Model of EAM and Treatment Regimen

Mice were randomly assigned to five groups (n=10 for each): (1) control; (2) non-treated EAM; (3) EAM+ taurine (5 mg/kg); (4) EAM+ taurine (10 mg/kg); (5) EAM+ taurine (20 mg/kg). Mice in EAM and EAM + taurine groups were injected subcutaneously with 300 μg of porcine cardiac myosin (Sigma-Aldrich, St. Louis, MO, USA) in a 1:1 emulsion with complete Freund's adjuvant (CFA) containing Mycobacterium tuberculosis H37Ra on days 1 and 8, as previously described[16]. Mice in control group received injections of equivalent volume of CFA. From day 1 after the first immunization, mice in EAM + taurine groups were intraperitoneal treated with taurine (5 mg/kg, 10 mg/kg, or 20 mg/kg) daily for consecutive 21 days. The doses were used based on our preliminary experiments. The mice in control and EAM groups were given equivalent volume of saline.

Blood Pressure, Heart Rate (HR) and Body Weight (BW)

Blood pressure, heart rate and body weight of all mice were measured on the last day of treatment. Blood pressure (systolic, diastolic and mean pressure), hear rate (HR) and body weight (BW) were determined in conscious mice by using a tail cuff system (BP-98A, Softron Co., Tokyo, Japan).

Echocardiogram

Transthoracic echocardiography was performed on animals anesthetized by intraperitoneal administration (0.1 mL/10 g body weight) of 3.6% chloral hydrate in saline on the last day of treatment. An echocardiographic machine with a 14-MHz transducer (Toshiba, Tokyo, Japan) was used for left ventricular echocardiographic recording. M-mode echocardiography was conducted first in the parasternal long-axis view to measure. Left ventricular end diastolic diameter (LVEDd), left ventricular end-systolic diameter (LVEDs). Left ventricular posterior wall thickness (LVPW) was determined by the leading-edge method. Left ventricular fractional shortening (LVFS) and left ventricular ejection fraction (LVEF) were calculated as previously described[15].

Histopathology

Hearts were harvested immediately after all mice were sacrificed by the cutting of the abdominal aorta under deep anesthesia on the last day of treatment. After measuring the heart weight, a mid-ventricular section was obtained and slices were stained with hematoxylin and eosin. The histopathological changes were observed by light microscopy.

Cytokines Measurement

Blood samples were collected by heart puncture immediately after echocardiographic measurements. Then, the serum levels of tumor necrosis factor-alpha (TNF-α), Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-10 (IL-10) and interferon-gamma (INF-γ) were determined by using enzyme-linked immunosorbent assay (ELISA) kits (Nanjing Jiancheng Co., Nanjing, Jiangsu, China) according to the manufacturer's instructions.

Detection of Biomarkers of Oxidative Stress in Heart Homogenates

The myocardium was homogenized in 9 (v/v) volumes of ice-cold phosphate buffer saline (PBS), and the homogenates were centrifuged at 3000 × g for 15 min at 4°C to obtain the supernatant. The malondialdehyde (MDA) content, superoxide dismutase (SOD) and glutathione peroxidases (GSH-Px) activities were measured by ELISA kits (Nanjing Jiancheng Co., Nanjing, Jiangsu, China) according to the manufacturer's instructions.

Statistical Analysis

Data statistical analysis was performed by SPSS 18.0 (SPSS Inc., Chicago, IL, USA) and all data were expressed as means ± SD. Changes between samples were compared by Student's *t*-test, and differences between groups were compared by the method of one-way ANOVA. LSD test was used to post-hoc test ANOVA. $p < 0.05$ was considered statistically significant.

Results

Effect of Taurine on HR, Blood Pressure and Heart Weight

As shown in Table I, although HR, SBP and DBP were not different among the five groups of mice ($p > 0.05$), the HW/BW ratio in non-treated EAM group was significantly increased than the control group ($p < 0.01$), taurine treatment markedly reduced the HW/BW ratio ($p < 0.05$).

Effect of Taurine on Echocardiographic Parameters

The heart function and cardiac structure in mice were examined using echocardiography. As demonstrated in Table II, LVFS and LVEF in non-treated EAM were greatly decreased compared with control mice ($p < 0.05$, $p < 0.01$), and treatment with 10 or 20 mg /kg taurine prevented the LV dysfunction. Moreover, LVEDs, LVEDd and LVPW were significantly increased in non-treated EAM group, treatment with 10 or 20 mg/kg taurine inhibited the increase in those parameters. These results showed that taurine treatment could prevent the development of LV remodeling and preserve cardiac function of EAM mice.

Taurine Treatment Ameliorates the Myocarditis

Twenty-one days after immunization, five serial sections from each heart were analyzed after hematoxylin and eosin staining. Myocarditis was assessed by identifying both inflammatory cells infiltrating and myocyte necrosis. As shown in Figure 1, tissue sections from control mice myocardium showed a normal myofibrillar structure with striations, a branched appearance, and continuity with adjacent myofibrils. Tissues from non-treatment mice revealed myocardial necrosis, degeneration, infiltrating inflammatory cells and destruction of myocardial fibers. Treatment with taurine ameliorated the inflammatory cells infiltrating and myocyte necrosis.

Effect of Taurine on Inflammatory Cytokines

In this study, we examined the levels of inflammatory cytokines. As shown in Figure 2, 21 days after EAM induction, the serum levels of IL-2 and INF-γ were significantly increased in mice of VMC group compared with control mice ($p < 0.01$), and TSA treatment decreased those cytokine production in

Table I. HR, blood pressure and heart weight in mice with EAM on day 21.

	Control (n = 12)	Non-treated EAM (n = 12)	EAM+ taurine 5 mg/kg (n = 12)	EAM+ taurine 5 mg/kg (n = 12)	EAM+ taurine 5 mg/kg (n = 12)
Body weight (g)	38.33 + 2.17	27.64 + 2.23**	29.64 + 1.17	30.72 + 1.99$^{\#}$	32.53 + 1.71$^{\#}$
Heart weight (g)	0.13 + 0.06	0.18 + 0.02	0.17 + 0.01	0.16 +0.02	0.15 + 0.13
HW/BW (mg/g)	3.39 + 0.18	6.51 + 0.32**	5.74 + 0.28$^{\#}$	5.21 + 0.27$^{\#}$	4.61 + 0.31$^{\#}$
HR (beats/min)	532 + 49	654 + 59	592 + 57	564 + 51	551 + 42
SBP (mmHg)	99.8 + 11.7	86.4 + 8.3	93.6 + 8.7	88.5 + 7.9	87.1 + 8.3
DBP (mmHg)	77.5 + 6.2	59.7 + 7.7	68.2 + 5.3	61.2 + 5.3	56.2 + 4.7

HW/BW, ratio of heart weight to body weight; Results are presented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$ *vs*. control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs*. non-treated EAM group.

Table II. Echocardiographic parameters in mice with EAM on day 21.

	Control (n = 12)	Non-treated EAM (n = 12)	EAM+ taurine 5 mg/kg (n = 12)	EAM+ taurine 5 mg/kg (n = 12)	EAM+ taurine 5 mg/kg (n = 12)
LVEDd (mm)	4.76 + 0.31	5.62 + 0.27*	5.53 + 0.21$^{\#}$	5.33 + 0.27$^{\#}$	5.11 + 0.29$^{\#}$
LVEDs (mm)	3.41 + 0.19	4.54 + 0.32*	4.49 + 0.43$^{\#}$	4.17 + 0.32$^{\#}$	3.89 + 0.23$^{\#}$
LVFS (%)	31.59 + 0.27	17.68 + 0.16*	21.09 + 0.31$^{\#}$	21.97 + 0.25$^{\#}$	26.22 + 0.25$^{\#}$
LVPWd (mm)	0.45 + 0.01	0.56 + 0.02*	0.57 + 0.02	0.47 + 0.03$^{\#}$	0.47 + 0.02$^{\#}$
LVEF (%)	57.09 + 3.17	38.18 + 5.41**	41.63 + 2.46$^{\#}$	44.65 + 2.12$^{\#}$	52.22 + 2.81$^{\#\#}$

Results are presented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$ *vs*. control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs*. non-treated EAM group.

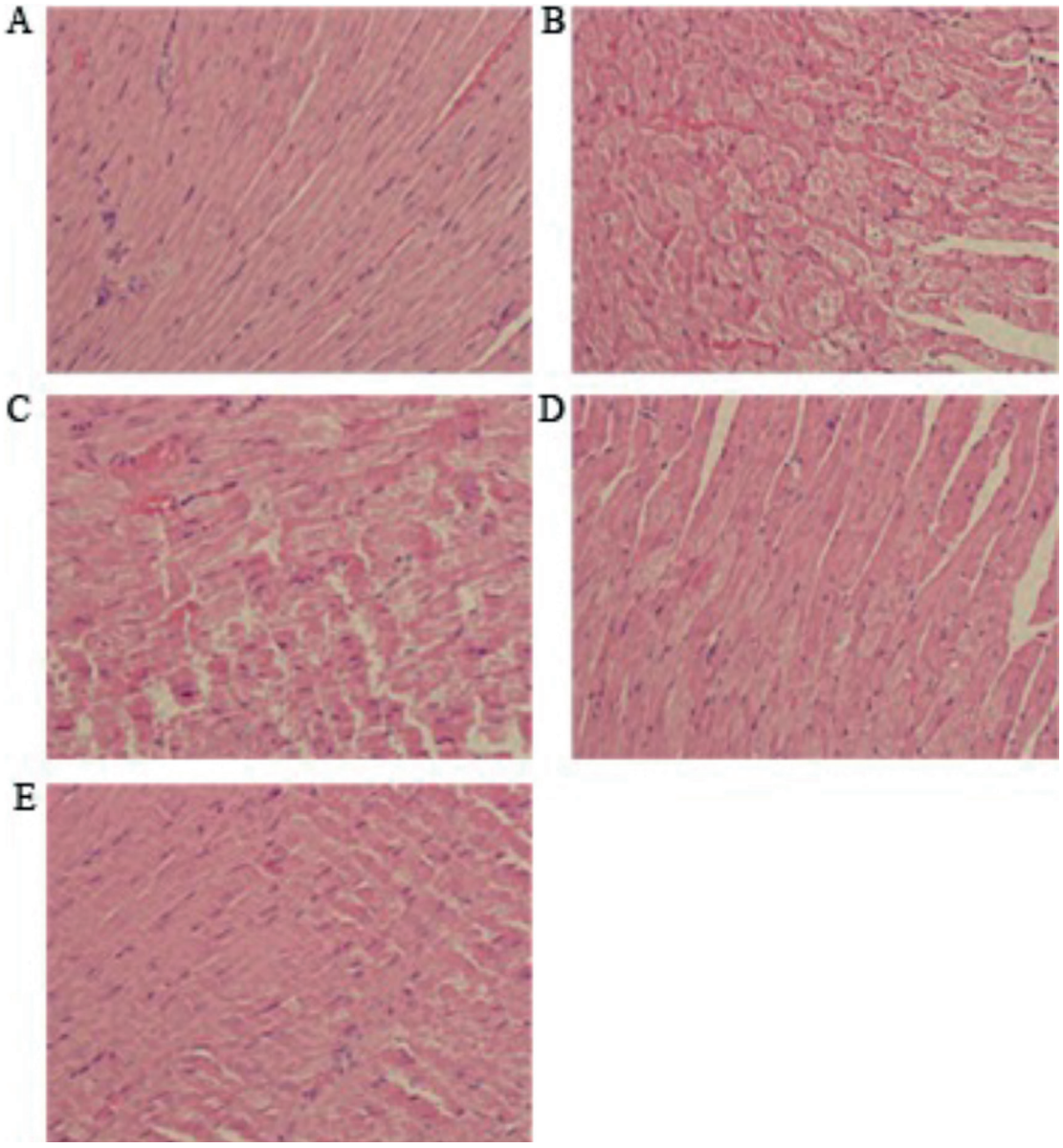

Figure 1. Effect of taurine on heart histopathologic changes in EAM mice (×200). ***(A)*** Control group; ***(B)*** non-treated EAM group; ***(C)*** EAM + 5 mg taurine group; ***(D)*** EAM + 10 mg taurine group; ***(E)*** EAM + 20 mg taurine group.

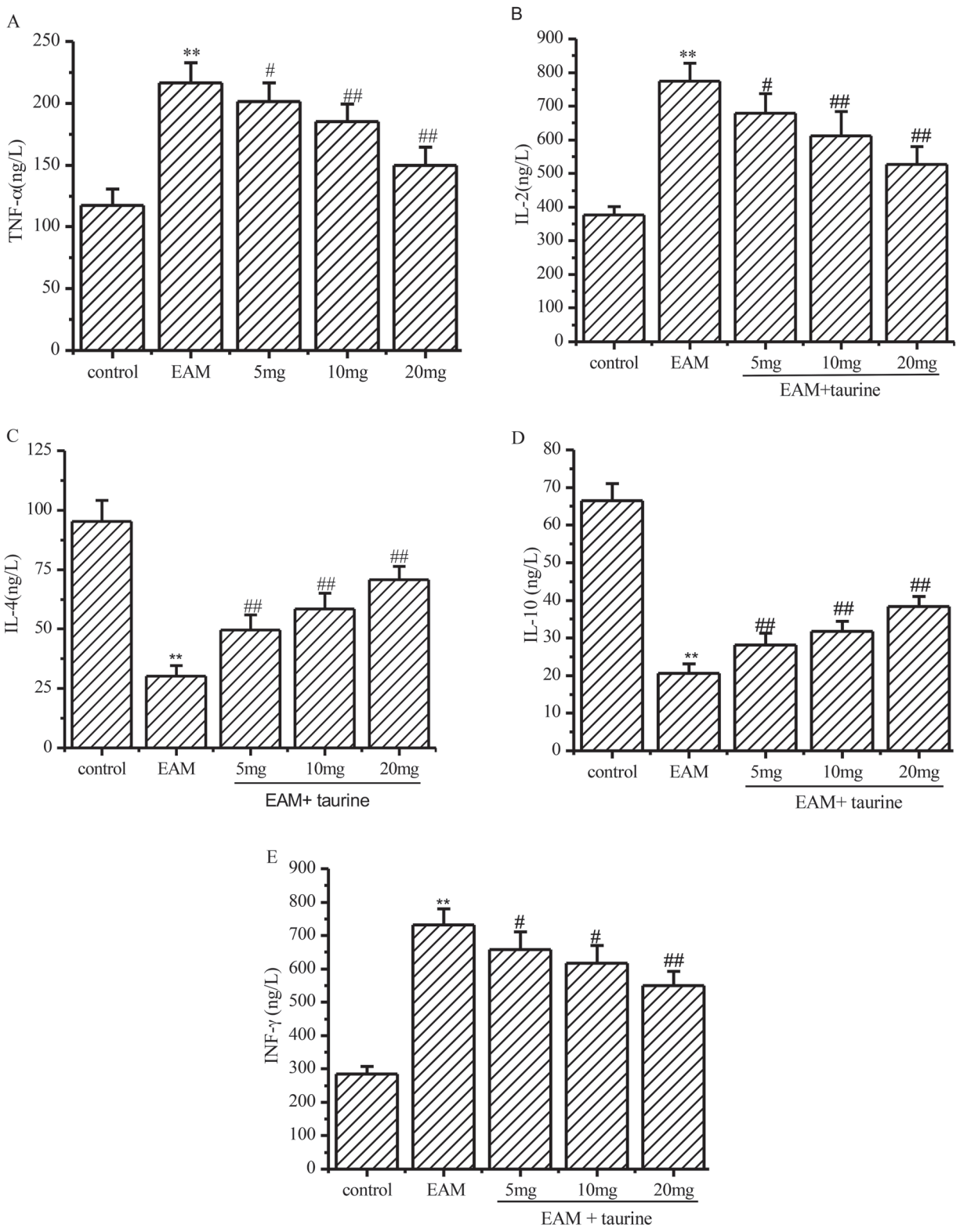

Figure 2. Effect of taurine on cytokines of Th1 (TNF-α, IL-2 and INF-γ) and Th2 (IL-4, IL-10) ***A-E,*** TNF-α, IL-2, IL-4, IL-10 and INF-γ, respectively. Date are presented as mean ± SD. $^{**}p < 0.01$ *vs.* control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* EAM + tauine group.

myocarditis mice. In contrast, the serum levels of IL-4 and IL-10 were significantly lowered in taurine-treated groups, either EAM or healthy control, compared with the non-treated groups.

Effect of Taurine on MDA Content, SOD and GSH-Px Activities

As shown in Figure 3, the MDA content in non-treated EAM mice was significantly higher than those in control mice ($p < 0.01$), taurine treatment decreased the MDA content. SOD and GSH-Px activities were decreased in non-treated EAM mice compared with control mice, and taurine treatment restored SOD and GSH-Px activities.

Discussion

Myocarditis is a severe, potentially lethal disease that is mostly induced by infectious agents[16]. Autoimmune mechanisms play a crucial role in myocarditis, and immunomodulatory therapy might be a potential strategy to reduce myocarditis damage[17]. This report demonstrated that taurine could attenuate the development of EAM, at least in part, through ameliorating deteriorated inflammation and oxygenation. Also, the taurine-induced shift from Th1 cells toward Th2 cells and the up-regulation of NF-κB may also be associated with inhibition of EAM. Previous reports have demonstrated that mice are visibly ill on day 21 after the first immunization, so we evaluated the effects of taurine on the onset and

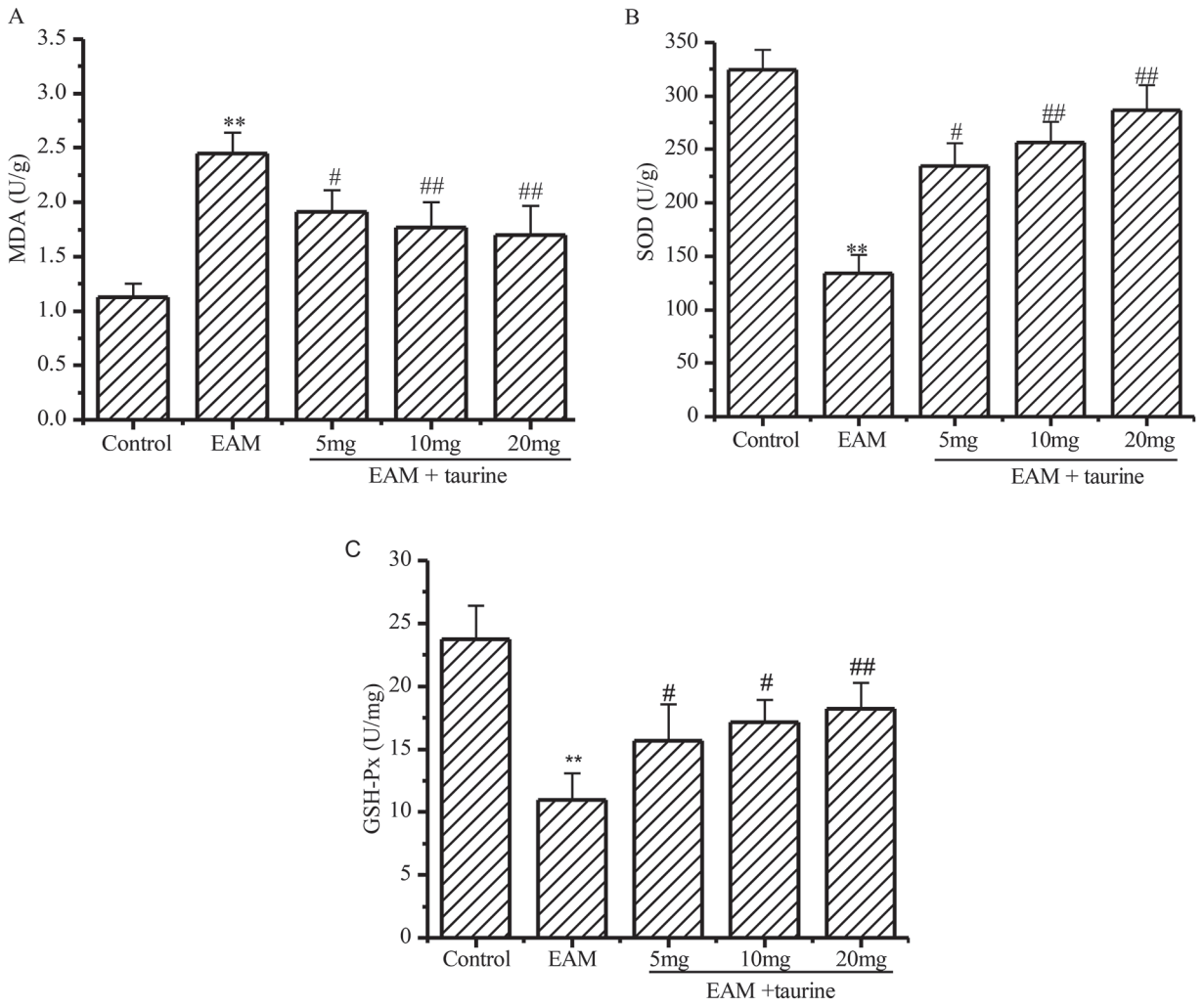

Figure 3. Effect of taurine on ***(A)*** MDA content, ***(B)*** SOD and ***(C)*** GSH-Px activity. Date are presented as mean ± SD. $^{**}p < 0.01$ *vs.* control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* EAM + tauine group.

ongoing EAM at this time. In the present study, mice in non-treatment EAM group exhibited a higher HW/BW ratio (one marker reflecting the severity of myocarditis), and HW/BW ratio decreased by taurine treatment. Meanwhile, histopathologic analysis indicated that infiltration of inflammatory cells and myocyte necrosis were markedly ameliorated by taurine treatment. These results suggested that taurine could effectively alleviate the severity of myocarditis. Additionally, echocardiography examination showed 10 or 20 mg/kg taurine prevented the LV dysfunction, and inhibited the increase of LVEDs, LVEDd and LVPW. The results indicated that treatment with taurine prevented the development of LV remodeling and the progression to heart failure. Several clinical studies[18,19] have implied that inflammatory cytokines play an important role in the pathogenesis of cardiac diseases and in the activation of detrimental autoimmune responses. In these studies, Th1 cytokine including TNF-α, IL-2 and INF-γ were significantly elevated, while Th2 cytokines including IL-4, IL-10 were significantly reduced in EAM mice. Thus, cytokines released by inflammatory cell exert a crucial role in the development of myocarditis, and myosin-induced EAM is associated with a Th1-polarized immunoresponse. This result was consistent with another reported study[15]. It is notable that, taurine treatment significantly down-regulated the levels of Th1 cytokine and significantly up-regulated Th2 cytokine in myocarditis mice, which indicated that taurine reduces the severity of EAM by suppression of Th1 immune response and augmentation of Th2 response. The imbalance between the occurrence of reactive oxygen species and the cellular antioxidant defense mechanism plays a key role in myocardial injury of viral myocarditis[20]. The critical role of both free radicals and oxidative stress in the development of heart failure have been demonstrated[21,22]. Some enzymes, such as SOD and GSH-Px, provide cellular protection against damage from oxygen-derived free radicals. The SOD is an important enzyme in maintaining the dynamic balance between O_2- production and elimination[23]. GSH-Px acts as an enzymatic antioxidant both intracellularly and extracellularly, in conjunction with various enzymatic processes that reduce hydrogen peroxide (H_2O_2) and hydroperoxides[24]. MDA is a major reactive aldehyde that appears during the peroxidation of biological membrane polyunsaturated fatty acids, so the content of MDA can be used as an indicator of heart tissue damage[25]. In our work, we found that MDA content significantly increased on day 21 after the first immunization, accompanied by a significantly decrease in SOD and GSH-Px activities, which proved that oxidative stress was elevated in EAM model. However, taurine treatment could ameliorate these abnormalities. These results indicated that taurine can protect heart tissues against harmful effects of oxidative stress by modulating SOD, GSH-Px and MDA activities.

Conclusions

The current study firstly demonstrated that taurine treatment could reduce the severity of EAM by regulation of Th1/Th2 cytokine production and inhibition oxidative stress. These results suggested that taurine might have a potential to be a novel method for the clinical treatment of myocarditis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Hirakawa H, Zempo H, Ogawa M, Watanabe R, Suzuki J, Akazawa H, Komuro I, Isobe MA. DPP-4 inhibitor suppresses fibrosis and inflammation on experimental autoimmune myocarditis in mice. PLoS One 2015; 10: e0119360.
2) Sukumaran V, Watanabe K, Veeraveedu PT, Ma M, Gurusamy N, Rajavel V, Suzuki K, Yamaguchi K, Kodama M, Aizawa Y. Telmisartan ameliorates experimental autoimmune myocarditis associated with inhibition of inflammation and oxidative stress. Eur J Pharmacol 2011; 652: 126-135.
3) Pando R, Barshack I, Raz A, Luboshits G, Haklai R, Maysel-Auslender S, Kloog Y, Keren G, George J. The Ras antagonist farnesylthiosalicylic acid ameliorates experimental myocarditis in the rat. Cardiovasc Pathol 2010; 19: 94-101.
4) Zempo H, Sugita Y, Ogawa M, Watanabe R, Suzuki J, Isobe M. A P2X7 receptor antagonist attenuates experimental autoimmune myocarditis via suppressed myocardial CD4+ T and macrophage infiltration and NADPH oxidase 2/4 expression in mice. Heart Vessels 2015; 30: 1-7.
5) Lee JH, Kim TH, Park HE, Lee EG, Jung NC, Song JY, Seo HG, Seung KB, Chang K, Lim DS. Myosin-primed tolerogenic dendritic cells ameliorate experimental autoimmune myocarditis. Cardiovasc Res 2014; 101: 203-210.

6) Lin YH, Chen XM, Zhang JW, He XQ, Dai WJ, Dai WJ, Chen MS. Preclinical study on induction of pluripotent stem cells from urine of dilated cardiomyopathy patients. Eur Rev Med Pharmacol Sci 2016; 20: 1450-1457.

7) Song ZC, Wang ZS, Bai JH, Li Z, Hu J. Emodin, a naturally occurring anthraquinone, ameliorates experimental autoimmune myocarditis in rats. Tohoku J Exp Med 2012; 227: 225-230.

8) MilenkoviĐ M, Arsenović-Ranin N, Stojić-Vukanić Z, Bufan B, Vučićević D, Jančić I. Quercetin ameliorates experimental autoimmune myocarditis in rats. J Pharm Pharm Sci 2010; 13: 311-319.

9) Cao H, Zhou XH, Zhang JP, Huang XL, Zhai Y, Zhang XJ, Chu L. Hydrogen sulfide protects against bleomycin-induced pulmonary fibrosis in rats by inhibiting NF-κB expression and regulating Th1/Th2 balance. Toxicol Lett 2014; 224: 387-394.

10) Sahan E, Sshan S, KaramanlioĐlu M, Gül M, Kalayci S, Boyaci A, Dereagzi F. The impact of the extent and severity of coronary artery disease on fractional flow reserve measurements. Eur Rev Med Pharmacol Sci 2016; 20: 3434-3439.

11) Shimada K, Uzui H, Ueda T, Lee JD, Kishimoto C. N-Acetylcysteine ameliorates experimental autoimmune myocarditis in rats via nitric oxide. J Cardiovasc Pharmacol Ther 2015; 20: 203-210.

12) Kim CK, Cha YN. Taurine chloramine produced from taurine under inflammation provides anti-inflammatory and cytoprotective effects. Amino Acids 2014; 46: 89-100.

13) Alhumaidha KA, Saleh DO, Abd El, Fattah MA, El-Eraky WI, Moawad H. Cardiorenal protective effect of taurine against cyclophosphamide-induced toxicity in albino rats. Can J Physiol Pharmacol 2015; 18: 1-9.

14) Maia AR, Batista TM, Victorio JA, Clerici SP, Delbin MA, Carneiro EM, Davel AP. Taurine supplementation reduces blood pressure and prevents endothelial dysfunction and oxidative stress in post-weaning protein-restricted rats. PLoS One 2014; 9: e105851.

15) Zhang S, Liu X, Sun C, Yang J, Wang L, Liu J, Gong L, Jing Y. Apigenin attenuates experimental autoimmune myocarditis by modulating Th1/Th2 cytokine balance in mice. Inflammation 2016; 39: 678-686.

16) Kishimoto C, Nimata M, Okabe TA, Shioji K. Immunoglobulin treatment ameliorates myocardial injury in experimental autoimmune myocarditis associated with suppression of reactive oxygen species. Int J Cardiol 2013; 167: 140-145.

17) Yuan J, Yu M, Li HH, Long Q, Liang W, Wen S, Wang M, Guo HP, Cheng X, Liao YH. Autophagy contributes to IL-17-induced plasma cell differentiation in experimental autoimmune myocarditis. Int Immunopharmacol 2014; 18: 98-105.

18) Martín R, Cordova C, San Román JA, Gutierrez B, Cachofeiroc V, Nietob ML. Oleanolic acid modulates the immune-inflammatory response in mice with experimental autoimmune myocarditis and protects from cardiac injury. Therapeutic implications for the human disease. J Mol Cell Cardiol 2014; 72: 250-262.

19) Sukumaran V, Veeraveedu PT, Gurusamy N, Lakshmanan AP, Yamaguchi K, Ma M, Suzuki K, Nagata M, Takagi R, Kodama M, Watanabe K. Olmesartan attenuates the development of heart failure after experimental autoimmune myocarditis in rats through the modulation of ANG 1-7 mas receptor. Mol Cell Endocrinol 2012; 351: 208-219.

20) Tajiri K, Shimojo N, Sakai S, Machino-Ohtsuka T, Imanaka-Yoshida K, Hiroe M, Tsujimura Y, Kimura T, Sato A, Yasutomi Y, Aonuma K. Pitavastatin regulates helper t-cell differentiation and ameliorates autoimmune myocarditis in mice. Cardiovasc Drugs Ther 2013; 27: 413-424.

21) Park H, Park H, Hwang HJ, Hwang HS, Kim H, Choi BR, Pak HN, Lee MH, Chung JH, Joung B. Alpha B-crystallin prevents ventricular arrhythmia by attenuating inflammation and oxidative stress in rat with autoimmune myocarditis. Int J Cardiol 2015; 182: 399-402.

22) Takamura C, Suzuki JI, Ogawa M, Watanabe R, Tada Y, Maejima Y, Akazawa H, Komuro I, Isobe M. Suppression of murine autoimmune myocarditis achieved with direct renin inhibition. J Cardiol 2016; 68: 253-260.

23) Fengqin L, Yulin W, Xiaoxin Z, Youpeng J, Yan C, Qing-qing W, Hong C, Jia S, Lei H. The heart-protective mechanism of Qishaowuwei formula on murine viral myocarditis induced by CVB3. J Ethnopharmacol 2010; 127: 221-228.

24) Abdel-Wahab BA, Metwally ME. Clozapine-induced cardiotoxicity: role of oxidative stress, tumour necrosis factor alpha and NF-κB. Cardiovasc Toxicol 2015; 15: 355-365.

25) Wang HB, Gao P, Jing LL, Qin XY, Sun XL. The heart-protective mechanism of nitronyl nitroxide radicals on murine viral myocarditis induced by CVB3. Biochimie 2012; 94: 1951-1959.