Industrial Health 2017, **55**, 108–118

Original Article

Taurine inhibits 2,5-hexanedione-induced oxidative stress and mitochondria-dependent apoptosis in PC12 cells

Shuangyue LI[1], Huai GUAN[2], Zhiqiang QIAN[1], Yijie SUN[1], Chenxue GAO[1], Guixin LI[3], Yi YANG[4], Fengyuan PIAO[1]* and Shuhai HU[5]**

[1]Department of Occupational and Environmental Health, Dalian Medical University, China
[2]Department of Obstetrics and Gynecology, No. 210 Hospital of PLA, China
[3]Clinical laboratory, the First Affiliated Hospital of Dalian Medical University, China
[4]Department of neurosurgery, General Hospital of Beijing Military Command, China
[5]College of Stomatology, Dalian Medical University, China

Received March 15, 2016 and accepted November 2, 2016
Published online in J-STAGE November 11, 2016

Abstract: 2,5-hexanedione (HD) is the ultimate neurotoxic metabolite of hexane, causing the progression of nerve diseases in human. It was reported that HD induced apoptosis and oxidative stress. Taurine has been shown to be a potent antioxidant. In the present study, we investigated the protection of taurine against HD-induced apoptosis in PC12 cells and the underlying mechanism. Our results showed the decreased viability and increased apoptosis in HD-exposed PC12 cells. HD also induced the disturbance of Bax and Bcl-2 expression, the loss of MMP, the release of mitochondrial cytochrome c and caspase-3 activation in PC12 cells. Moreover, HD resulted in an increase in reactive oxygen species (ROS) level and a decline in the activities of superoxidedismutase and catalase in PC12 cells. However, taurine pretreatment ameliorated the increased apoptosis and the alterations in key regulators of mitochondria-dependent pathway in PC12 exposed to HD. The increased ROS level and the decreased activities of the antioxidant enzymes in HD group were attenuated by taurine. These results indicate that pretreatment of taurine may, at least partly, prevent HD-induced apoptosis via inhibiting mitochondria-dependent pathway. It is also suggested that the potential of taurine against HD-induced apoptosis may benefit from its anti-oxidative property.

Key words: Taurine, 2,5-hexanedione, Oxidative stress, Apoptosis, Mitochondrial pathway

Introduction

N-hexane, one of organic solvents which are prominent environmental pollutants, has been shown to cause neurotoxicity in vivo and in vitro. Central-peripheral neuropathy induced by n-hexane exposure has been a major health concern and occupational health hazard. In living beings, 2,5-hexanedione (HD) is the ultimate neurotoxic metabolite of n-hexane and mediates the neurotoxicity of the parent compound[1, 2]. Some studies suggest that HD induces apoptosis in neurons and neuron-like cell lines, and the increased apoptosis is being the major cause for the pathophysiological changes induced by HD[3–6]. It is well known that apoptosis could be mediated by several different pathways and the mitochondrial pathway is a major signaling leading to apoptosis. It has been documented that mitochondria-mediated pathway includes the

To whom correspondence should be addressed.
*E-mail: piao_fy_dy@163.com
**E-mail: shuhaihu4141@yahoo.com.cn
The first and second authors contributed equally to this work.
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disturbed expression of Bax and Bcl-2, loss of mitochondrial transmembrane potential (MMP), release of mitochondrial cytochrome c (Cyt C), activation of caspase-3 and ultimately triggers apoptosis. It was reported that HD up-regulated Bax expression and caspase-3 activity in rat nerve tissue[5]. Mishra *et al.*[7] showed the loss of MMP in HD-exposed spermatogenic cells. In previous studies, we also found that HD altered the key regulators of mitochondria-dependent apoptosis[8, 9]. These studies indicate that the mitochondria-dependent pathway may involve in HD-induced apoptosis.

Environmental toxicants induce the production of reactive oxygen species (ROS). ROS elicit oxidative stress causing a state of imbalance between the antioxidant defense system levels and the production of free radicals. It is known that ROS plays a key role in cell signaling to affect many pathological and physiological processes[10]. Especially, it has been reported that oxidative stress, as a trigger for cell death, is involved in the apoptotic signaling mechanism. Chang *et al.*[11] showed that Cd-induced ROS resulted in pancreatic β apoptosis. Lu *et al.*[10] reported that arsenic significantly induced ROS and apoptosis in Neuro-2a cells. Pretreatment with the antioxidant N-acetyl cysteine effectively reversed the arsenic-induced responses. These results indicated that ROS leads to apoptosis in various toxicant-exposed cells and tissues. Rashid *et al.*[12] found that ROS triggered the loss of MMP and the release of mitochondrial Cyt C after alloxan treatment. It was also reported that the increased ROS promoted several characteristics of mitochondria-dependent apoptotic events, including loss of MMP and alteration of Bax and Bcl-2 expression, in the testis of arsenic-treated rat[13]. Kim *et al.*[14] reported that exposure to HD significantly increased ROS level in murine neural progenitor cells and in the hippocampus of ICR mice. McDermott *et al.*[15] also showed a concentration-dependent increase in ROS formation in Jurkat T-cell exposed to n-hexane, the parent of HD. These studies implied that HD might exert the pro-apoptotic action via ROS-regulated mitochondrial-dependent pathway.

Taurine, the major free amino acid, is normally present at high concentrations in nerve tissue and has no side-effects even if administered at high doses[16]. It has been reported that taurine is an antioxidant and protects against oxidative stress-induced pathology in nervous system[17]. Taurine also possesses anti-apoptotic properties in neurons and neuron-like cells[18−20]. In recent, Chang *et al.*[21] reported that taurine effectively suppressed the disturbance of Bax and Bcl-2 in human proximal tubular epithelial cells exposed to oxidized LDL. Taurine treatment also significantly inhibited endosulfan-induced the disturbance of MMP and caspase-3 activation in rat testis[22]. These results indicate that taurine may have protective effect on the important regulators of mitochondria-dependent apoptosis altered by toxicants. Therefore, we are interested in whether taurine prevent HD-induced apoptosis via inhibiting mitochondria-dependent pathway.

In the present study, the viability and apoptosis of HD-exposed PC12 cells were observed with or without taurine pretreatment. Moreover, we also investigated the expression of Bax and Bcl-2, MMP, Cyt C translocation and caspase-3 activity. To assess the effect of taurine on the oxidative stress and the activities of major antioxidases in HD-exposed PC12 cells, the ROS level and activities of superoxidedismutase (SOD) and catalase (CAT) were further examined. This study aimed at investigating the prevention of taurine against HD-induced apoptosis in PC12 cells and its underlying mechanism. Output of this study may offer an interesting therapeutic approach in HD-induced neurotoxicity.

Materials and Methods

Cell culture

PC12 cells, a rat pheochromocytoma cell line, possess properties of neurons and exhibit cellular responses to toxicants that are similar to those found in human primary cultures. It has been used extensively as a model for neurotoxicity studies[23]. In this study, PC12 cells were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM with 10% fetal bovine serum, 100 U/mlpenicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2.

MTT assay

To establish the exposure dose and time of HD necessary for maximum damage in PC12 cells, cells were treated with five different doses of HD (0, 2.5, 5, 10 and 20 mM) and each dose experiments were carried out at for 6 h, 12 h and 24 h, respectively. To determine the optimal dose of taurine, PC12 cells were treated with six different doses of taurine (0, 6.25, 12.5, 25, 50 and 100 mM) followed by HD intoxication (20 mM). Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 24 h after the final dose of HD intoxication, all cells were incubated with 0.5 mg/ml MTT (Sigma, USA) for 4 h. The medium was then removed and replaced

with 750 μL DMSO. The plate was shaken for 10 min and the absorbance was measured at 570 nm using a microplate reader. The effect of HD and taurine on cell viability was expressed as percentage to the control.

Cell treatment

Cells were divided into four groups: control group, cell received neither taurine nor HD; taurine group, cells received 100 mM taurine for 0.5 h; HD group, cells received 20 mM HD for 24 h; taurine+HD group, cells received 100 mM taurine for 0.5 h, then removed and replaced with HD for 24 h .

ROS quantization

The formation of ROS was estimated with dichlorohydrofluoresce indiacetate (DCFH-DA, Beyotime, China). Cells were washed with PBS and suspended in DMEM loaded with DCFH-DA for 20 min at 37°C. Fluorescence dye was measured with a fluorescence plate reader (TECAN, Austria) at excitation and emission wave lengths of 485 and 530 nm, respectively.

SOD assay

Cells were lysed with RIPA buffer (Beyotime, China) with 1% tyrosine phosphatase inhibitor and 1% phenylmethylsulfonyl fluoride. Cells were centrifuged at 8,000 g for 15 min at 4°C. SOD activity in the proteins was determined by using the SOD analysis kit (Beyotime, China).

CAT activity assay

Cells were lysed as above and centrifuged at 13,000 rpm for 20 min at 4°C. The activity of CAT in the proteins was determined by using the CAT analysis kit (Beyotime, China).

Flow cytometry with AnnexinV/PI double staining

After treatment as described above, the cells were gently digested with trypsin without EDTA, washed thrice with PBS and collected 4×10^6 cells. Then were suspended in 500 μL annexin V Binding buffer and incubated with PI and annexin V (KeyGEN, China) at room temperature in dark for 30 min. Fluorescence analysis was carried out using a flow cytometer (BD FACS Calibur, USA).

MMP measurement

JC-1 is a sensitive and relatively mitochondrion-specific lipophilic cationic probe fluorochrome. JC-1 accumulates and emits red fluorescence in the mitochondria of higher membrane potentials, yet dissociates into monomers and emits green fluorescence in those that lose cross-membrane electrochemical gradient. The ratio of green to red fluorescence intensity (MFI) therefore provides a reliable estimate of impairment of MMP. For this assay, PC12 cells were washed with PBS, then incubated with JC-1 (5 μmol/L) in DMEM at 37°C for 20 min and analyzed with confocal microscope (TCS SP5, Leica, Mannheim, Germany) and Image-Pro Plus 6.0 (Labsystems, MA, USA) to determine the ratio of green (excitation/emission wavelength=485/538 nm) to red (excitation/emission wavelength=485/590 nm) fluorescence, both normalized to baseline value.

Western blot

Cells were homogenized in ice-cold RIPA Tissue Protein Extraction Reagent (Beyotime, China) supplemented with 1% proteinase inhibitor mix. The proteins were separated by SDS-PAGE and then electrotransferred to Hybond-P PVDF membrane (Millipore, France). The membrane was incubated with appropriate primary antibodies overnight at 4°C. Antibodies used were Bcl-2 and Bax (1:500, Cell Signaling Technology, USA), Cyt C and β-actin (1:500, Beyotime, China), VDAC (1:1,000, Cell Signaling Technology, USA). Immunoreactivity was visualized by second horseradish peroxidase-conjugated antibody (1:5,000, Sigma, USA) and enhanced chemoluminescence (Beyotime, China). Quantified densitometric analysis was using with UVP BioSpectrum Multispectral Imaging System (Ultra-Violet Products Ltd. USA).

Caspase-3 activity detection

Caspase-3 activities were determined with a caspase-3 activity assay kit (Beyotime, China), which detects the production of the chromophore p-nitroanilide after its cleavage from the peptide substrate DEVD-p-nitroanilide and LEHD-p-nitroanilide. Colorimetric reaction was developed and measured at 405 nm in a BioRad plate reader.

Statistical analysis

Data were presented as mean±standard deviation (SD). All data were analyzed with SPSS 11.0 for windows. Difference in mean values between groups was tested with the one-way ANOVA and LSD test. P values less than 0.05 were considered significant.

Results

Dose and time dependent effect of HD intoxication

To determining the dose and time necessary for HD to

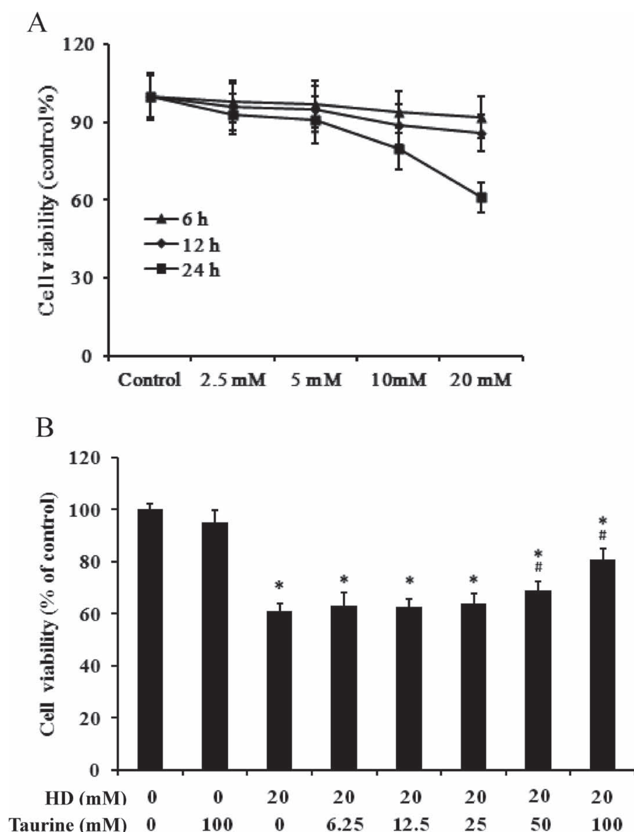


Fig. 1. The viability of PC12 cells received HD alone or with taurine. A. Dose- and time-dependent effect of HD on cell viability. PC12 cells were exposed to different concentrations (5, 10, 20, 40 mM) of HD for 0, 6, 12 and 24 h; B. PC12 cells were treated with different concentrations of taurine (0–100 mM) for 30 min, followed by 20 mM HD for 24 h. Cell viability was assessed by MTT assay. Each column represents mean±SD, n=6. $^{*}p<0.05$, compared with control group; $^{\#}p<0.05$, compared with HD group.

induce maximum cellular damage, we carried out the dose and time dependent assays by MTT. As evidenced from Fig. 1A, HD intoxication decreased the cell viability linearly up to a dose of 20 mM for 24 h in a dose- and time-dependent. This dose and time were, therefore, chosen as the optimum dose and time of HD throughout the study.

Taurine protects against hd-induced toxicity in a dose-dependent manner

To determine the optimal levels of taurine exposure, PC12 cells were preincubated with serial concentrations of taurine (0, 6.25, 12.5, 25 and 100 mM) for 0.5 h before being exposed to 20 mM HD for 24 h. Figure 1B indicates that taurine protected against HD-induced neurotoxicity in a dose-dependent manner. Preincubation with 100 mM taurine resulted in optimal recovery from neuronal injury (Fig. 1B, lane 8). This optimal concentration of 100 mM taurine was used in the later experiments.

Taurine attenuates HD-induced apoptosis in PC12 cells

PC12 cells were treated with 100 mM taurine for 0.5 h, followed by exposed to 20 mM HD for 24 h (Fig. 2A). To determine the anti-apoptotic efficacy of taurine, Hochest 33342 staining was used to characterize the morphological changes of HD-intoxicated-PC12 cells with or without taurine. As shown in Fig. 2B, the nuclei of control cells were of a rounded shape with homogeneous intensity. However, the HD-exposed PC12 cells showed crescent-shaped nuclei and fragmentation with heterogeneous intensity in the nuclei, suggesting that these cells underwent gross morphological change indicative of apoptosis. Interestingly, compared with HD group, taurine pretreatment markedly decreased the number of cells with condensation and fragmentation in HD-intoxicated cells, indicating that taurine ameliorated apoptotic morphological changes induced by HD. To further determine the number of apoptotic cells, the cells were stained with annexin V and propidium iodide and were then analyzed via flow cytometry. We observed a significant increase in apoptotic cell numbers in the HD-treated cells (Fig. 2C). The pretreatment of cells with taurine, however, the apoptosis rate in HD-exposed PC12 cells was reduced from about 55% to 18%, suggesting taurine attenuated HD-induced apoptosis in PC12 cells.

Taurine inhibited the disruption of Bax and Bcl-2 expression in HD-treated PC12 cells

As taurine suppressed HD-induced apoptosis, we assessed the effects of taurine on the expression levels of Bax and Bcl-2 proteins. As shown in Fig. 3A-C, compared with control group, HD intoxication increased the pro-apoptotic protein expression of Bax in PC12 cells, which was significantly attenuated once HD-intoxicated cells were pretreated with taurine. On the contrary, HD intoxication decreased the anti-apoptotic protein expression of Bcl_2 in PC12 cells, which was markedly inhibited once HD-intoxicated cells were pretreated with taurine. It was shown that the ratio of Bax to Bcl-2 (Bax/Bcl-2) determines the susceptibility of a cell to apoptosis5). Bax/Bcl-2 ratio was also assessed in this study. As seen in Fig. 3D, consistent with Bax protein expression, taurine significantly mitigated the elevation of Bax/Bcl-2 ratio induced by HD, indicating that taurine inhibited the disruption of Bax and Bcl-2 expression in HD-treated PC12 cells.

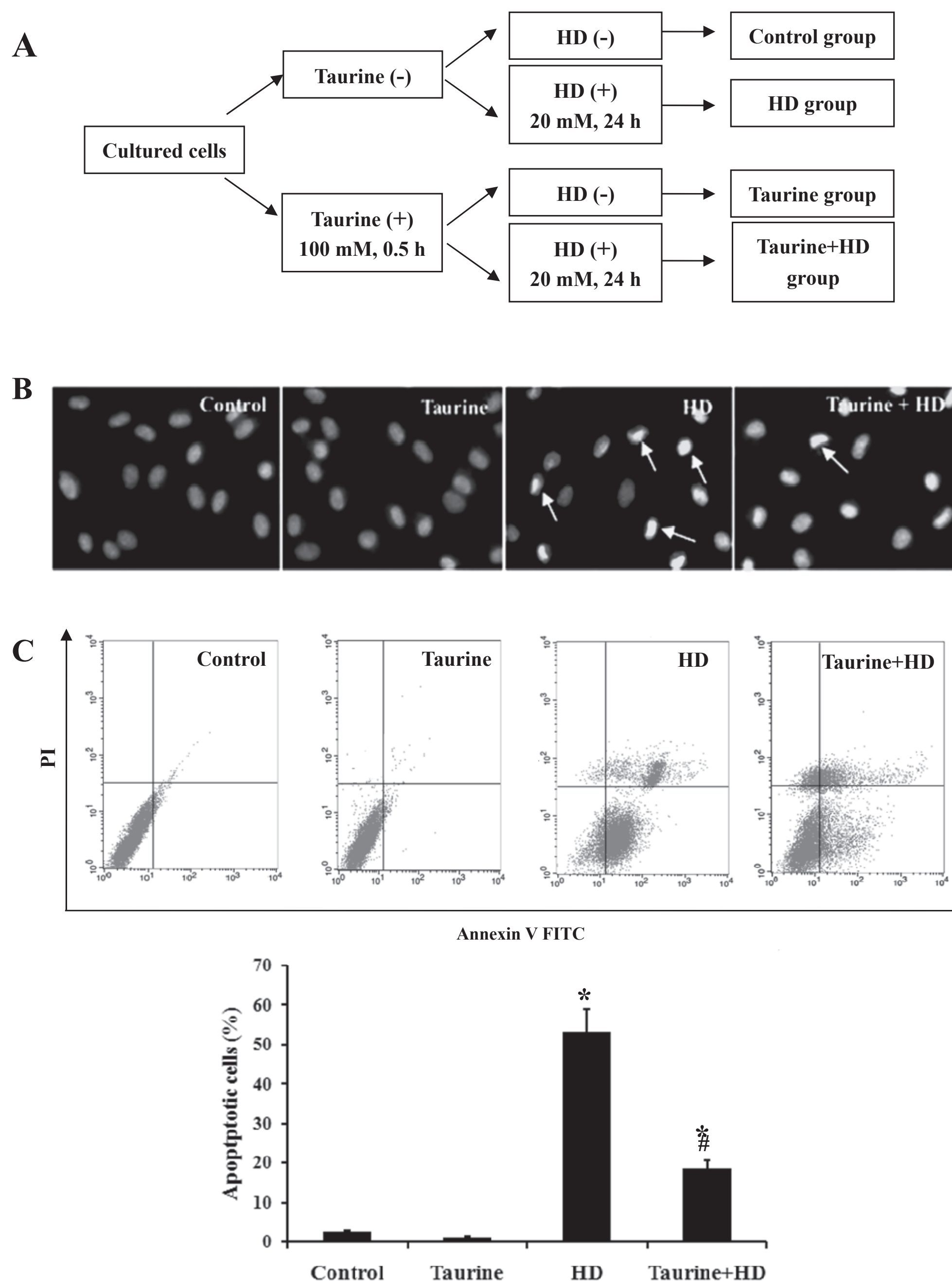


Fig. 2. Effect of taurine on apoptosis induced by HD in PC12 cells. A. Hoechst 33342 staining was used to detect the morphological changes (400×); B. Flow cytometric analysis of effects of taurine on PC12 cells apoptosis and necrosis induced by HD. Representatively original data of flow cytometry and the apoptotic rate in different groups. Data were presented as mean±SD, n=6. *$p<0.05$, compared with control group; #$p<0.05$, compared with HD group.

Taurine blocked the collapse of MMP in HD-intoxicated PC12 cells

Increasing of Bax protein but decreasing of Bcl-2 protein would trigger the dissipation of MMP in *mitochondria*-mediated pathway[8, 9]. As shown in Fig. 4, compared with the control, HD exposure markedly reduced MMP in PC12 cells. In contrast, in the presence of taurine, HD failed to interfere with MMP in PC12 cells, indicating that taurine inhibite blocked HD-induced collapse of MMP in PC12 cells.

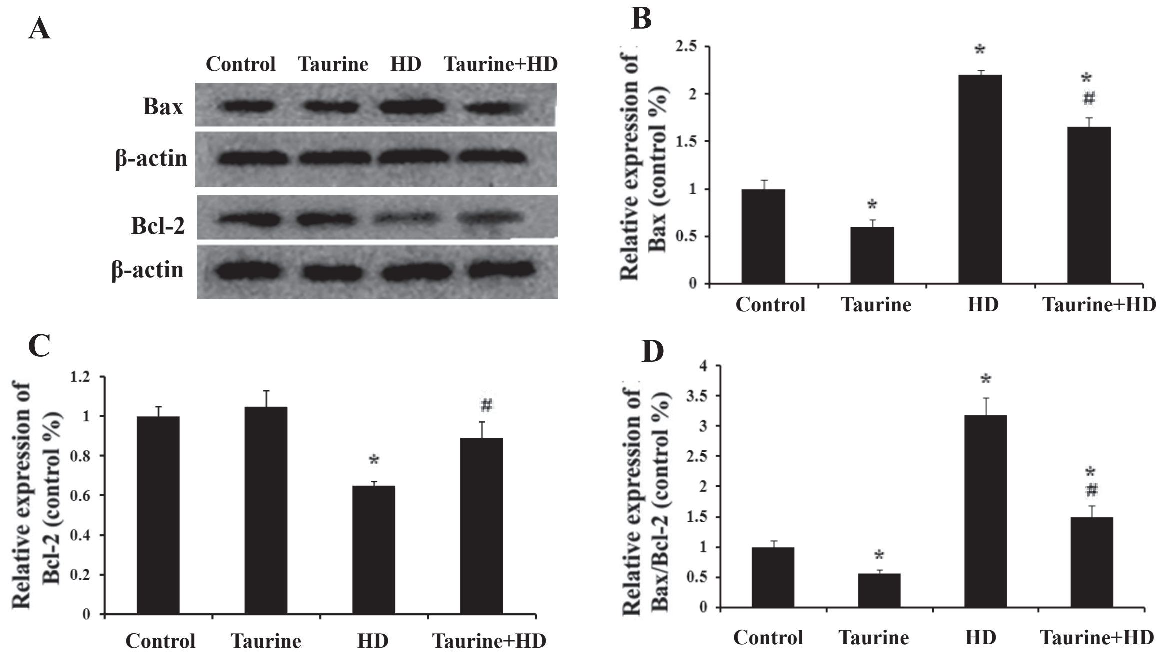
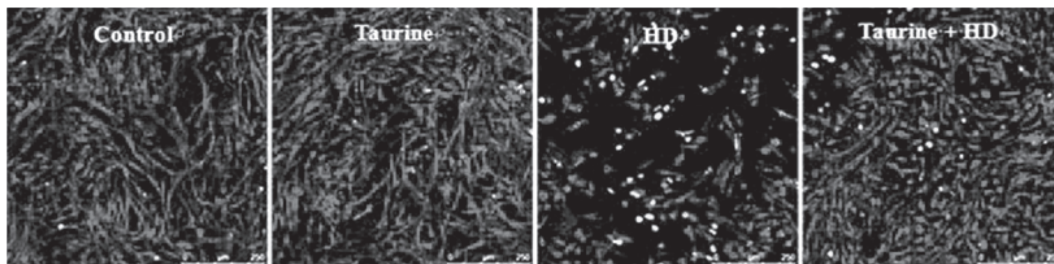


Fig. 3. Effect of taurine on the expression of Bax and Bcl-2 in HD-exposed PC12 cells. Western blot analysis was used to detect Bax and Bcl-2 (A-C) protein levels. The ratio of Bax/Bcl-2 was analyzed (D). Representatively WB images were shown and quantified data were presented as mean ± SD. $^{*}p < 0.05$, compared with control group; $^{\#}p < 0.05$, compared with HD group.



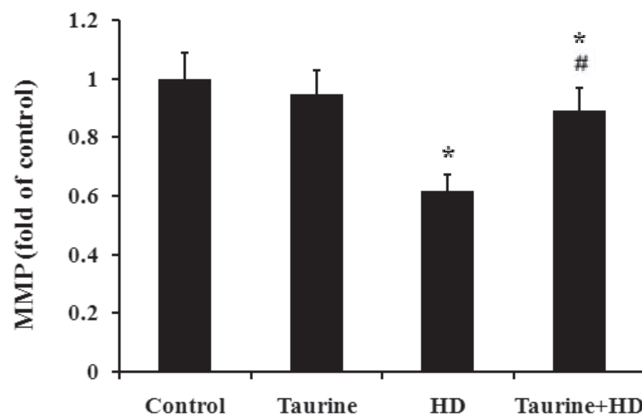


Fig. 4. Effect of taurine on MMP in HD-exposed PC12 cells. $^{*}p < 0.05$, compared with control group; $^{\#}p < 0.05$, compared with HD group.

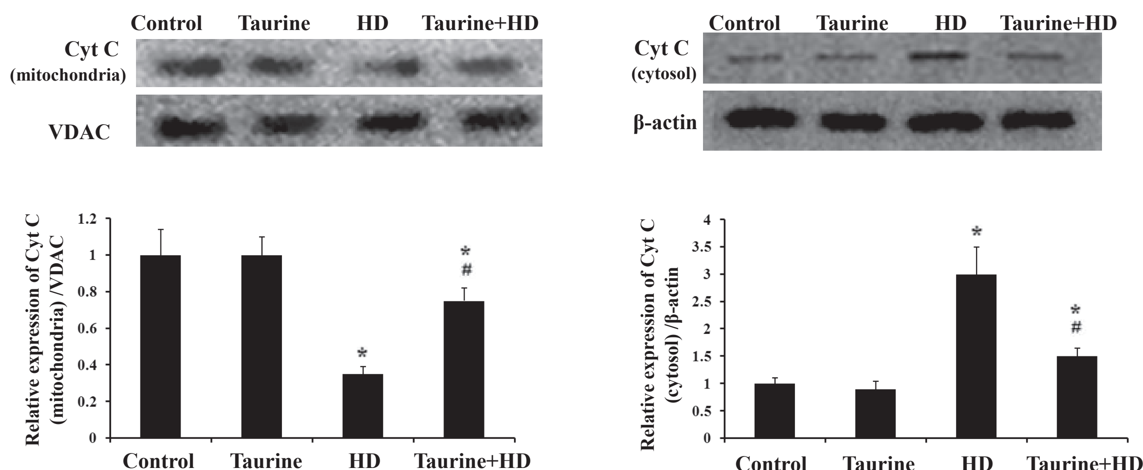


Fig. 5. Effect of taurine on the release of cytochrome c in HD-exposed PC12 cells. Western blot was used to detect cytochrome c levels in mitochondrial fraction (A) and cytosolic fraction (B). Data were presented as mean±SD. $^{*}p<0.05$, compared with control group; $^{\#}p<0.05$, compared with HD group.

Taurine suppressed the Cyt c release from mitochondria in HD-treated PC12 cells

Disruption of MMP is one of the earliest intracellular events that occur following the onset of apoptosis, which leads to the subsequent release of Cyt C from mitochondria and consequently triggers other apoptotic factors[8, 9]. Our results showed that compared with vehicle controls, the protein level of Cyt C in mitochondria was significantly decreased (Fig. 5A), while markedly increased in cytosolic fraction in HD-intoxicated PC12 cells (Fig. 5B). Interestingly, taurine markedly suppressed HD-induced Cyt C release by showing elevated expression of mitochondrial Cyt C and reduced expression of cytosol Cyt C in taurine/HD-treated cells compared with HD alone group (Fig. 5).

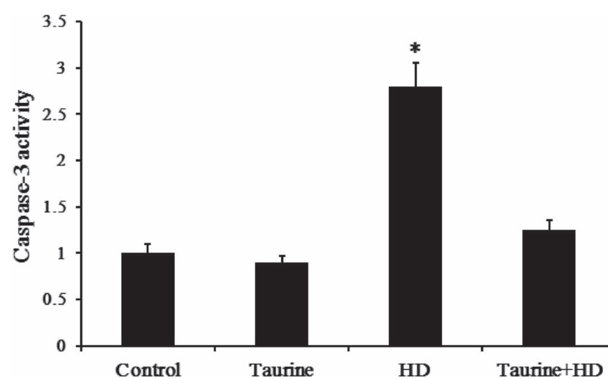


Fig. 6. Effect of taurine on caspase-3 activity in HD-exposed PC12 cells. Data were presented as mean±SD, n=6. $^{*}p<0.05$, compared with control group; $^{\#}p<0.05$, compared with HD group.

Taurine prevented HD-induced activation of caspase-3 in PC12 cells

Caspase-3 is the effector of mitochondria-mediated apoptosis pathway. In the present study, the effect of taurine on the activity of caspase-3 protein in PC12 cells was shown in Fig. 6. As expected, compared with control group, a significant increase in caspase-3 activity was observed in HD-intoxicated PC12 cells, which was significantly repressed once HD-intoxicated cells were pretreated with taurine, suggesting that HD-induced caspase-3 activation was prevented by taurine.

Taurine lowered ROS production in HD-treated PC12 cells

Oxidative stress seems to responsible for the activation of mitochondria-mediated pathway 12). ROS is assumed to be the one of the important parameters of oxidative stress, therefore, we determined the ROS production in taurine/HD-treated PC12 cells. As seen in Fig. 7A, HD treatment significantly increased the production of ROS, indicating an oxidative stress to cells. However, such effect was reversed when HD-intoxicated cell was pretreated with taurine.

Taurine ameliorated the decreased activities of antioxidant enzymes induced by HD

The depletion of the activities of antioxidant enzymes would result in ROS overproduction. The activities of CAT and SOD were tested in PC12 cells exposed to HD in the presence of taurine or not. As shown in Fig. 7B and C, HD-induced decrease of CAT and SOD activities in HD-intoxicated cells was significantly *ameliorated* in the presence of taurine.

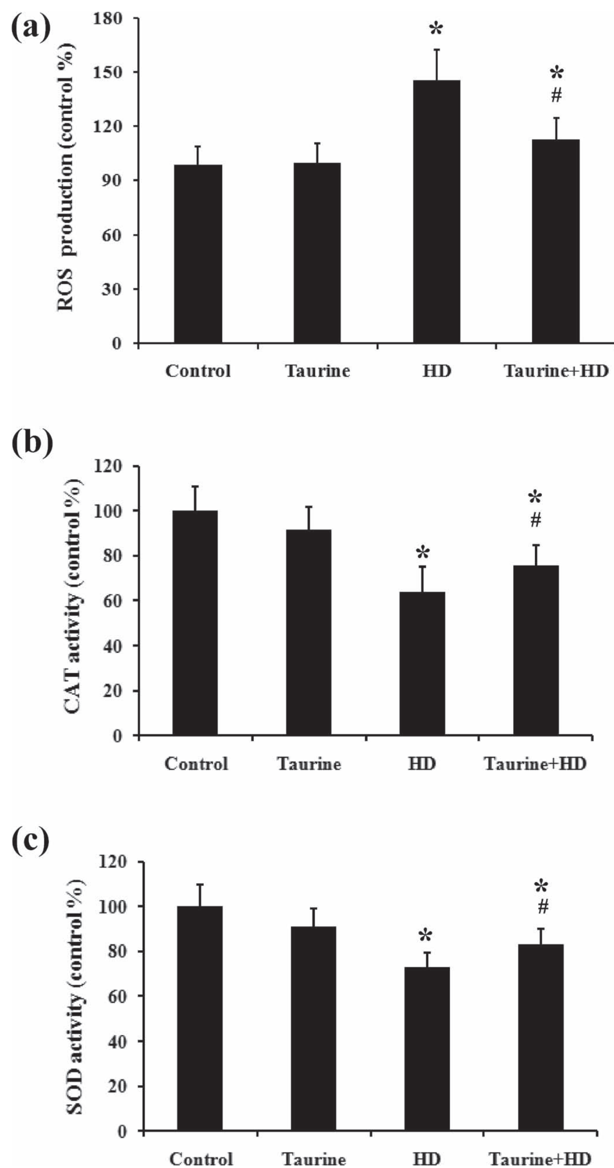


Fig. 7. Effect of taurine on ROS production (A), SOD activity (B) and CAT activity (C) in HD-exposed PC12 cells. Data were presented as mean±SD, n=6. *$p<0.05$, compared with control group; #$p<0.05$, compared with HD group.

Discussion

Apoptosis is a phenomenon of programmed cell death and plays an important role during neuronal development and in the homeostasis of the adult nervous system. The disruption of this process can lead to abnormal neuronal apoptosis and the increased apoptosis may contribute to the pathophysiology of nervous system disorders[24, 25]. Several studies have demonstrated that an abnormal increase in apoptosis is the main form of cell death caused by HD, and the increased apoptosis of neurons directly involved in HD-induced neurotoxicity[3–6]. These findings indicate that apoptosis may be a potential therapeutic target in neuropathy induced by HD. Taurine possesses anti-apoptotic properties in neurons and neuron-like cells[18–20]. Therefore, our study focuses mainly on the protection of taurine against HD-induced apoptosis and its underlying mechanism.

In the present study, the viability and apoptosiswere observed in PC12 cells received HD alone or with taurine. The results showed that HD significantly decreased the viability of PC12 cells and increased the number of apoptotic cells. However, the decreased the viability and the increased apoptosis in HD-exposed PC12 cells were significantly ameliorated in the presence of taurine. Das *et al.*[13] reported that the increased apoptosis in primary cardiomyocytes exposed to doxorubicin was reduced by taurine administration. Rashid *et al.*[12] also reported that taurine reduced the increased apoptosis in the hepatic tissue of diabetic rats, supporting our results. These results indicate that taurine pretreatment can prevent HD-induced apoptosis in PC12 cells.

Mitochondrial pathway is the major signaling leading to apoptosis. Bcl-2 family plays critical roles in the regulation of mitochondria-mediated apoptosis. Bax and Bcl-2 are representative members of the Bcl-2 family. The former is pro-apoptotic molecule and the latter is anti-apoptotic molecule. Bax induces the permeabilization of mitochondrial outer membrane, causes the efflux of Cyt C from mitochondria to cytosol and leads to caspase-3 activation. Bcl-2 plays a role in controlling the integrity of the mitochondrial membrane and forms heterodimers with Bax to prevent the mitochondria dysfunction and the activation of caspase-3. Therefore, a shift in the balance between anti- and pro-apoptotic Bcl-2 family proteins could lead to mitochondria-dependent caspase-3 activation and apoptotic cell death. In the present study, the results showed that HD down-regulated Bcl-2 expression, up-regulated Bax expression, promoted the disruption of MMP and mitochondrial release of Cyt C and increased the activity of caspase-3 in PC12 cells, indicating that HD induced dysregulation of Bax and Bcl-2 and the activation of mitochondria-dependent apoptosis pathway in PC12 cells. However, pretreatment with taurine significantly reversed the activated mitochondria-dependent pathway in HD-exposed PC12 cells. Chang *et al.*[11] reported that taurine effectively suppressed the disturbance of Bax and Bcl-2 as well as the enhancement of MMP in human proximal tubular epithelial cells exposed to oxidized LDL. Aly and Khafagy[22] showed that taurine pretreatment prevented the increased activity of caspase-3 in adult rat testis exposed to endosulfan. These studies and

our results indicate that taurine represses mitochondrial apoptosis pathway and the inhibited mitochondria-dependent pathway may be involved in the prevention of taurine against HD-induced apoptosis in PC12 cells. In addition, whether there is any extra mitochondrial pathway regulating HD-induced apoptosis has to be studied further.

Studies indicate that oxidative stress involved in the apoptotic signaling mechanism. ROS elicit oxidative stress causing an imbalance between pro-oxidant and anti-oxidant mechanisms[14]. The present study showed that HD exposure induced a significant decline in the activities of SOD and CAT and a significant increase in ROS production in PC12 cells. However, such changes were notably blocked by taurine pretreatment. Incubation with taurine and other toxins showed the similar results[12, 13]. SOD and CAT are the key antioxidant enzymes to scavenge ROS and defense the oxidative stress. Antioxidant activity of SOD is mediated by dismutation reaction where SOD scavenges highly reactive superoxide radical and converts it to oxygen molecule and less reactive H_2O_2 molecule. CAT then catalyses the decomposition of this H_2O_2 to water and O_2. Therefore, inhibition of these antioxidant enzymes or decrease in their activities would result in an excessive production of ROS and pathological damage. The over-production of ROS in HD-treated PC12 cells may result from the inhibited activities of SOD and CAT. Meanwhile, taurine pretreatment protected the efficiency of the antioxidant enzymes, and may also quench and detoxify ROS intermediates which all contributed to its protection against ROS-induced toxicity in HD-exposed PC12 cells.

ROS are involved in the activation of mitochondrial pathway, which is mediated by various signaling. The present study showed that HD induced oxidative stress and apoptosis in PC12 cells, which was accompanied by the characteristic changes of mitochondrial pathway. Das *et al.*[25] reported that ROS overproduction induced the disturbance of Bax and Bcl-2 during oxidative death in nerve cells. Rashid *et al.*[12] found that oxidative stress induced apoptosis via mitochondria-dependent pathway. These results indicate that HD-induced oxidative stress lead to the apoptosis of PC12 cells via activating mitochondria-dependent pathway. On the other hand, pretreatment with taurine significantly reversed the induced ROS and the increased apoptosis as well as the activated apoptotic pathway in HD-exposed PC12 cells. Our results indicate that taurine prevents oxidative stress-promoted apoptosis in HD-exposed PC12 cells via inhibiting mitochondria-dependent pathway. Moreover, the attenuated oxidative stress may be mainly responsible for the protection of taurine. Although protective mechanisms of taurine against oxidative stress have not been clearly elucidated, several mechanisms may play a role in taurine-mediated reduction in oxidative stress. Taurine could quench and detoxify several ROS, like hypochlorous acid, nitric oxide, H_2O_2 and hydroxyl radical[26−29]. It also could upregulate the activities of antioxidant enzymes. Among the antioxidant enzymes, SOD and CAT defend cells against the toxic effect of oxygen metabolism by eliminating ROS, which attributed to the protection of taurine against HD toxicity in this study. Moreover, it could enhance the level of the non-enzymatic radical scavenger glutathione (GSH) by directing cysteine into the GSH synthesis pathway. GSH scavenges residual free radicals escaping decomposition by the antioxidant enzymes, as the second line of defense. Sulfhydryl groups of GSH easily react with free radicals, which protects the cell against oxidative attack by chemicals[28].

Our results showed that pretreatment with taurine significantly ameliorated the increased apoptosis and the alteration in major regulators of mitochondria-dependent pathway in HD-exposed PC12. However, these abnormal changes in HD group could not completely be reversed by taurine pretreatment. These results indicate that taurine pretreatment may partly prevent HD-induced apoptosis in PC12 cells via mitochondria-dependent pathway, and the partial protection of taurine may be associated with that it could not completely inhibited HD-induced ROS, as shown in Fig 8. In addition, apoptotic cell death is mainly regulated by two signaling pathways: mitochondria-dependent pathway and FasL/Fas pathway. It was reported that HD induced germ cell apoptosis via FasL pathway[30]. Therefore, the mechanism of apoptosis via FasL pathway may affect the protection of taurine against HD-induced apoptosis.

In conclusion, the present study showed the decreased viability and the increased apoptosis in the PC12 cells exposed to HD. HD also induced the disturbance of Bax and Bcl-2 expression, the loss of MMP, Cyt C release and caspase-3 activation in PC12 cells. Moreover, HD resulted in an increase in ROS level and a decline in the activities of SOD and CAT. It indicates that HD elicits oxidative stress and mitochondrial-dependent apoptosis in PC12 cells. However, taurine pretreatment ameliorated the increased apoptosis and the alterations in key regulators of mitochondria-dependent pathway in PC12 exposed to HD. The increased ROS level and the decreased activities of the antioxidant enzymes in HD group were attenuated by taurine pretreatment. These results indicate that pretreatment with taurine may at least partly prevent HD-

induced apoptosis from HD-induced apoptosis via inhibiting mitochondria-dependent pathway. It is also suggested that the potential of taurine against HD-induced apoptosis may benefit from its anti-oxidative property. Although the anti-apoptotic effect of taurine in HD-exposed PC12 cells has been linked to the inhibition of oxidative stress, the direct mechanisms involved have remained largely elusive. As for the more precise mechanism, further studies are required.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 81273038, No. 81102160), China Postdoctoral Science Foundation funded project (No. 2015M581338) and Dalian Municipal Science and Technology Plan Project (No. 2013E15SF163).

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