Taurine Ameliorates Arsenic-Induced Apoptosis in the Hippocampus of Mice Through Intrinsic Pathway

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Abstract Our group previously reported that arsenic (As) exposure induced apoptosis in hippocampus neurons. The aim of the present study was to clarify the protective capacity of taurine (Tau) on As-induced neuronal apoptosis and the related mechanism in mouse hippocampus. Mice were divided into: control group, Tau control group, As exposure group and Tau protective group, randomly. The apoptotic rate of mouse hippocampus was determined by TUNEL staining. The levels of Bcl-2 and Bax gene and protein were analyzed by real time RT-PCR and WB, respectively. Furthermore, cytochrome c (Cyt C) release, and the activity of caspase-8 and caspase-3 were also determined. The results showed that Tau treatment induced the decrease of TUNEL-positive cells, prohibited the disturbance of Bcl-2 and Bax expression, and inhibited Cyt C release and caspase-8 and caspase-3 activation significantly. The results indicated that Tau supplement markedly ameliorates As-induced apoptosis by mitochondria-related pathway in mouse hippocampus.

Keywords Arsenic • Taurine • Apoptosis • Hippocampus • Intrinsic pathway

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Abbreviations

As	Arsenic
Cyt C	Cytochrome c
Tau	Taurine

1 Introduction

Arsenic (As), a naturally toxicant existing in both organic and inorganic forms, is universal found in food, groundwater, ambient air and dust. As toxin, being inorganic and/or organic compound, is widely spread in water and environment which was resulted by the increasing pollution. Several researches indicated that inhalated- or ingested-exposure of As involved in the more and more chronic diseases which include diabetes mellitus, cardiovascular disease, peripheral vascular disease and various cancers, and one of the major targets is nerve system (Ferreccio et al. 2000, Chen and Ahsan 2004, Meliker et al. 2007, Huang et al. 2008, Das et al. 2012a, b). It was illustrated that As exposure induces dysfunctions in nervous system or severe signs of neuropathy in animal and human models (Meliker et al. 2007; Sinczuk-Walczak 2009; Zhang et al. 2013). Impaired learning ability and neural behaviors were observed in many As-intoxicated rodents at environmental relevant levels (Sinczuk-Walczak 2009; Zhang et al. 2013).

The hippocampus, one of the most important components of brain, is responsible for several vital functions, including behaviours, mental and intellectual activities, in both rodents and human. Moreover, the hippocampus is very susceptible to the toxicities of some neurotoxins such as ethanol, lead, $MnCl_2$ and CuO (Sharifi et al. 2002; Oliveira-da-Silva et al. 2009; Sinczuk-Walczak 2009; Wang et al. 2012; Zhang et al. 2013). It was reported that the gene levels of caspase-3 and 9, the effectors of apoptosis, were markedly elevated in hippocampus of As-intoxicated rats (Zhang et al. 2013). Our group recently found that As intoxication caused apoptosis in hippocampus neurons by showing the increasing number of Tunel-positivity cells (Wang et al. 2015). These researches indicated abnormal apoptosis induced by As exposure in hippocampus may involve in the neurotoxicity induced by As exposure.

Apoptosis, the basic process under both physiological and pathological condition, regulates cell death according to surrounding environmental (Namgung and Xia 2000). Disruption or over-activation of this process, the programmed cell death, may lead to the pathogenesis of nervous system. Thus, minimizing neuronal apoptosis and promoting injured neuron recovery may be the most effective and direct therapeutic approach to reduce the neurotoxic effects of As.

Taurine, a natural amino acid, exists in mammalian brain and acts as a functionally neurotransmitter or neuromodulator. It modifies protein phosphorylation, regulates calcium transport and maintains the structural integrity of membrane in nerve tissue (Zhou et al. 2011). It has been shown that Tau protected nervous system against injury, acting

as an antioxidant and apoptosis inhibitor. However, the protective capacity of Tau on As-induced apoptosis in hippocampus and its related mechanism is unknown.

In the present study, Tunel assay was used to examine the capacity of Tau on As-induced apoptosis in hippocampus. To clarify the mechanism of anti-apoptotic potential of Tau, Bax and Bcl-2 expression in hippocampus was examined by real-time PCR and Western blotting (WB). The release of cytochrome c (Cyt C) was examined by WB, and the activity of caspase-8 and caspase-3 were analyzed by commercial kits. The study aimed at illustrating the neuroprotection of Tau on As-induced apoptosis in mouse hippocampus and exploring its related mechanism.

2 Materials and Methods

2.1 Animal and Treatment

19.2 ~ 24.7 g male mice were provided by Animal Center, Dalian Medical University. During experiments, all mice were raised under 20 ~ 24 °C temperature, 55% humidity, 12 h dark-light cycle environment with an ad libitum diet and water. The 30 mice were divided into control group, As exposure group and Tau protection group randomly. As exposure group exposed to 4 mg/L As_2O_3 in double-distilled water orally; tau protection group received 4 mg/L As_2O_3 in double-distilled water orally and 150 mg/kg Tau once daily by gavage; control group only received double-distilled water. After 60-day treatment, all model mice were sacrificed and samples were collected carefully. The animal experiments were carried out according to the guidelines of the committee of Dalian Medical University.

2.2 Tunel Assay

In Situ Cell Death Detection Kit (Roche, Germany) was used to perform Tunel assay according to instructions. DAPI staining was performed to counter the number of cells. To quantify the apoptotic cells, 6 fields were randomly selected per slide under fluorescence microscope and calculated the apoptosis index (AI). AI score equals the percentage of number of Tunel-positive cells on total number of cells.

2.3 Real Time RT-PCR

Trizol® reagent (Takara, China) was used to extract RNA sample according to the instructions. Transcriptor First Strand cDNA Synthesis Kit (Roche,\USA) was used to perform RT reactions. TP800 System and SYBR Green PCR kit (Takara, Japan) were used to carry out real time RT-PCR. 95″C 5 min, followed by 95″C for 30 s, 40 cycles,

then 55″C 30 s, 72″C 30 s were used as reaction conditions. The primers for genes of interest and β-actin are as followed: Bcl-2, GACTGAGTACCTGAACCGG CATC, CTGAGCAGCGTCTTCAGAGACA; Bax, CGAATTGGCGAT GAACTGGA, CAAACATGTCAGCTGCCACAC; β-actin, GGAGAT TACTGCCCTGGCTCCTA, GACTCATCGTACTCCTGCTTGCTG.

2.4 Western Blot

Total proteins were extracted from liver tissue with lysis buffer. BCA method was used to qualify protein concentration. SDS-polyacrylamide gel electrophoresis was carried out with same gram of loading sample protein, and the protein samples were transferred to a PVDF membrane. After blocking, the membrane was incubated with Bax, Bcl-2 (1:800), Cyt C (1:1000) and β-actin (1:500) primary antibodies, respectively. The blots were treated with HRP-conjugated secondary antibodies, and then detected by Bio-Rad imaging system (Bio-Rad, USA), and then qualified with the Gel-Pro software.

2.5 Caspase-8 and Caspase-3 Activity Detection

Caspase-8 and Caspase-3 Colorimetric Assay Kit (Beyotime, China) was used to detect caspase activities of liver according to the manufacturer's manipulations. The liver lyses were incubated in ice-cold lysis buffer for 20 min, then centrifuged at 10,000 × g 2 min. The related results were showed as a ratio to control.

2.6 Statistical Analysis

Statistical analysis was performed with SPSS 11.0 statistical software. Data were analyzed using one-way ANOVA and expressed as means ± SD in triplicate.

3 Results

3.1 Protective Capacity of Tau on As-Induced Apoptosis in Mouse Hippocampus

Tunel staining was performed to detect the apoptotic cells in hippocampus. As shown in Fig. 1, an elevated quantity of TUNEL-positive cells were observed in As-intoxicated mouse hippocampus, whereas almost no Tunel-positive cells was observed in both saline and Tau alone-treated mouse hippocampus, indicating As intoxication induced apoptosis in hippocampus. Interestingly, compared with As

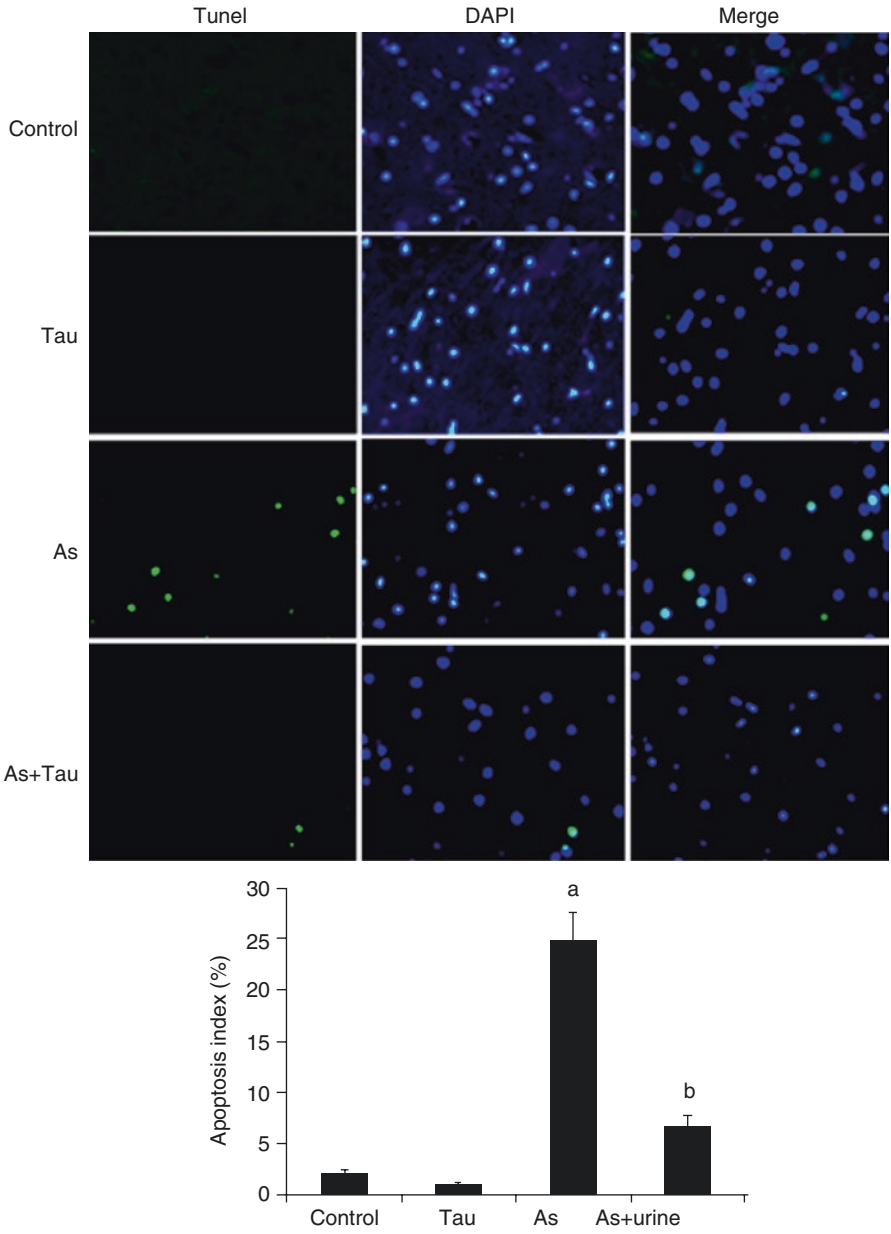

Fig. 1 Effect of Tau on apoptosis induced by As in mouse hippocampus. The apoptotic rates were determined by TUNEL staining. Green color indicates apoptosis. Counterstaining with DAPI, blue color, suggests cell nuclei. TUNEL-positive cell percent was quantified as apoptotic index. $^{a}p < 0.05$, vs. control group; $^{b}p < 0.05$, vs. As group

alone group, Tau treatment markedly reduced the quantity of Tunel-positive cells. The increased apoptosis induced by As was significantly reversed by Tau treatment, suggesting Tau attenuates As-induced apoptosis in mouse hippocampus.

3.2 *Protective Capacity of Tau on Bax and Bcl-2 Expression in As-Exposed Hippocampus*

Level of Bax and Bcl-2 were examined by both real time RT-PCR (Fig. 2a, b) and WB (Fig. 2c, d). As Fig. 2a showed that Bax gene expression was markedly increased in As group than that in control group. However, after treating with Tau, the gene expression of Bax was markedly decreased. On the other hand, compared with control group, As intoxication markedly decreased the gene expression of Bcl-2 in hippocampus, which was significantly reversed in protective groups (Fig. 2b). The similar effects also observed in protein level of Bax and Bcl-2 in Tau protect group (Fig. 2c, d).

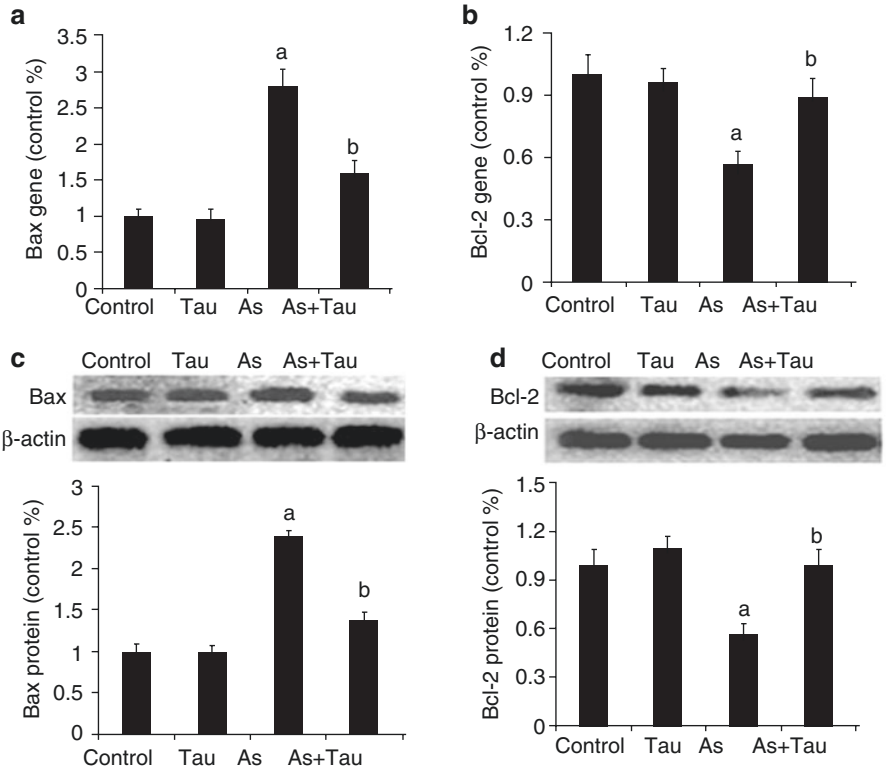

Fig. 2 Effect of Tau on Bax and Bcl-2 expression in As-exposed hippocampus. Real time RT-PCR (**a**, **b**) and WB (**c**, **d**) were used to examine Bax and Bcl-2 expression. $^{a}p < 0.05$, vs. control group; $^{b}p < 0.05$, vs. As group

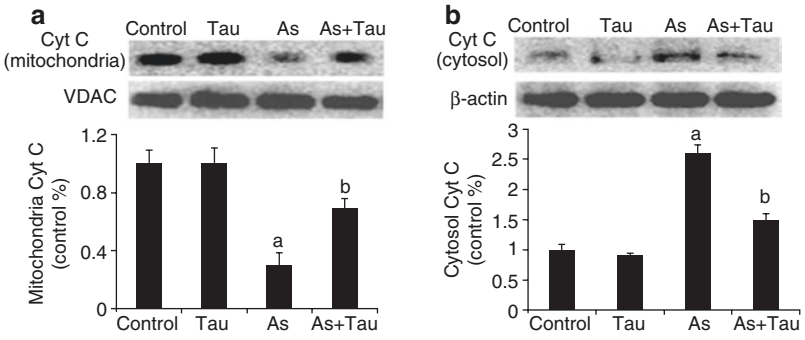

Fig. 3 Effect of Tau on cytochrome c level in mitochondria (**a**) and cytosol (**b**) in hippocampus of As-intoxicated mice. [a]$p < 0.05$, vs. control group; [b]$p < 0.05$, vs. As group

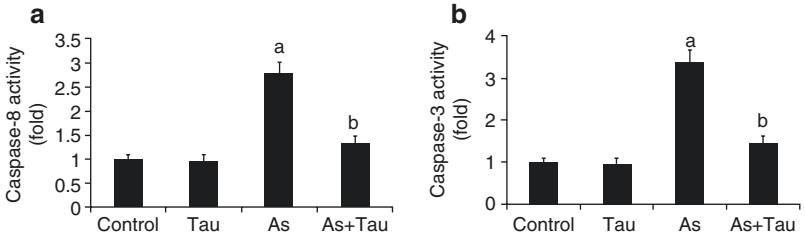

Fig. 4 Effect of Tau on caspase-8 and caspase-3 activity in As-exposed hippocampus. [a]$p < 0.05$, vs. control group; [b]$p < 0.05$, vs. As group

3.3 *Protective Capacity of Tau on Cyt C Expression in Hippocampus of As-Exposed Mice*

As Fig. 3a showed the protein level of Cyt C in mitochondria significantly decreased in As-exposed hippocampus compared to control group. On the other hand, the protein level of Cyt C in cytosol significantly increased in As-exposed hippocampus (Fig. 3b). These results indicate As exposure caused mitochondrial Cyt C release. Interestingly, the release was abolished when As-intoxicated mice were treated with Tau (Fig. 3a, b).

3.4 *Protective Capacity of Tau on Activities of Caspase-8 and Caspase-3 in As-Intoxicated Hippocampus*

Activities of caspase-8 and caspase-3 in As-intoxicated hippocampus were shown in Fig. 4. Compared with control group, caspase-8 activity in As-exposed hippocampus was markedly higher. Interestingly, Tau reversed the activation of caspase-8 in As-treated mice compared with As alone group. Consistent with that of caspase-8, As intoxication also induced the activation of caspase-3, which was blocked by Tau treatment (Fig. 4b).

4 Discussion

Increasing evidences showed that taking an apoptosis inhibitor may be a practical approach for the protection against nervous system disorders. Currently, the anti-apoptotic potential of Tau has been highlighted in many disorders (Hsu et al. 2008; Leon et al. 2009; Das et al. 2010; Taranukhin et al. 2010; Gao et al. 2011; Das et al. 2012a, b). Moreover, it was reported that Tau treatment could also prevent from apoptosis induced by various toxicants in vitro (Leon et al. 2009; Zhou et al. 2011; Chang et al. 2014). In the present study, the anti-apoptotic capacity of Tau was assayed in hippocampus of mice received As alone or with Tau by Tunel staining. As expected, As-induced elevation of Tunel-positivity cells in hippocampus was markedly blocked by Tau treatment, indicating that Tau has the efficacy to protect against As-induced apoptosis in hippocampus of mice.

Intrinsic pathway is the major intracellular signaling leading to apoptosis, which is accompanied by the dysregulation of Bcl-2 family protein and Cyt C release, where Cyt C eventually activates caspase-3 (Mikhailov et al. 2003; Wang et al. 2013). Bax and Bcl-2 are the representative regulators of Bcl-2 family, which play a vital role in mediating apoptosis process (Braun 2012). It was reported that As intoxication increased Bax level and decreased Bcl-2 level in cultured mesenchymal cells (Zhou et al. 2007). However, there are no reports that the MSCs or Tau treatment mediates the disturbed expression of Bax and Bcl-2 in As-intoxicated nerve cells. In the present study, the results suggested that Tau significantly inhibited the disrupted expression of Bax and Bcl-2 in hippocampus. Bax/Bcl-2 ratio was also assessed in this study, which is markedly decreased in hippocampus once As-intoxicated mice were treated with Tau. These results indicate a potential link between mediating the disturbed expression of Bax and Bcl-2 and the anti-apoptotic capacity of Tau in hippocampus of As-exposed mice.

Several evidences illustrated that the disturbance of Bax and Bcl-2 expression led to the efflux of mitochondrial Cyt C, which activates the downstream caspase cascade (Wang et al. 2013). Among the identified caspases, caspase-8 and caspase-3 are the important enzymes that induce the activation of apoptosis process (Braun 2012; Kadeyala et al. 2013). It was reported that Tau treatment significantly inhibited endosulfan-induced the activation of caspase-3 in rat testis (Aly and Khafagy 2014). In the present stud, we found that Tau significantly suppressed Cyt C release and the activation of caspases in As-intoxicated mouse hippocampus, indicating the trigger of apoptosis in As-intoxicated hippocampus was blocked by Tau. These studies and our results indicate that Tau represses intrinsic apoptosis pathway and the inhibited intrinsic pathway may take part in the prevention of Tau against As-induced apoptosis in mouse hippocampus.

5 Conclusion

In conclusion, the results indicated that taruine inhibits As-induced apoptosis in hippocampus of mice. Moreover, treatment of Tau significantly inhibited the disturbed expression of Bax and Bcl-2, the release of Cyt C and the activated caspases in

As-exposed hippocampus. These results indicate that Tau may protect against apoptosis in hippocampus via mediating As-disturbed intrinsic pathway. As for the more precise mechanism, further studies are required.

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