Taurine Protects Against Arsenic-Induced Apoptosis Via PI3K/Akt Pathway in Primary Cortical Neurons

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Abstract Arsenate, a well known toxicant, can induce injury in nerve system via oxidative stress and apoptosis. This study was designed to explore the protective effect of taurine against arsenite-induced neurotoxicity and its related mechanism in primary cortical neurons. The cells were treated with arsenite with or without taurine. Twenty-Four hours later, cell viability was examined using the MTT assay. The activity of caspase-3 was analyzed and the level of Akt and p-Akt were examined by western blot. The results show that taurine treatment significantly attenuates the decrease in cell viability of arsenite-exposed primary cortical neurons. Taurine also reversed the arsenite-induced increase in caspase-3 activity. The decrease in p-Akt levels induced by arsenite exposure was prevented by taurine treatment. Thus, taurine attenuated the effect of arsenite on primary cortical neurons, an effect that may involve the Akt pathway.

Keywords Taurine · Arsenic · PI3K/AKT · Apoptosis

Abbreviations

As	arsenic
DMSO	dimethyl sulfoxide

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1 Introduction

Being a well known environmental contaminant, arsenic (As) is widely distributed, being present in nearly every country of the world. The metalloid was contained in contaminated drinking water, soil, fish and occupational poisons (Carlin et al. 2016). Both organic and inorganic arsenic compounds threaten the health of millions of people and as such has become a major public health issue throughout the world (Hata et al. 2012). Various epidemiologic investigations and lab studies have shown that As exposure affects a myriad of targets with multiple endpoints, such as cancer, neurologic deficits, psychiatric problems, kidney disease, diabetes, cardiovascular disease, respiratory outcomes, and reproductive abnormalities (Kuo et al. 2013; Navas-Acien et al. 2005; Peters et al. 2015). Recent evidence indicates that As exposure is linked to damage of neurons (Yorifuji et al. 2016; Chen et al. 2015; Liu et al. 2012). Several research studies show that apoptosis takes part in the onset and development of As-induced neurotoxicity.

Taurine, a sulfur-containing-β-amino acid, is present in many mammalian tissues as a free intracellular amino acid (Batista et al. 2013). A wide range of studies have shown that taurine treatment reduces inflammation, fibrosis, apoptosis and hyperplasia in various organs (Li et al. 2017; Zhang et al. 2014; Men et al. 2010). It protects many tissues and organs against oxidative stress and toxicity induced by various poisonous substances (Das et al. 2009; Sun et al. 2014; Men et al. 2010). Taurine is considered an attractive candidate to relieve As-induced neurotoxicity.

In the present study, the effect of arsenite and taurine on the viability of primary cortical neurons was assessed using the MTT assay. Apoptosis was assessed by measuring caspase-3 activity in taurine/arsenite-treated cells. The aim of the study was to investigate the beneficial effect of taurine on As-induced neurotoxity in primary cortical neurons.

2 Methods

2.1 Primary Cortical Neuron Culture and Treatment

Primary cortical neurons were obtained from embryonic rat brains and characterized according to methods reported previously (Teng et al. 2013) with slight modifications. Briefly, embryos were collected from pregnant rat at day 16–18. Brains were isolated and kept in basal media eagle containing 26.8 mM glucose, 2 mM glutamine, 20% fetal bovine serum at 37 °C for 10 min with a gentle shaking. Then, the cortices were passed through a 14-G cannula and dissociated. The resulting suspension was centrifuged at 200 × g for 5 min and cell pellet was collected. Cells were resuspended and seeded on poly-D-lysine (5 ug/ml) precoated dish in an incubator. Sodium arsenite was exposed on the second day with or without taurine for 24 h.

2.2 MTT Assay

Cell viability was assessed by examining the level of MTT reduction. Briefly, 10 μl of 5 mg/ml MTT solution (in PBS) was added to each well of a 96-well plate, and the cells were incubated at 37 °C for 4 h. Then, 100 μl of dimethyl sulfoxide (DMSO) was added to each well to dissolve formazan crystals. Following incubation for 30 min, the absorbance was read at 570 nm. Cellular viability was expressed as a percentage relative to the control (Control %).

2.3 Caspase-3 Activity Assessment

Caspase-3 activity was examined with a commercial kit (Beyotime, China) according to the protocols. The absorbance was measured at 405 nm and expressed as a percentage relative to the control (control %).

2.4 Western Blot

Cells were homogenized in lysis buffer with 1% proteinase inhibitors. The total cell lysis was loaded for SDS-PAGE to separate various proteins, then transferred to a PVDF membrane. The membrane was incubated with Akt or p-Akt primary antibodies (1:1000, Cell Signaling Technology, USA) overnight at 4 °C. Second horseradish peroxidase-conjugated antibody (1:5000, Sigma, USA) was used for visualizing.

2.5 Statistic Analysis

Data were analyzed with SPSS 11.0. Difference between various groups was analyzed by one-way ANOVA and LSD test.

3 Results

3.1 Effect of as Exposure on Cell Viability

To confirm the toxicity of As on primary cortical neurons, neurons were incubated with medium containing 0, 1, 3, 5, 7, 9 μM arsenite for 24 h. As shown in Fig. 1, viability of neurons was decreased in a dose-dependent manner by As exposure. Cell viability was reduced nearly 55% at a concentration of 5 μM arsenite. Therefore, 5 μM arsenite was used in subsequent experiments.

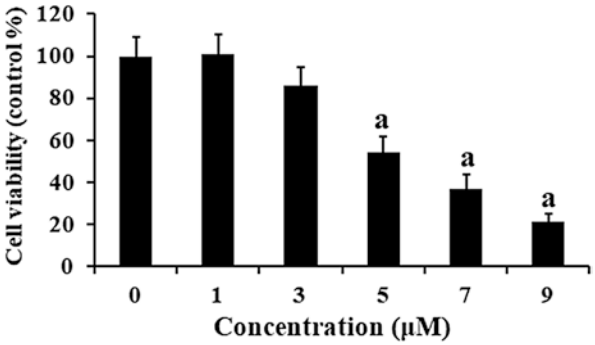

Fig. 1 The viability of arsenite-exposed primary cortical neurons. Data are represented as means ± SD. [a]$p < 0.05$, compared with the control group

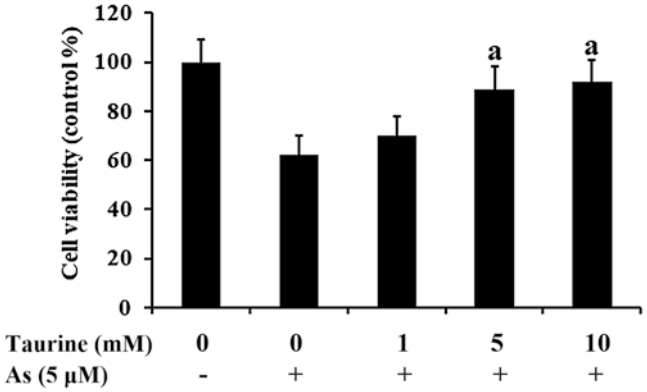

Fig. 2 Effect of taurine on arsenite-mediated drop in cell viability. Primary cortical neurons cells were co-treated with taurine and arsenite for 24 h. The MTT assay was performed to examine cell viability. Data represent means ± SD. [a]$p < 0.05$, compared with arsenite group

3.2 Effect of Taurine Treatment on Cell Viability

To assess the action of taurine on arsenite-induced neurotoxicity, primary cortical neurons were co-treated with taurine and arsenite. As shown in Fig. 2, cell viability decreased after 24 h of arsenite exposure, indicating that arsenite is toxic to neurons. However, taurine treatment significantly attenuated the decline in viability mediated by arsenite, suggesting that taurine functions as a cytoprotective agent against arsenite toxicity.

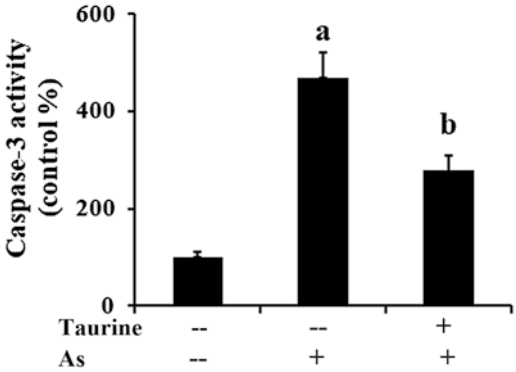

Fig. 3 Effect of taurine treatment on caspase-3 activity in arsenite-exposed primary cortical neurons. Data represent means ± SD. [a]$p < 0.05$, compared with control group; [b]$p < 0.05$, compared with arsenite group

3.3 Effect of Taurine on Caspase-3 Activity

We assessed the activity of caspase-3 using a commercial kit. As shown in Fig. 3 arsenite exposure markedly increased caspase-3 activity, an effect significantly attenuated by taurine treatment.

3.4 Effect of Taurine Treatment on Akt and p-Akt Levels

We subsequently detected the effect of taurine on the levels of total Akt ltersand phosphorylated Akt (p-Akt) in primary cortical neurons. Figure 4 shows that neither arsenite exposure nor taurine treatment alters total Akt content. Compared with the control group, the level of p-Akt decreased significantly in arsenite-exposed primary neurons. Taurine treatment markedly reduced the response of arsenite, indicating that the Akt pathway may contribute to the beneficial effect of taurine on arsenite-induced neurotoxicity.

4 Discussion

Apoptosis plays an important role in the neurotoxicity of various toxicants (Li et al. 2017; Mao et al. 2016; Ghooshchian et al. 2017; Dai et al. 2017). It has been reported that the regulation of apoptosis is a positive means to protect disturbed nerves. Taurine, a well-known antioxidant, is potentially anti-apoptotic (Li et al. 2017; Das et al. 2009). In this study, the effect of taurine on arsenite-exposed neurons was assessed. Primary cortical neurons were exposed for 24 h to arsenite with or without taurine, and the cell viability was examined using MTT assay. When compared with the control cells, cell viability of arsenite-treated primary cortical neurons decreased in a dose-dependent manner. Interestingly, taurine co-treatment significantly

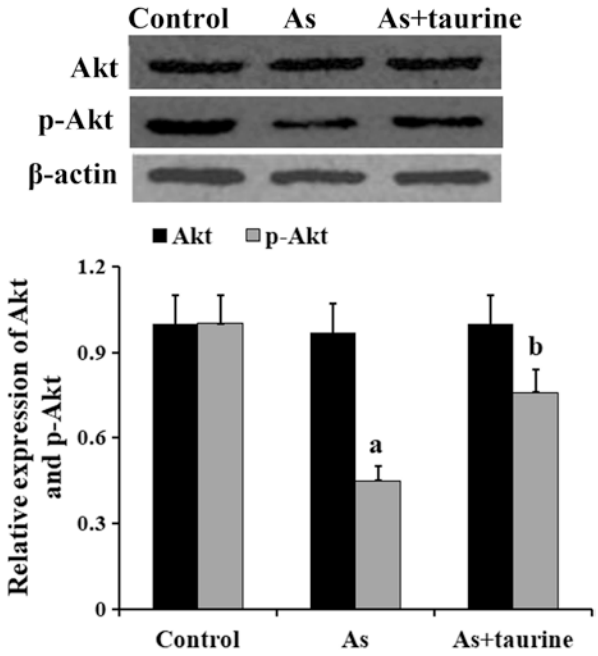

Fig. 4 Effect of taurine on Akt and p-Akt levels in arsenite-exposed primary cortical neurons. Data represent means ± SD. [a]$p < 0.05$, compared with control group; [b]$p < 0.05$, compared with As group

inhibited the decrease of cell viability in arenite-exposed neurons. Das et al. reported that taurine protected against $NaAsO_2$-induced apoptosis and oxidative stress in rat testes (Das et al. 2009). It was reported that taurine attenuated apoptosis in the lung of a limb ischemia reperfusion rat (Men et al. 2010), supporting our results.

To establish the effect of taurine on arsenite-induced apoptosis, caspase-3, a mediator of apoptosis, was examined. The results show that arsenite exposure is associated with the increase in caspase-3 activity, while the effect was prevented by taurine. We also found that arsenite exposure induced a decrease in Akt phosphorylation level in primary cortical neurons. While the inhibited effect of arsenite in neurons was abolished by taurine treatment. These results suggest a possible link between the Akt pathway and the anti-apoptotic effect of taurine in arsenite-exposed primary cortical neurons. In future, we will block the activation of Akt and assess the cytoprotective activity of taurine to provide more evidence.

5 Conclusion

In summary, the present study shows that taurine treatment largely prevents the decrease in cell viability of arsenite-exposed primary cortical neurons. Taurine also attenuates arsenite-induced activation of caspase-3 activity and the decrease in Akt

phosphorylation. Thus, taurine treatment protects against arsenite-mediated apoptosis, an effect that may involve the activation of the Akt pathway.

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