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ORIGINAL ARTICLE

Taurine prevented cell cycle arrest and restored neurotrophic gene expression in arsenite-treated SH-SY5Y cells

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Abstract The study investigated the effect of taurine on cell viability and neurotrophic gene expression in arsenite-treated human neuroblastoma SH-SY5Y cells. Arsenite-induced intracellular reactive oxygen species (ROS) and interrupted cell cycle in SH-SY5Y cells. In addition, arsenite reduced mitochondria membrane potential (MMP) and decreased neurotrophic gene expressions such as n-myc downstream-regulated gene 4 (NDRG-4), brain-derived neurotrophic factor (BDNF) and sirtuin-1 (SIRT-1) in SH-SY5Y cells. In parallel, taurine prevented cell cycle, restored MMP and reduced the intracellular ROS level, and taurine recovered NDRG-4, BDNF and SIRT-1 gene expressions in arsenite-treated SH-SY5Y cells while taurine alone has no effect on these parameters.

Keywords Arsenite · Taurine · NDRG-4 · BDNF · SIRT-1 · Neurotrophic

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Introduction

The environmental arsenic (Ars) from fungicides, industrial processing and especially drinking water has caused a huge impact on human society. Ars leads people to a variety of diseases such as neurodegenerative disease and results in loss of short-term memory (Bolla-Wilson and Bleecker 1987). Disruption of cell cycle in primary embryonic neuroepithelial cells by Ars was verified (Sidhu et al. 2006). In addition, Ars inhibited pyruvate and succinate dehydrogenase activity that resulted in mitochondria dysfunction and increase of intracellular reactive oxygen species (ROS) (Hughes 2002). Furthermore, Ars would accumulate in the brain and even transfer across the placenta barrier into offspring when pregnant rats are exposed to Ars-containing water (Xi et al. 2010).

There are several reports that indicated the relationship between Ars and Alzheimer's disease (AD). Hyper-phosphorylation of the tau protein is observed in Ars-treated Chinese hamster ovary cells (Giasson et al. 2002). In addition, activation of c-Jun *N*-terminal kinases (JNKs) and p38-mitogen-activated protein kinase (MAPK) by Ars-induced apoptosis in the cortical neurons of rats was observed. This is similar to β-amyloid-induced neuronal apoptosis through JNKs activation (Namgung and Xia 2001; Troy et al. 2001).

In mammals, the n-myc downstream-regulated gene (NDRG) family is classified to four related proteins with a 53–65 % conserve sequences (Okuda et al. 2008). The distribution of NDRG family is recognized in a variety of neuronal cells in the central nervous system (CNS). NDRG family is important for neuron development such as maintenance of myelin sheaths in peripheral nerves and differentiation of dendritic cell (Okuda et al. 2004; Choi et al. 2003). The latest identified protein in the NDRG

family, NDRG-4, is expressed in specific tissues such as brain and heart and crucial for neuron survival. In the NDRG-4-deficient mice, brain-derived neurotrophic factor (BDNF) is significantly decreased in the brain (Yamamoto et al. 2011). Similarly, this phenomenon is also observed in AD patients (Phillips et al. 1991). Notably, BDNF is crucial for increasing the density of synapses in neuron cells and is required for long-term memory storage (Bekinschtein et al. 2008; Hu et al. 2005). The aforementioned studies have suggested that NDRG-4 may be a key point in neurodiseases.

Taurine is an amino-acid-containing sulfur group and exists in a variety of organs in mammals. It has received considerable attention in regard to its versatile roles in the brain. Taurine protects neurons against glutamate-induced cytotoxicity (Leon et al. 2009). Recently, sirtuin-1 (SIRT-1), a kind of nicotinamide adenine dinucleotide-dependent deacetylase, is restored by taurine in zebra fish with hepatic steatosis (Hammes et al. 2012). In addition, SIRT-1 promotes mitochondria biogenesis and regulates the gene involved in survival-related genes in neuron cells (Tang and Chua 2008). Over-expression of SIRT-1 attenuated β-amyloid production in the AD mouse model (Donmez et al. 2010).

Neuroprotective effect of taurine in Ars-treated rats is proved (Das et al. 2009). However, the protective mechanism of taurine is still not well known. Furthermore, there are few evidences to demonstrate effect of taurine on neurotrophic expression. To investigate protective mechanism of taurine, we examined whether taurine prevented cell cycle arrest and restored mitochondria membrane potential (MMP) and neurotrophic gene expressions such as NDRG-4, BDNF and SIRT-1 in Ars-treated SH-SY5Y cells.

Materials and methods

Chemicals

DMEM/F12 medium and trypsin–EDTA were purchased from Gibco (Carlsbad, CA, USA). Fetal bovine serum was purchased from Hyclone Laboratories, Inc. (Logan, UT, USA). The purelink^{TM} RNA mini kit was purchased from Invitrogen (Carlsbad, CA, USA). Taurine, arsenite, hydrogen peroxide, 2,7-dichlorofluorescin diacetate (DCFDA), Rhodamine 123, ribonuclease A (RNase A) and propidium iodide (PI) were purchased from Sigma (St. Louis, MO, USA). A HiScrip I^{TM} first-strand cDNA synthesis kit system was purchased from Bionovas Biotechnology (Toronto, Ontario, Canada). WST-1 was purchased from Clontech (Mountain View, CA, USA). Oligo (dT) 15 primer was purchased from Promaga (Madison, WI, USA). The iQ SYBR Green Supermix was purchased from Bio-Rad (Hercules, CA, USA). Primers in the study were purchased from Mission Biotech (Taipei, Taiwan). Other chemicals used in the study were purchased from common sources and cell culture grade.

Cell culture and experimental setup

The human neuroblastoma SH-SY5Y cell line was obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA) and incubated with DMEM/F12 (1:1 ratio) medium with 10 % fetal bovine serum, antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin), 2 mM L-glutamine, 1 mM sodium pyruvate and 0.1 mM nonessential amino acids in a 75 cm^2 flask, and maintained in a humidified incubator with 5 % CO_2 at 37 °C.

Determination of cell viability

Cell viability was examined by cell proliferation agent WST-1 (Vu et al. 2012). A total of 4×10^4 cells were seeded into a 96-well plate. The next day, the medium was removed and fresh medium was added with or without a different concentration of Ars and 25 mM taurine for 24 h. After the incubation period, 10 μL WST-1 solution was added to the wells containing 100 μL medium, and the plate was incubated in 37 °C for another 2 h. The cell viability was represented with absorbance at 450 nm by ELISA reader (Biotek, USA) and was expressed as a percentage of untreated cells (control group).

Determination of cell cycle

Cell cycle was examined by PI staining (Schilling et al. 2009). A total of 8×10^5 cells were seeded into a 6-well plate. The next day, the medium was removed and fresh medium was added with or without 25 mM taurine and 20 μM Ars for 24 h. After the incubation period, cells were fixed in 70 % alcohol at −20 °C overnight. Then, the cells were resuspended in 500 μL propidium iodide staining solution (50 μg/mL PI, 0.1 mg/mL RNase A, 0.05 % Triton X-100) for 40 min at 37 °C. After the incubation period, the cell pellet was washed with PBS and resuspended in 500 μL PBS for FACScan flow cytometer analysis through the FL1 channel (Becton–Dickinson, USA).

Determination of intracellular ROS

Intracellular oxidative stress was estimated with 2,7-dichlorofluorescin diacetate fluorescence by inverted fluorescence microscopy and flow cytometry. DCFDA was converted to fluorescent DCH by peroxides and lost its

cell-permeant ability as well as retained in cells (Roy and Sil 2012). A total of 8×10^5 cells were seeded into a 6-well plate. The next day, the medium was removed and fresh medium was added with or without 25 mM taurine and 20 μM Ars for 24 h. Then, SH-SY5Y cells were incubated for 30 min with 10 μM DCFDA in serum-free DMEM-F12 medium at 37 °C. After the staining period, medium was removed and the cells were washed with ice-cold PBS. Then, cells were observed through inverted fluorescence microscopy (Nikon, Japan) or harvested with 0.25 % trypsin and resuspended in PBS for analysis of FACScan flow cytometry through the FL1 channel.

Determination of mitochondria membrane potential (MMP)

MMP was determined by fluorescent rhodamine 123 (Song et al. 2012). Briefly, a total of 8×10^5 cells were seeded into a 6-well plate and fresh medium with or without Ars and taurine was added the next day. After 24 h incubation, the medium was removed and the cells were stained with 10 μM rhodamine 123 for 30 min at 37 °C. Then, the cells were harvested with 0.25 % trypsin and resuspended in PBS for analysis of FACScan flow cytometry through the FL1 channel.

Quantitative real-time PCR

Gene expression was examined by quantitative real-time PCR (Schilling et al. 2009). Total RNA was extracted by the purelink^{TM}RNA mini kit, and 1 μg RNA was used for cDNA synthesis using the Hiscrip I^{tm} first-strand cDNA synthesis kit system for quantitative real-time PCR (qPCR). QPCR of n-myc down-regulation gene 4 (NDRG-4), BDNF, sirtuin-1 and β-2-microglobulin (B_2M) was carried out using the iQ SYBR Green Supermix system (Bio-Rad, USA). The results of NDRG-4, BDNF and SIRT-1 were normalized by B_2M levels. The sequences of the primers were NDRG-4-forward: 5′-GGAGGTTGTCTC T TTGGTCAAGGT-3′; NDRG-4-reverse: 5′-CTCATGAC AGCAGCCACCAGAAT-3′; BDNF-forward: 5′-AAACA TCCGAGGACAAGGTG-3′; BDNF-reverse: 5′-AGAAG AGGAGGCTCCAAAGG-3′; SIRT-1-forward: 5′-TGCTG GCCTAA TAGAGTGG CA-3′; SIRT-1-reverse: 5′-CTCA GCGCCATGGAAAATGT-3′; B_2M-forward: 5′-GAGGTT TGAAGATGCCGCATT-3′; B_2M-reverse: 5′-TGTGGAG CAACCTGCTC AGATA-3′.

Statistical analysis

The results were represented as mean ± SEM of three independent experiments and statistically analyzed by ANOVA followed by the Duncan test.

Results

Effect of taurine on Ars-induced cytotoxicity in SH-SY5Y cells

Figure 1 shows effect of Ars on cell viability in SH-SY5Y cells at concentration 0–20 μM and in combination of 25 mM taurine in the presence of all arsenite concentrations for 24 h. The cell viability is significantly reduced by Ars in a dose-dependent manner in Ars 10 and 20 μM group and is significantly restored when co-treated with 25 mM taurine from 80.6–91.3 % to 46–67 %, respectively. In addition, taurine alone has no effect on cell viability in SH-SY5Y cells as compared to control group.

Effect of taurine on cell cycle in Ars-treated SH-SY5Y cells

Paralleling to the cell viability experiment, we investigated whether taurine prevented cell cycle arrest in Ars-treated SH-SY5Y cells (Fig. 2a). In accordance with the expected result, the subG1 phase cells were significantly increased from 9.6 to 42.3 % and G1 phase cells remarkably decreased from 58.2 to 24.9 % in the 20 μM Ars-treated group. Co-treatment of 25 mM taurine resulted in a decrease of subG1 phase cells from 42.3 to 31.2 % and recovered G1 phase cells from 24.9 to 33.2 % in the 20 μM Ars-treated group. Quantitative data revealed a significant decrease of subG1 cells and restore of G1 cells by taurine and indicated that taurine prevented cell cycle arrest in Ars-treated SH-SY5Y cells while taurine alone has no

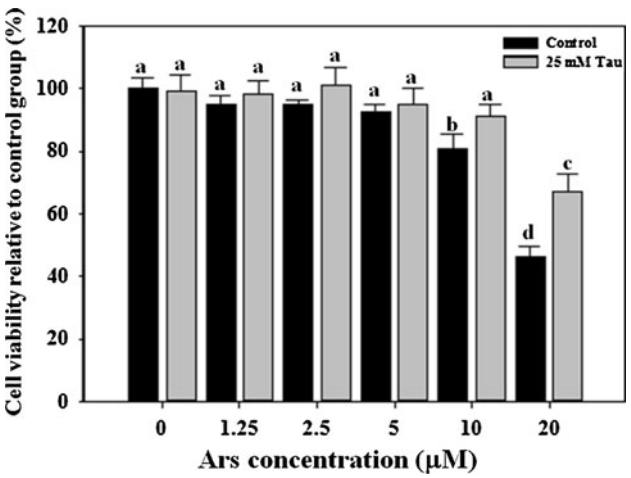

Fig. 1 Effect of taurine on Ars-induced cytotoxicity in SH-SY5Y cells. Cells were incubated with or without the indicated concentration of Ars and 25 mM taurine (Tau) for 24 h. Quantitative data are represented as the mean ± SEM from three independent experiments. The *different letters a–d* represent a significant difference with $p < 0.05$ among each group

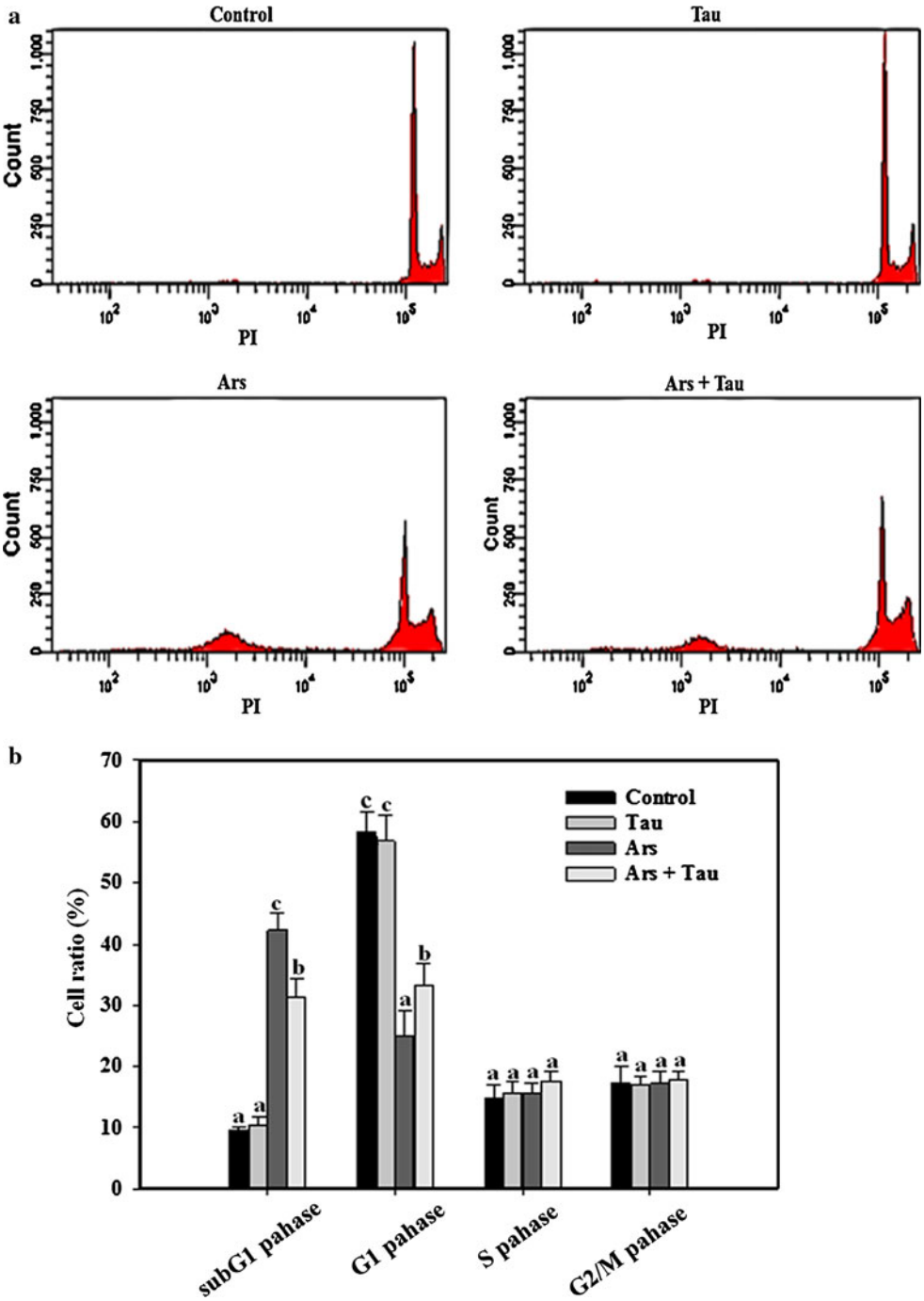

Fig. 2 Effect of taurine on cell cycle in Ars-treated SH-SY5Y cells. Cells were treated with or without 20 μM Ars and 25 mM taurine (Tau) for 24 h. (**a**) Cell cycle was examined by flow cytometry. (**b**) Quantitative data are represented as the mean ± SEM from three independent experiments. The *letters a–c* represent the significant difference with $p < 0.05$ among the groups

effect on cell cycle distribution as compared to control group.

Effect of taurine on intracellular ROS in Ars-treated SH-SY5Y cells

The intracellular oxidative stress is quantitatively determined by flow cytometry. As compared with control group, the high ROS cells have an obvious increase from 27.6 to 61.2 % in 20 μM Ars-treated cells at 24 h. Addition of taurine to Ars group resulted in a decrease from 61.2 to 44.8 % (Fig. 3a). Quantitative data revealed a significant inhibition of intracellular ROS level by taurine and indicated that taurine protected SH-SY5Y cells from Ars-induced oxidative stress since taurine alone do not influence the basal ROS level (32.3 %) (Fig. 3b).

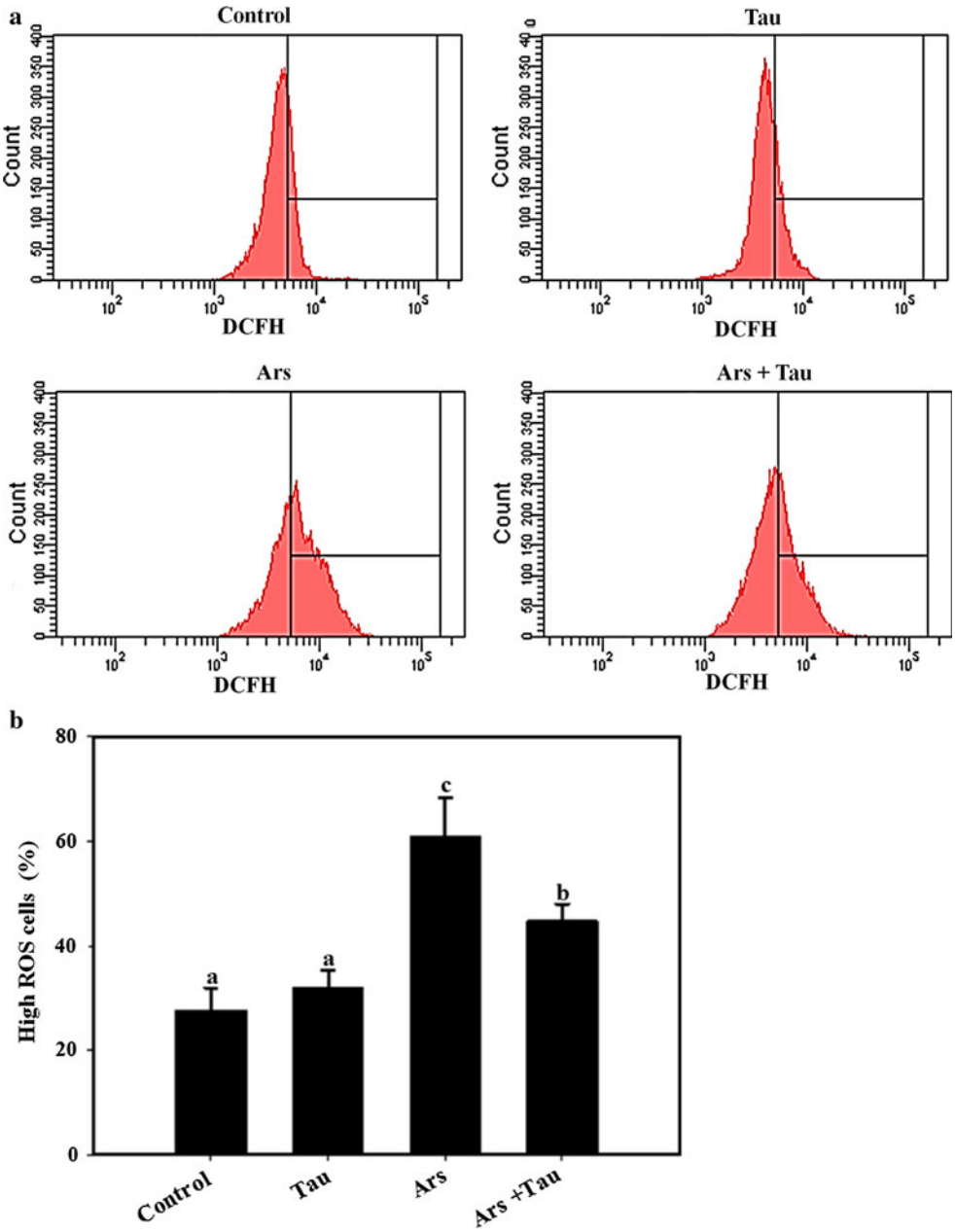

Fig. 3 Effect of taurine on intracellular ROS level in Ars-treated SH-SY5Y cells. Cells were treated with or without 20 μM Ars and 25 mM taurine (Tau) for 24 h. **a, b** Quantitative data of intracellular ROS level are represented as the mean ± SEM from three independent experiments. The *letters a–c* represent the significant difference with $p < 0.05$ among the groups

Effect of taurine on MMP in Ars-treated SH-SY5Y cells

As compared with the control group, a significant increase of low MMP cells from 28.0 to 77.9 % is observed in 20 μM Ars group at 24 h. Co-treatment with 25 mM taurine decreased the ratio of low MMP cells (59.8 %) (Fig. 4a). Quantitative data revealed a significant recovery of MMP by taurine and indicated that taurine prevented MMP loss in Ars-treated SH-SY5Y cells while taurine alone has no effect on MMP (26.4 %) (Fig. 4b).

Effect of taurine on neurotrophic gene expression in Ars-treated SH-SY5Y cells

To investigate whether taurine regulates neurotrophic gene expression, qPCR was used to examine the NDRG-4, BDNF and SIRT-1 gene expressions. The results showed that NDRG-4, BDNF and SIRT-1 gene expressions were significantly reduced after treatment of Ars for 24 h. Co-treatment with 25 mM taurine exhibited a significant restoring of NDRG-4, BDNF and SIRT-1 gene expressions, and indicated that taurine regulated neurotrophic gene expression in Ars-treated SH-SY5Y cells. In

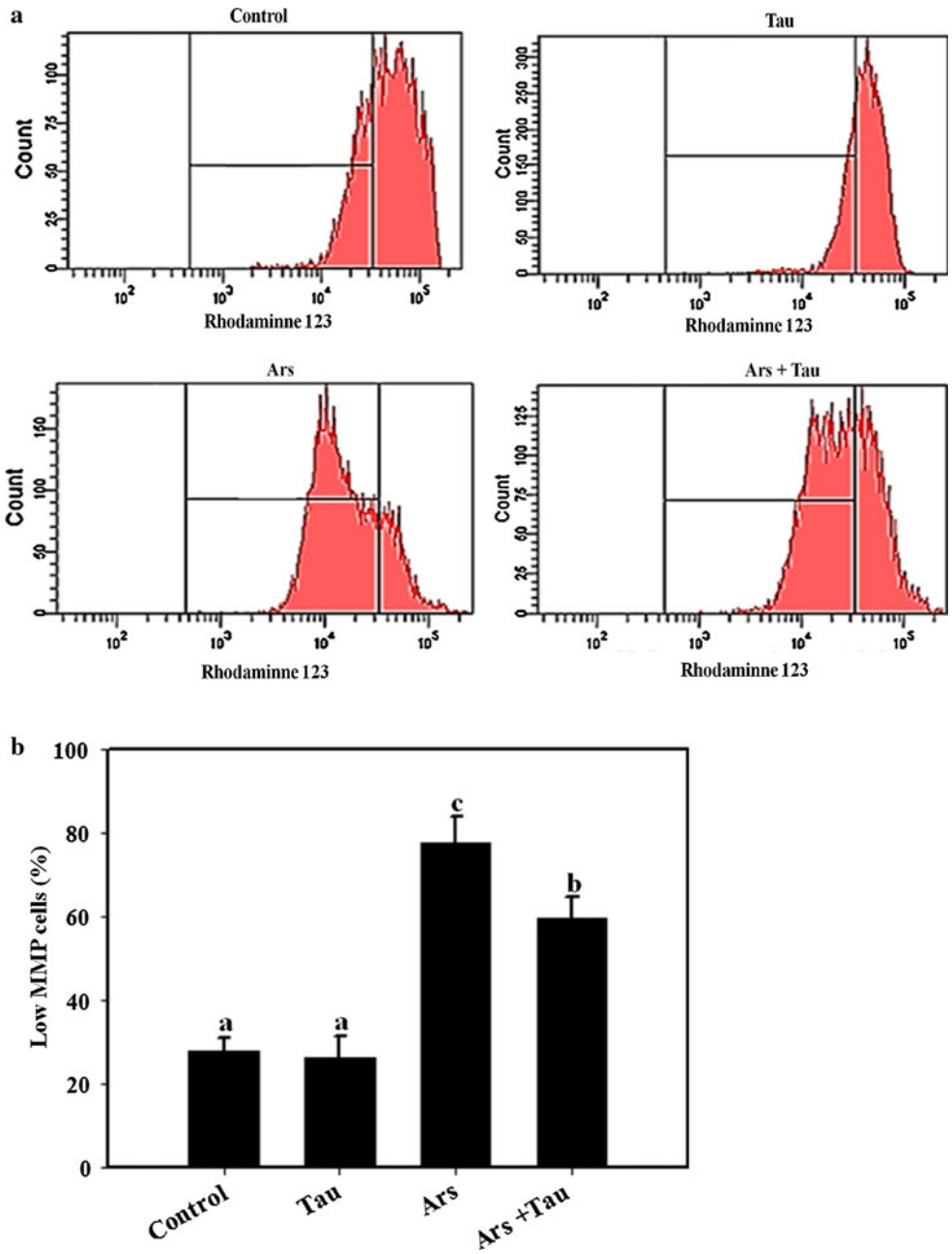

Fig. 4 Effect of taurine on intracellular MMP in Ars-treated SH-SY5Y cells. Cells were treated with or without 20 μM Ars and 25 mM taurine (Tau) for 24 h. **a** MMP was examined by flow cytometry. (**b**) Quantitative data are represented as the mean ± SEM from three independent experiments. The *letters a–c* represent the significant difference with $p < 0.05$ among the groups

taurine-treated group, all the gene expressions examined in current study have no significant change as compared to control group.

Discussion

As a natural antioxidant, taurine protected the cerebellar granule cells against glutamate-induced cytotoxicity by modulating the intracellular calcium homeostasis and energy metabolism (Idrissi and Trenkner 1999). Besides, taurine prevented β-amyloid-induced neurotoxicity (Louzada et al. 2004). Beyond the multiple protective routes of taurine, it is one of abundant free amino acids in the CNS and even reaching up to 50 mM in leukocytes (Fukuda et al. 1982). Although taurine is a hydrophilic compound and can not diffuse quickly across the cell membrane, taurine transporter could maintain the ratio of intra- and extracellular concentration of taurine to even 600:1 (Della Corte et al. 2002). Administration of taurine by i.p. injection significantly raised taurine level that reached up to about 14 mM in the hippocampus of rats (Lallemand and De Witte 2003). Hence, these exerted us to examine the protective effect of taurine in Ars-treated

SH-SY5Y cells. In this study, we demonstrated that taurine prevented cell cycle arrest, reduced intracellular ROS level and restored MMP, NDRG-4, BNDF and SIRT-1 gene expressions in Ars-treated SH-SY5Y cells.

Oxidative stress is involved in neurodegenerative disease and facilitates inflammatory progression (Perry et al. 2002). In our results, increased intracellular ROS level was observed in Ars-treated SH-SY5Y cells (Fig. 3). Thus, we examined whether taurine could reduce intracellular oxidative stress and these parallel experiments attested that taurine could reduce the intracellular ROS level in Ars-treated SH-SY5Y cells. The antioxidant activity of taurine may contribute to its neuroprotective effect. In order to explore other new routes, we examined neurotrophic gene expression, including SIRT-1, NDRG-4 and BDNF by qPCR. In Ars-treated SH-SY5Y cells, NDRG-4 gene expression is also significantly decreased and cell cycle is arrested. Similarly, BDNF gene expression is also down-regulated by Ars. The results supported our hypothesis that Ars abated NDRG-4 gene expression and resulted in a decrease of the BDNF gene expression in SH-SY5Y cells. NDRG-4 is required for cell survival and differentiation in neuron cells. Inhibition of NDRG-4 in rat pheochromocytoma PC12 cells resulted in disruption of differentiation (Ohki et al. 2002). Importantly, NDRG-4 is responsible for cell cycle progression in glioblastoma cells (Schilling et al. 2009). These may explain why Ars-induced neurotoxicity accompanied the memory loss. With respect to energy metabolism, SIRT-1 is imperative for neuron survival and regulates energy metabolism through promoting mitochondria biogenesis. Furthermore, activation of SIRT-1 stimulates protein expression of anti-stress and anti-apoptotic protein (Tang and Chua 2008). Ars reduced SIRT-1 gene expression and this may involve in the MMP decrease. In addition, Ars diminished MMP by inhibiting pyruvate and succinate dehydrogenase activity (Hughes 2002). Therefore, we evaluated MMP as an indicator of mitochondria function (Fig. 4) and demonstrated that taurine possessed capacity to restore MMP in Ars-treated SH-SY5Y cells.

Various studies investigated the relationship between BDNF and neurodegenerative diseases. BDNF is one of the important neurotrophins associated with memory storage and learning ability (Bekinschtein et al. 2008). In addition, BDNF prevents cortical neurons death in amyloid-transgenic mice (Nagahara et al. 2009). Notably, NDRG-4 is essential for maintaining the BDNF level in the cortex (Yamamoto et al. 2011). NDRG-4-deficient mice exhibited a significant decrease of BDNF gene expression, symptom of poor memory and spatial cognitive impairment (Yamamoto et al. 2011). Hence, we hypothesized that Ars inhibits NDRG-4 and BNDF gene expressions, both deficient in the brain of AD (Phillips et al. 1991; Zhou et al. 2001). However, NDRG-4 is required for cell cycle in neuron cells. Decrease of NDRG-4 gene expression supported that cell cycle progression was inhibited in Ars-treated SH-SY5Y cells (Fig. 5). Meanwhile, taurine significantly restored NDRG-4 and BDNF gene expressions. In addition, we found that SIRT-1 gene expression was also down-regulated by Ars and was recovered by taurine (Fig. 5). This result is similar to that of Hammes et al. (2012). Although the current study used higher concentration in arsenite-treated SH-SY5Y cells, we provide a putatively protective mechanism of taurine against arsenic-induced oxidative cerebral disorders in the rats brain (Das et al. 2009). In addition, taurine could restore cell viability, MMP and neurotrophic gene expression under arsenite-induced cell cytotoxicity, taurine alone has no effect of on cell proliferation and neurotrophic gene expression as compared to control group in SH-SY5Y cells. We concluded that taurine prevented SH-SY5Y from oxidative stress induced by arsenite rather than that taurine promoted SH-SY5Y

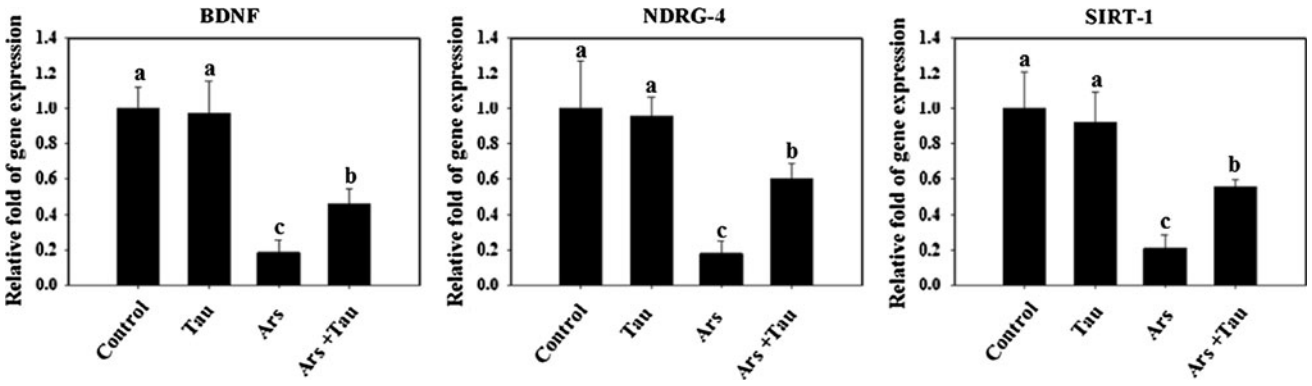

Fig. 5 Effect of taurine on gene expression of NDRG4, BDNF and SIRT-1 in Ars-treated SH-SY5Y cells. Cells were treated with or without 20 μM Ars and 25 mM taurine (Tau) for 24 h. The gene expression was determined by quantitative PCR and normalized to B_2M expression. Quantitative data are represented as the mean ± SEM from three independent experiments. The *letters a–c* represent the significant difference with $p < 0.05$ among the groups

proliferation or enhanced neurotrophic gene expression by itself.

Conclusion

In summary, the results of this study proved that taurine (1) prevented cell cycle arrest, (2) reduced the intracellular ROS level, (3) restored MMP and (4) recovered NDRG-4, BNDF and SIRT-1 gene expressions and in Ars-treated SH-SY5Y cells. Furthermore, we first revealed that Ars inhibited neurotrophic gene expression. This study suggested the regulatory effect of taurine on the neurotrophic gene in Ars-treated SH-SY5Y cells.

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Conflict of interest The authors declare that they have no conflict of interest.

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