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ORIGINAL ARTICLE

Taurine resumed neuronal differentiation in arsenite-treated N2a cells through reducing oxidative stress, endoplasmic reticulum stress, and mitochondrial dysfunction

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Abstract The goal of the study is to investigate the preventive effect of taurine against arsenite-induced arrest of neuronal differentiation in N2a cells. Our results revealed that taurine reinstated the neurite outgrowth in arsenite-treated N2a cells. Meanwhile, arsenite-induced oxidative stress and mitochondrial dysfunction as well as degradation of mitochondria DNA (mtDNA) were also inhibited by co-treatment of taurine. Since oxidative stress and mitochondrial dysfunction is closely associated with endoplasmic reticulum (ER) stress, we further examined indicators of ER stress, 78 kDa glucose-regulated protein (GRP78), and C/EBP-homologous protein (CHOP) protein expression. The results demonstrated that taurine significantly reduced arsenite-induced ER stress in N2a cells. In the parallel experiment, arsenite-induced disruption of intracellular calcium homeostasis was also ameliorated by taurine. The proven bio-function of taurine preserved a preventive effect against deleteriously cross-talking between oxidative stress, mitochondria, and ER. Overall, the results of the study suggested that taurine reinstated neuronal differentiation by inhibiting oxidative stress, ER stress, and mitochondrial dysfunction in arsenite-treated N2a cells.

Keywords Arsenite · Neuronal differentiation · Taurine · ER stress · Oxidative stress

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Introduction

Arsenic (As), one of the most abundant elements in the earth, widely exists in the environment such as industrial waste, fungicide, and ground water. In the natural environment, As level is estimated as 1–10 ppm in soil and 1.6 ppb in seawater (Emsley 2011). The amount of As compared with other elements ranks twelfth in the human body, fourteenth in seawater, and twentieth in the earth crust (Mandal and Suzuki 2002). Notably, Agency for Toxic Substances and Disease Registry ranked As as the most hazard element in USA based on toxicity and hazard to human in 2011 (ATSDR 2011). Although As compounds could be used as respiratory metabolites in few species of bacteria, there are accumulating studies indicating that As is tremendously poisonous to human, and increased environmental As level has become more and more an emergent issue in the world (Sarkar et al. 2013).

Arsenic is one of the five identified industrial metals with strong neurotoxicity (Grandjean and Landrigan, 2014). Various studies have proven that As exposure led children to brain-related disorders such as delaying of intelligent development and decline of cognitive function during rapid brain-developing times in human. As caused unusual migration of neurons and postponed neuronal maturation during brain-developmental period of rats (Dhar et al. 2007). Abnormal changes of morphology of neuronal structure and axons were also found in another

study (R´ros et al. 2009). Recent study revealed that As exposure interrupted neuronal differentiation in N2a cells (Wang et al. 2010a). Epidemiological study also reported impaired cognitive performance in Mexican school children who exposed to As (Rosado et al. 2007). According to neuropsychological tests, children exposed to As in school-age showed significant decline of general intelligence and intelligence quotient score (Wright et al. 2006). Indeed, neuronal differentiation is an essential process for brain development, especially in children. During the development, neural stem and progenitor cells differentiated into specialized neurons and formed axons and synapses which made neural network and plasticity in brain (Ladran et al. 2013). Once neuronal differentiation is interfered by environmental toxic factors in early development, conformational change and disturbed synapse activity of neurons are found and these led to faults of behaviors and cognitive function in human (Heath and Picciotto 2009). On the other hand, As-induced degeneration of axon structure may also be the contributor for delaying brain development. Obvious decline of the neurofilament light chain in sciatic nerve is found in As-treated rats, and this contributed to degeneration of the nervous integrity (Vahidnia et al. 2006). Besides, As intoxication gave rise to reduction of nerve conduction velocity and might involve axonal degeneration (Goebel et al. 1990). These studies elucidated that As has serious deleterious effects on neuronal differentiation during brain-developmental period and leads to neurological dysfunction in children.

The As-induced neuro-toxic mechanism is closely associated with mitochondrial dysfunction, ER stress, and oxidative stress. These pathological signs are also considered as major contributors to neurological disease. Indeed, As interrupted neuronal differentiation by inducing oxidative stress in N2A cells (Wang et al. 2010a). On the other hand, As inhibited metabolism-related enzymes such as pyruvate dehydrogenase via attacking with their thiol groups and altered balance of cellular energy (Samikkannu et al. 2003). Consequently, disruption of mitochondrial metabolism not only resulted in failure of energy production, but also an increase in intracellular reactive oxygen species (ROS). In addition, As caused oxidative modification of proteins such as nitrosylation in the brain of As-treated rats and induced ER stress as well as disturbed homeostasis of intracellular calcium, which caused neuronal dysfunction and death (Florea et al. 2007; Fan et al. 2010; Yen et al. 2012). Overall, it is noteworthy to concern that the complicated cross-talking between mitochondria, ER, and oxidative stress may synergistically augment the As-induced neurotoxicity (Brookes et al. 2004).

Looking for a naturally occurring candidate with multi-physiological functions may be a good strategy to cease the burden of the As-induced neurological disorders on human. For instance, administration of taurine could reduce oxidative and nitrosative stress in the brain of the As-treated rats (Fan et al. 2010). Recovery of neurotransmitter level was also found in further studies (Liu et al. 2013). Our previous study has demonstrated that taurine reduced oxidative stress and restored mitochondria function and memory-related gene expression (Chou et al. 2013). These reports provided the potential of taurine against the As-induced neurological disorders. Therefore, we are going to examine whether taurine prevented failure of neuro-differentiation in the aspect of the relationship between mitochondria, ER stress, and oxidative stress.

Materials and methods

Chemicals

DMEM medium and trypsin–EDTA were purchased from Gibco (Carlsbad, CA, USA). Fetal bovine serum was purchased from Hyclone Laboratories, Inc. (Logan, UT, USA). Taurine, arsenite, 2,7-dichlorofluorescin diacetate (DCFDA), rhodamine 123, all-trans retinoic acid, radioimmunoprecipitation assay buffer, protease inhibitor cocktail, and fluo-3 acetoxymethyl (fluo-3 AM) were purchased from Sigma (St. Louis, MO, USA). Rabbit-anti-mouse GRP78, CHOP, β-actin antibodies, goat-anti-rabbit IgG secondary antibody-conjugated HRP, and electrochemiluminescence kit were purchased from Santa Cruz Biotechnology, Inc (Ave, CA, USA). Geno Plus™ genomic DNA extraction miniprep system was purchased from Viogene Biotek, Corp (New Taipei City, Taiwan, ROC). 2 × long range PCR DNA polymerase master was purchased from Bionovas Biotechnology, Ltd (Toronto, Ontario, Canada).

Methods

Cell cultures and N2a cell differentiation

Mouse neuroblastoma N2a cell was obtained from American Type Culture Collection (Manassas, VA, USA) and maintained in DMEM composed of 10 % fetal bovine serum, 2 mM L-glutamine, antibiotics (penicillin: 100 U/mL; streptomycin: 100 μg/mL) in a humidified incubator at 37 °C and with 5 % CO_2. The cell differentiation was carried on in the growth medium containing 2 % fetal bovine serum and 20 μM all-trans retinoic acid for 48 h.

Cell morphology after neuronal differentiation

2×10^5 N2a cells were seeding in the 6-well plate overnight. Next day, N2a cells were co-treated with or without different concentrations of As (0, 0.5, 2.5, 5 μM) and

25 mM taurine in a 6-well plate for 48 h. After the experimental period, cells were washed by PBS and fixed in ice-cold methanol for 15 min at −20 °C. To make a clear image and cell morphology, cells were then stained by 0.5 % crystal violet solution for 30 min at 25 °C (Wang et al. 2010a). After that, cells were washed by PBS twice and pictured using inverted microscope at magnification 200×.

Determination of intracellular ROS

The intracellular ROS level was examined by staining with DCFDA as described before (Chou et al. 2013). In brief, 2×10^5 N2a cells were seeding in the 6-well plate overnight. Then, the medium was removed and fresh differentiating medium with or without 2.5 μM As and 25 mM taurine were added to well for another 48 h of incubation. After the differentiating period, the medium was replaced by serum-free DMEM with 10 μM DCFDA for another 30 min at 37 °C. Then, cells were washed by PBS twice and harvested by 0.25 % trypsin. Cells were centrifuged and resuspended in PBS immediately for the analysis of FACScan by the FL1 channel. The quantitative results of fluorescence were presented as mean value from three independent experiments.

Determination of cytosolic calcium concentration

The cytosolic calcium level is determined by calcium-sensitive fluorescent dye, fluo-3 AM (Zhang et al. 2004). Briefly, a total of 2×10^5 N2a cells were seeded into a 6-well plate, and differentiating medium with or without 2.5 μM As, and 25 mM taurine was added the following day. After the differentiating period, cells were washed twice by calcium-free PBS and then harvested by trypsinization. After centrifugation, cells were resuspended in calcium-free PBS with 2.5 μM fluo-3 AM and incubated at 37 °C for 10 min. Then, the fluorescence was immediately measured at FL-1 in a flow cytometer. The quantitative results of fluorescence were presented as mean value from 3 independent experiments.

Determination of mitochondria membrane potential (MMP)

MMP was determined by fluorescent rhodamine 123 as described before (Chou et al. 2013). Briefly, a total of 2×10^5 N2a cells were seeded into a 6-well plate, and differentiating medium with or without 2.5 μM As, and 25 mM taurine was added the following day. After 48 h incubation, the medium was replaced by DMEM with 10 μM rhodamine 123 for another 30 min at 37 °C. After the staining period, cells were washed by PBS twice and harvested with 0.25 % trypsin for the analysis of FACScan flow cytometry through the FL1 channel. The quantitative results were presented as mean value from three independent experiments.

Determination of mtDNA integrity

mtDNA integrity is determined by PCR (Wang et al. 2010a, b). Briefly, total DNA is extracted by Geno Plus™ genomic DNA extraction miniprep system and used amount of 30 ng DNA for PCR reactions. The monitored long fragment of 10-kb product is the indicator of mtDNA integrity, and a short 117-bp product is used for internal control to normalize the level of 10-kb products. The primer sequences are as following: 117-bp product: forward primer 5′-CCCAGCTACTACCATTCAAGT-3′, reverse primer 5-ATGGTTTGGGAGATTGGTTGATG-3′; 10-kb product, forward primer 5′-GCCAGCCTGACC CATAGCCATAATAT-3′, reverse primer 5′-GAGAGATTTTATGGGTGTAATGCGG-3′. PCR cycling conditions are presented as following, respectively. 10-kb product: denaturation for 15 s at 94 °C and then 12 min at 68 °C for annealing and extension; 117-bp PCR, denaturation for 30 s at 94 °C, annealing for 45 s at 60 °C, extension for 45 s at 72 °C. The amplifying cycle is run twenty times and finally extended at 72 °C for 10 min. The PCR products were separated by electrophoresis in an agarose gel with HealthView nucleic acid stain. The band area is quantified by Quantity One software and considered as degree of mtDNA integrity. The quantitative results were presented as mean value from 3 independent experiments.

Western blotting

After the experimental period, cells were lysed by radioimmunoprecipitation assay buffer containing 1 % (v/v) protease inhibitor cocktail on ice for 15 min. After centrifugation (20,800*g*) for 10 min, the supernatant is collected as protein extraction. Protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Pan et al. 2010). After that, the membrane was blocked in Tris buffer saline (pH 7.5) containing 0.1 % tween-20 (TBST) and 5 % defatty milk powder for 2 h at room temperature. Then, membrane was washed twice by TBST and incubated corresponding primary antibody (1:500) overnight and further incubated with the HRP-conjugated secondary antibody (1: 5,000) at room temperature for another 2 h. Finally, membrane was washed by TBST twice and visualized by electrochemiluminescence kit in an image system. The band area is quantified by Quantity One software and is considered as the amount of CHOP and GRP78 expression. The quantitative results were presented as mean value from three independent experiments.

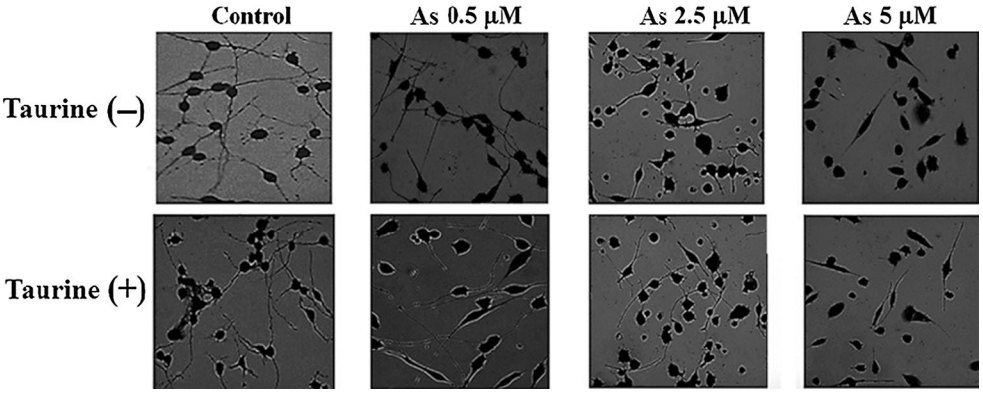

Fig. 1 Effect of taurine on the As-induced arrest of neuronal differentiation in N2a cells. Cells were incubated with or without indicated concentration of As and 25 mM taurine for 48 h. Cell morphology is observed in invert microscopy at ×200 magnification

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 the As-induced arrest of neuronal differentiation in N2a cells

Figure 1 showed the effect of As on cell morphology in differentiated N2a cells at concentrations 0.5, 2.5, and 5 μM and in combination of 25 mM taurine in the presence of all As concentrations for 48 h. The neurite length is significantly reduced by As in a dose-dependent manner (2.5 and 5 μM group). Co-treatment with 25 mM taurine obviously restored neurite outgrowth in 2.5 μM As-treated group, but no significant effect in 5 μM As-treated group (Fig. 1). In addition, taurine alone has no effect on basal neuronal differentiation in N2a cells, as compared to control group. Hence, the following experimental condition used the 2.5 μM As group as a negative control to investigate the protective routes of taurine against As-induced arrest of neuronal differentiation.

Effect of taurine on the As-induced intracellular ROS level in N2a cells after differentiation

After experimental period, the intracellular ROS level of N2a cells is quantitatively examined by flow cytometry, and the ROS signal is presented as fluorescent unit (FU). To compare with the control group (3,596 FU), 2.5 μM As-treated N2a cell exhibited a significant increase of intracellular ROS level (7,256 FU) after the differentiation period. Co-treatment of 25 mM taurine to As group resulted in an obvious inhibition of ROS signal from 7,526 to 5,795 FU as compared to As-treated alone group (Fig. 2a). Quantitative data demonstrated that a significant reduction of intracellular ROS level by taurine, which elucidated that taurine protected N2a cells from As-induced oxidative stress after differentiation. However, taurine alone does not influence the basal ROS level (3,848 FU) (Fig. 2b).

Effect of taurine on As-induced mitochondrial dysfunction in N2a cells after differentiation

After the differentiation of N2a cells, MMP was quantitatively examined by flow cytometry as the indicator of mitochondria function. Impaired MMP is found in As-treated N2a cells since rhodamine 123 fluorescence is dropped from 21,254 to 12,894 FU as compared with the control group. Meanwhile, MMP is restored by co-treatment of taurine from 12,894 to 16,566 FU, (Fig. 3a). Quantitative data demonstrated that a significant recovery of MMP by taurine, which elucidated that taurine prevented N2a cells from the As-induced mitochondrial dysfunction after differentiation. Similarly, taurine alone does not influence the basal MMP (Fig. 3b). Meanwhile, the integrity of mtDNA is also examined by PCR as another parameter for monitoring mitochondria function. mtDNA integrity is remarkably reduced by As, and co-treatment with taurine significantly restored mtDNA integrity in the As-treated N2a cells (Fig. 4). Quantitative data demonstrated that a significant recovery of MMP and mtDNA by taurine, which elucidated that taurine prevented N2a cells from the As-induced mitochondrial dysfunction after differentiation, while taurine alone does not influence the basal condition of MMP and mtDNA (Figs. 3, 4).

Effect of taurine on the As-induced disruption of intracellular calcium homeostasis in N2a cells after differentiation

In the parallel experiment, we consistently monitored intracellular calcium homeostasis by flow cytometry in the As-treated N2a cells. Figure 5a shows a significant increase of intracellular calcium level from 11,527 to 23,235 FU

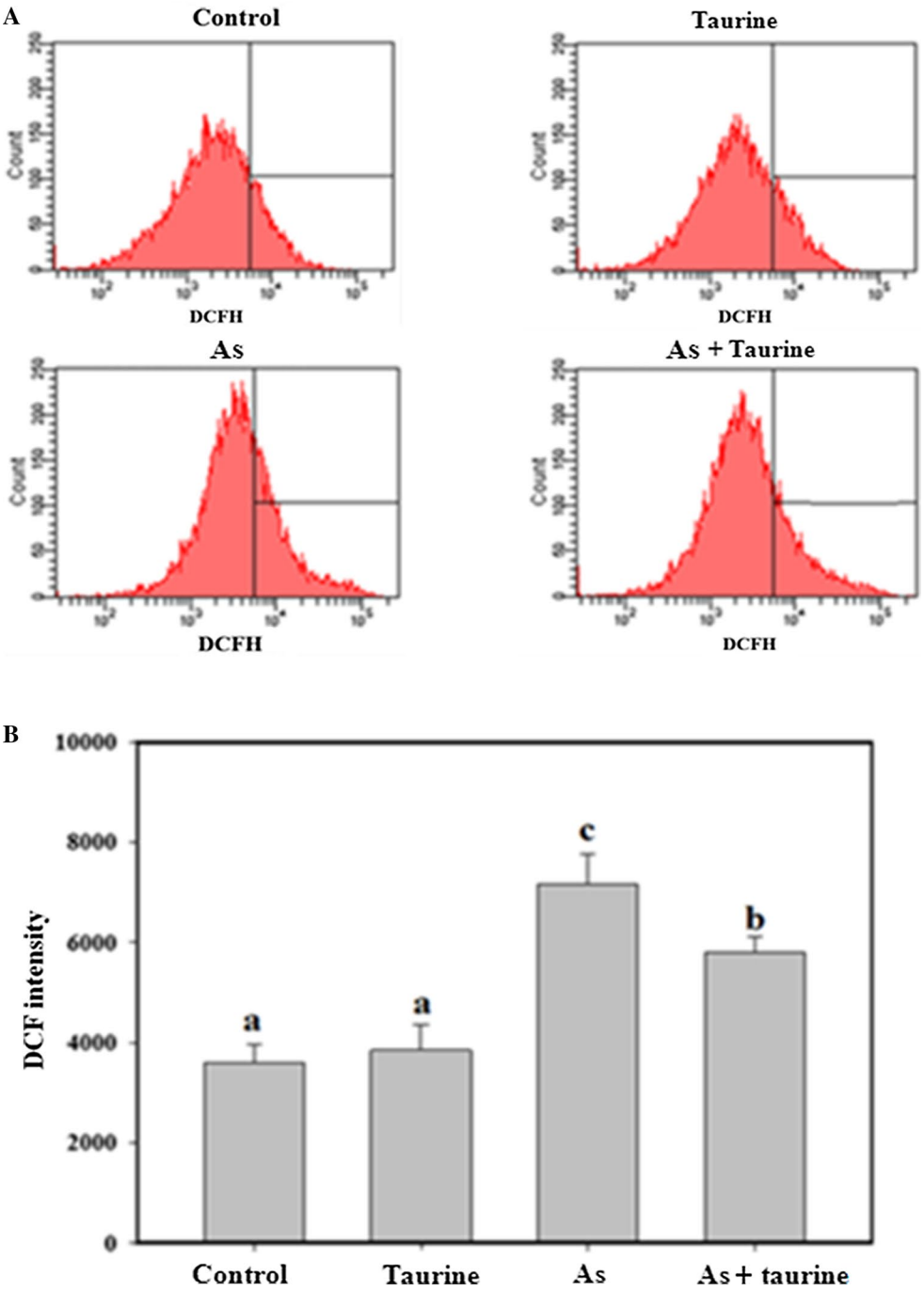

Fig. 2 Effect of taurine on intracellular oxidative stress in the As-treated N2a cells. Cells were incubated with or without indicated concentration of As and 25 mM taurine for 48 h. (**a**) Intracellular oxidative stress is examined by flow cytometry. (**b**) The quantitative results were presented as mean value from three independent experiments. The *letters a–c* mean the significant difference with $p < 0.05$ among each group

in the As-treated N2a cells after the differentiation period. Co-treatment of taurine in As-treated N2a cells showed an obvious reduction of intracellular calcium level from 23,235 to 18,105 FU as compared to As-treated alone group (Fig. 5a). Quantitative data demonstrated that a significant reduction of intracellular calcium level by taurine in the As-treated N2a cells after differentiation elucidated that taurine regulated intracellular calcium homeostasis in As-treated N2a after differentiation period, while taurine alone does not influence the basal intracellular calcium level (12,633 FU) (Fig. 5b).

Effect of taurine on the As-induced ER stress in N2a cells after differentiation

Since ER is an organelle for the storage of intracellular calcium, we followed to investigate whether As induces ER stress in N2a cells after differentiation. The

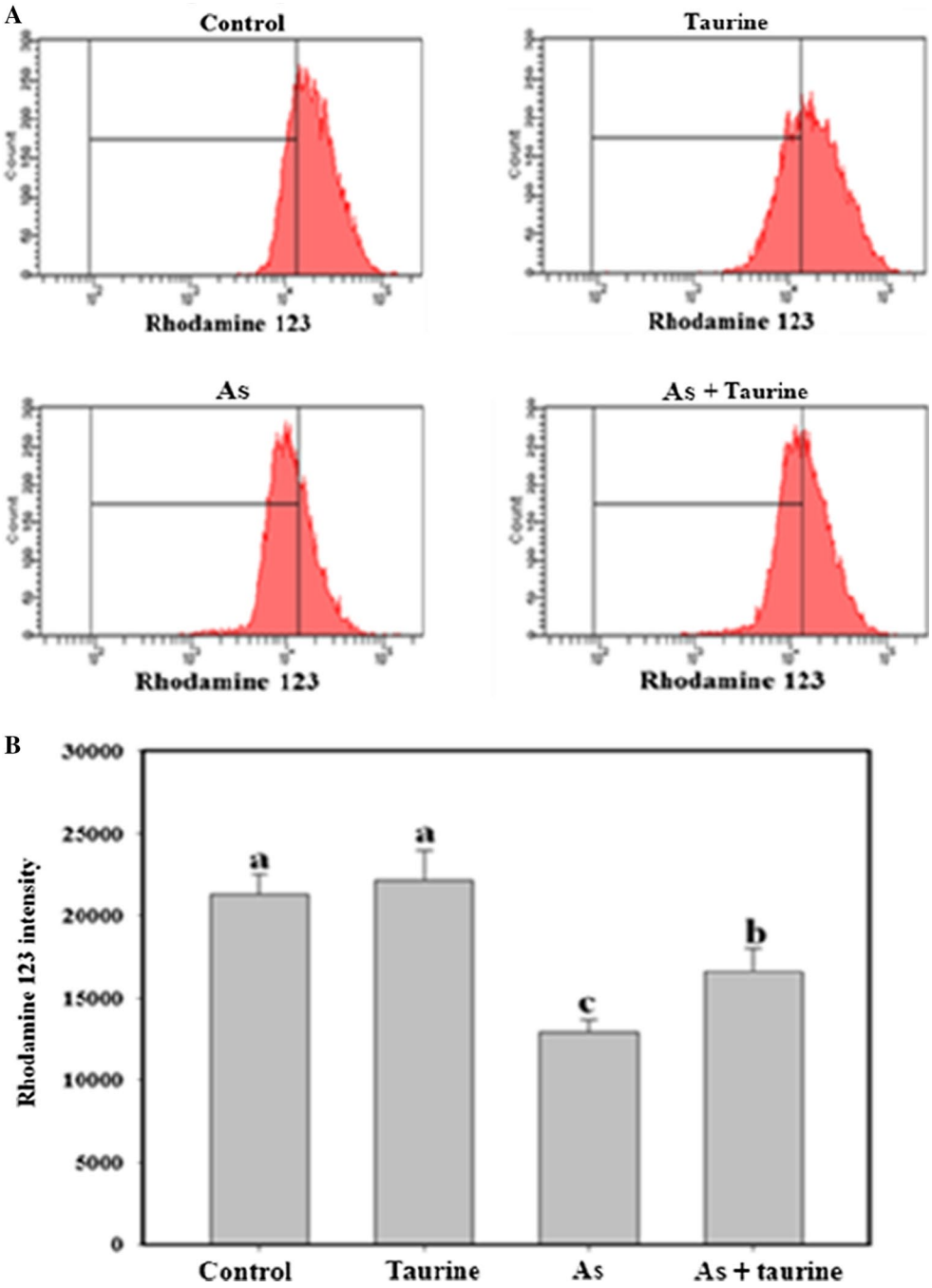

Fig. 3 Effect of taurine on MMP in the As-treated N2a cells. Cells were incubated with or without indicated concentration of As and 25 mM taurine for 48 h. (**a**) MMP is examined by flow cytometry. (**b**) The quantitative results were presented as mean value from three independent experiments. The *letters a–c* mean the significant difference with $p < 0.05$ among each group

examined indicator included ER stress-responsible chaperone, GRP78, and downstream protein of unfold protein response, CHOP. The results of Western blot analysis showed that both protein expressions of GRP78 and CHOP significantly increased (254 and 217 %, respectively) in the As-treated N2a cells as compared to control group. Meanwhile, co-treatment with taurine inhibited GRP78 and CHOP expression (171 and 162 %, respectively). Quantitative data demonstrated that a significant reduction of GRP78 and CHOP expression by taurine, which elucidated that taurine reduced ER stress in As-treated N2a after differentiation, while taurine alone does not influence the basal protein expression of GRP78 and CHOP (Fig. 6).

Discussion

There are growing consensus indicating that As-induced neurotoxicity is closely associated with oxidative stress and mitochondrial dysfunction. As-induced ROS formation and subsequently caused mitochondrial dysfunction and ER stress in neuronal cells (Lu et al. 2014). Also, As resulted

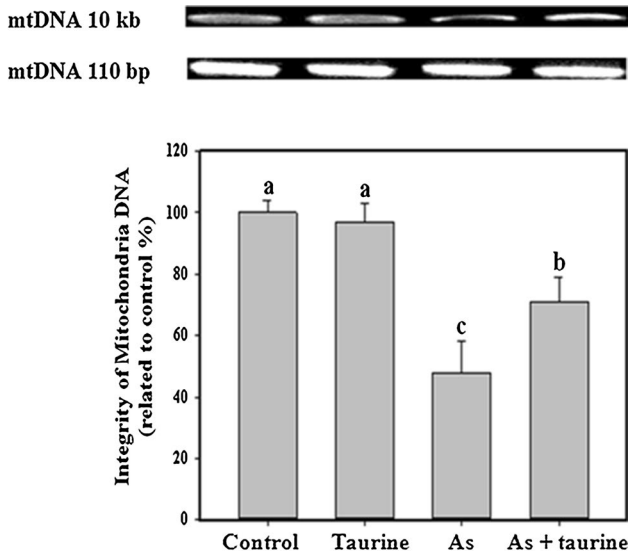

Fig. 4 Effect of taurine on mtDNA in the As-treated N2a cells. Cells were incubated with or without indicated concentration of As and 25 mM taurine for 48 h. (**a**) mtDNA is examined by PCR. The quantitative results were presented as mean value from three independent experiments. The *letters a–c* mean the significant difference with $p < 0.05$ among each group

in the release of iron from ferritin, producing lots of free radical through Fenton's reaction (Ahmad et al. 2000). Recently, it is proven that As-induced arrest of neuro-differentiation through ROS-dependent pathway since co-treatment with NAC resumed neuro-differentiation in the As-treated N2a cells (Wang et al. 2010a, b). Similarly, we demonstrated that co-treatment with taurine reinstated neurite outgrowth in As-treated N2a cells after differentiated period (Fig. 1). In the meanwhile, increase of intracellular oxidative stress is also found, which is significantly reduced by taurine (Fig. 2). These results suggested that taurine resumed neuro-differentiation in the As-treated N2a cells through reducing intracellular ROS level.

It is evident that mitochondrial dysfunction is associated with neuro-disorders and has been considered as early cyto-pathological sign of Alzheimer's disease (Dragicevic et al. 2010). For instance, β-amyloid impaired mitochondria function, resulting in the failure of respiratory chain and production of excessive ROS (Abramov et al. 2004). Enhancement of mitochondria function ameliorated cognitive ability in the mouse model with Alzheimer's disease, which indicated that mitochondria is crucial for maintaining cognitive function in Alzheimer's disease (Dragicevic et al. 2011). Hence, we consistently examined the effect of taurine on mitochondria function. The results indicated that taurine restored MMP in the As-treated differentiated N2a cells (Fig. 3). In the parallel experiment, we found that As-induced degradation of mtDNA is also reduced by taurine (Fig. 4). However, mitochondria function and mtDNA integrity are crucial for neuronal differentiation. The mtDNA integrity is crucial for maturation of mitochondria in differentiating neural stem cells (Wang et al. 2010b). Consistent study revealed that mutation of 8-oxoguanine DNA glycosylase, a DNA repair enzyme for maintaining mtDNA integrity, resulted in a shift of differentiation toward astrocytic lineage rather than neuronal lineage in neural stem cell (Wang et al. 2011). Also, blockage of complex III of respiratory chain in mitochondria inhibited neuronal differentiation in embryonic stem cells (Sandro et al. 2013). On the other hand, the physiological role of taurine in mitochondria is wildly discussed. Taurine-modified-uridine is found in mitochondrial tRNA and its deficiency is relevant to mitochondrial disease, which indicated taurine involved translation of respiratory chain protein in mitochondria (Suzuki et al. 2002). Moreover, the concentration of taurine in the water phase of mitochondria is estimated to be about 30–40 mM (Hansen et al. 2010). Therefore, the high concentration and specialized location of taurine in mitochondria may preserve beneficial effects in physiological meaning. These studies illustrated how important mitochondria is in neuro-differentiation and supported our hypothesis that taurine reinstated neuro-differentiation by reducing oxidative stress and restoring mitochondrial function in As-treated differentiated N2a cells.

ER is the organelle for protein synthesis and kinetic balance in the plasma, and ER stress would inhibit neuronal differentiation in neural stem cells (Kurosawa et al. 2007). When ER stress occurred, ER stress-response chaperone GRP78 would dissociate from double-stranded RNA-dependent protein kinase-like eukaryotic initiation factor 2α kinase and, consequently, induce unfold protein response-related gene ex pression such as CHOP (Wang et al. 2009). It has been verified that As would induce ER stress in neuronal cells. The disruption of intracellular calcium homeostasis is found in As-treated SY-5Y neuroblastoma cells (Florea et al. 2007). Very recent study elucidated that As-induced protein expression of GRP78 and CHOP through induction of intracellular oxidative stress and mitochondrial dysfunction in N2a cells (Lu et al. 2014). Hence, we further examined the effect of taurine on As-induced ER stress in N2a cells after differentiation period. Our results found that As-induced ER stress and disturbed homeostasis of intracellular calcium, whereas these phenomena were significantly counteracted by taurine in As-treated N2a cells (Figs. 5, 6). These corresponded to that As causes degeneration of neurofilament light chain through post-translational modification such as activating calcium-sensitive protease rather than gene modulation in sciatic nerve (Vahidnia et al. 2006). Accordingly, we surmised that taurine resumed neuronal differentiation by suppressing ER stress and intracellular ROS level since oxidative stress was

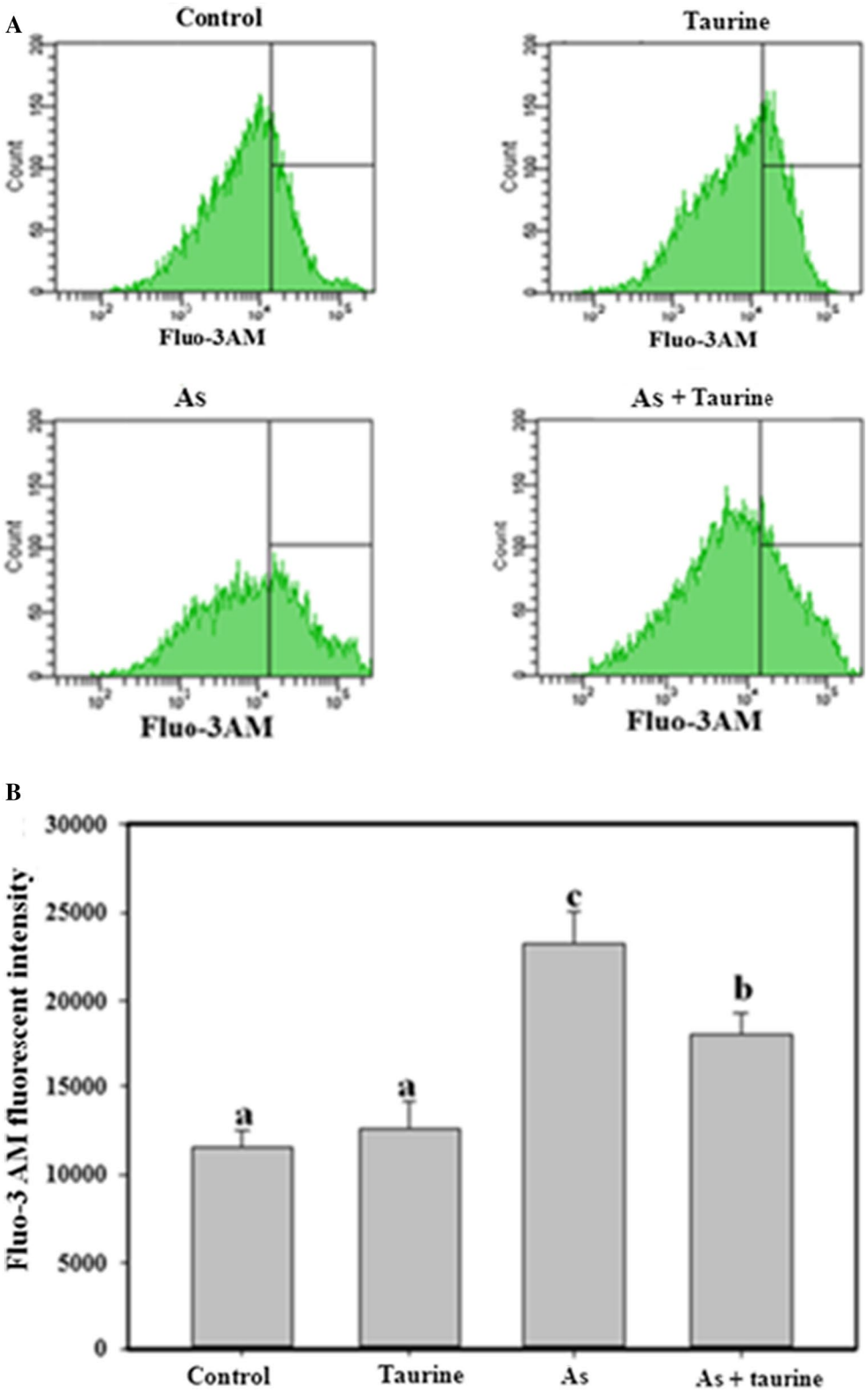

Fig. 5 Effect of taurine on intracellular calcium level in the As-treated N2a cells. Cells were incubated with or without indicated concentration of As and 25 mM taurine for 48 h. (**a**) The intracellular calcium level is examined by flow cytometry. (**b**) The quantitative results were presented as mean value from three independent experiments. The *letters a–c* mean the significant difference with $p < 0.05$ among each group

reduced by taurine in the parallel experiment (Fig. 2). The argument is also supported by the fact that taurine could reduce H_2O_2-induced ER stress in PC-12 cells (Pan et al. 2010).

To consider the linkage between ER stress and mitochondrial dysfunction, As-induced oxidative stress may act as a connective point in its neurotoxic mechanism. Indeed, oxidative stress not only altered intracellular

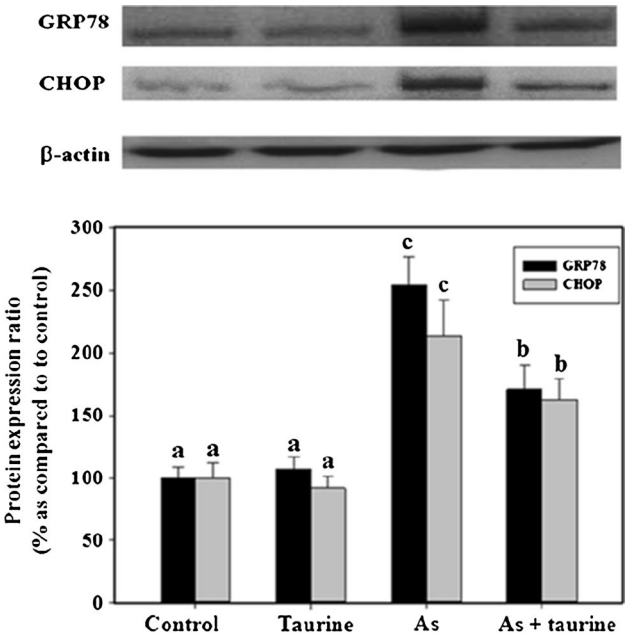

Fig. 6 Effect of taurine on GRP78 and CHOP expression in the As-treated N2a cells. Cells were incubated with or without indicated concentration of As and 25 mM taurine for 48 h. (**a**) GRP78 and CHOP expression is examined by Western blotting. The quantitative results were presented as mean value from three independent experiments. The *letters a–c* mean the significant difference with $p < 0.05$ among each group

signaling and led to arrest of neuronal differentiation, but also impaired mitochondrial function and mtDNA integrity (Wang et al. 2010a, b, 2011). In addition, oxidative products would also abnormally modified structure of cytoskeleton such as nitrosylation and result in conformational changes of cytoskeleton (Fan et al. 2010). Then, induction of ER stress and CHOP expression lead to further oxidative stress and disruption of calcium homeostasis (Florea et al. 2007; Song et al. 2008). Importantly, calcium homeostasis is crucial for regulating mitochondrial function and regulating activity of calcium-dependent enzymes. For instance, increased intracellular calcium activated calpain which destabilized mitochondria by activating Bid protein and leading to dissociation of cytochrome c from mitochondria (Mandic et al. 2002). As a consequence, cells are disable to repair damage and keep normal function in many perspectives of energy production, protein synthesis, and anti-oxidant system. Returning to central point, all of these routes consistently increased oxidative stress and aggravate deleterious cycle between mitochondria and ER inside neurons. Taken together, the proven bio-function of taurine such as mitochondrial stabilization, inhibition of ER stress, and regulation of calcium homeostasis may preserve beneficial effects to reduce As-induced neurotoxicity during neuro-differentiation. These viewpoints matched our hypothesis that taurine reduced As-induced arrest of neuronal differentiation by reducing oxidative stress and preventing deleteriously complicated cross-talking between mitochondria and ER in the As-treated N2a cells.

Conclusion

In summary, failure of neuronal differentiation in As-treated N2a cells is resumed by co-treatment of taurine, through recovering MMP, integrity of mtDNA, and reducing ER stress and intracellular ROS level. The results suggested the protective routes of taurine against As-induced arrest of neuronal differentiation through reducing oxidative stress and preventing further vicious cycle between mitochondria, ER and oxidative stress.

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

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