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EFFICACY OF TAURINE AGAINST ALUMINUM MALTOLATE-INDUCED APOPTOSIS IN SH-SY5Y CELLS VIA REDUCTION OF OXIDATIVE STRESS, ENDOPLASMIC RETICULUM STRESS, AND MITOCHONDRIAL DYSFUNCTION

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Key words: aluminum maltolate, taurine, apoptosis, oxidative stress, ER stress, mitochondrial dysfunction.

ABSTRACT

Aluminum (Al) is one of the most abundant elements on the earth's crust and is used in various industrial applications. However, Al is known to be associated with various neurodegenerative diseases. Taurine is a free amino acid presents at high concentrations in the brain and is crucial for neuron development. Here, the protective effects of taurine against Al-induced neurotoxicity were investigated. Al, at a concentration of 600 μM, induced apoptosis of and cell cycle arrest in human neuroblastoma SH-SY5Y cells. Additionally, Al induced a 55% increase in the levels of reactive oxygen species and inhibited mitochondrial membrane potential up to 60%. Al treatment also stimulated caspase 9 activities and Grp78 production. Furthermore, the expressions of neurotrophic genes including NDRG-4, BDNF and SIRT1 were inhibited. Hence this study revealed that taurine attenuates oxidative stress, ER stress and mitochondrial dysfunction and consequently protects against Al-induced cytotoxicity and neuronal apoptosis.

I. INTRODUCTION

Aluminum (Al) is a ubiquitous element encompassing approximately 8.3% of earth's crust, and is abundantly distributed in the environment as an industrial toxicant. Al has been applied in daily life in medicine, food processing, agriculture and water treatment (Bertholf et al., 1989; Becariaet al., 2002; Donmez et al., 2010). Al pollution disrupts the homeostasis of several metals, including magnesium, calcium and iron, and thereby inducing Al toxicity which affects biological functions and triggers many biochemical alterations (Hansen et al., 2010). These influences cause renal failure and systemic toxicity in various tissues including the brain, bone, liver and kidney. Epidemiological studies have provided evidence of the relationship between high Al levels and increased risk of numerous neurodegenerative disorders including Alzheimer's disease (AD) and Parkinson's disease (PD) (Berthon, 1996; Chen et al., Becaria et al., 2002; Yokel, 2002; Yumoto et al., 2009; Chen et al., 2011; Chou et al., 2013; Wasi et al., 2013).

1. Al Induces Neurodegenerative Diseases

Al reportedly causes an overproduction of reactive oxygen species (ROS) which can induce mitochondrial DNA mutation, mitochondrial respiratory chain damage, and membrane permeability alteration as well as influence calcium homeostasis and mitochondrial defense systems to cause apoptosis. Furthermore, ROS was also reported to affect endoplasmic reticulum (ER) stress via activation of caspase-12, thereby causing cell death (Perl and Moalem, 2006). Based on these studies, the role of Al has been implicated in neurodegenerative diseases (Kumar and Gill, 2009). AD is characterized by neuropathological hallmarks, namely Tau/neurofibrillary tangle and amyloid-β (beta)/amyloid plaques, which are accompanied by synaptic and neuronal loss as well as astrocytic gliosis (Tang and Chua, 2008). Additionally, this disease induces progressive loss of memory and cognitive dysfunction.

2. Al causes Neuronal Cell Apoptosis

Moreover, neurotrophic factors (NTFS) such as the N-myc

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downstream regulated gene (NDRG), brain-derived neurotrophic factor (BDNF) and sirtuin-1 (SIRT-1) are small, versatile proteins that maintain neuronal cell survival and function. NDRG family consists of four related proteins. NDRG-1~3 proteins are expressed in human umbilical vein endothelial cells, cortical pyramidal neurons and outer layers of the seminiferous epithelium, respectively (Schilling et al., 2009; Kawahara and Kato-Negishi, 2011; Yamanoto et al., 2011), whereas NDRG-4 is expressed in the brain and heart. Especially, NDRG-4 is a stress-related protein, which protects cells against neurological disorders and cerebrovascular disease. Notably, BDNF and SIRT-1 play an important role in cognition, learning and memory formation by modulating synaptic plasticity (Takahashi et al., 2005). An *In vivo* study revealed that BDNF level is decreased in the brain of NDGR-4 deficient mice. Similarly, this phenomenon is also observed in AD patients (Takahashi et al., 2005; Pan et al., 2010). Additionally, SIRT-1 promotes mitochondria biogenesis and regulates survival-related genes in neuronal cells. Indeed, the over-expression of SIRT-1 attenuates β-amyloid production in an AD mouse model (Das et al., 2009; Song et al., 2012).

3. Taurine Plays a Protective Role by Preventing Al-Insult

Taurine (2-aminoethane sulfonic acid), a nonprotein amino acid exists in a variety of organs in mammals and is present at high concentration (5% in dried weight) in seafood, especially squid and mussel. On the other hand, the concentration of taurine in the mature central nervous system (CNS), immature CNS, and embryonic neocortex was reportedly 25 μM, 75 μM and 1 mM, respectively (Benitez-Diaz et al., 2003; Molchanova et al., 2004; Furukawa et al., 2014). Various experimental studies have shown that taurine is an essential neuromodulator that plays a role in neuronal development via gamma-aminobutyric acid (GABA) and glutamate neurotransmitters (Kilb and Fukuda, 2017). $GABA_A$ receptors showed a rather low taurine affinity above an EC_{50} value of 10 mM (Kibl and Fukuda, 2017). *In vivo* studies have revealed that taurine has inhibitory effects on the mature CNS, including anticonvulsive and antinociceptive effects, and also plays a role in memory formation (El Idrissi and L'Amoreaux, 2008; Hara et al., 2012; Neuwirth et al., 2013). *In vitro* studies showed that 25 mM taurine has neuroprotective effects on arsenic-induced neurotoxicity by reducing ER stress and glutamate excitotoxicity (Pan et al., 2010; Chou et al., 2013; Prentice et al., 2017). Hansen et al. (2010) reported that the taurine level in the mitochondria of a rat heart is approxiunately 25 mM. Several studies in recent years have suggested that taurine protects against oxidative stress, neurotoxicity and hepatic steatosis (Idrissi and Trenkner, 1999; Das et al., 2009; Kumar and Gill, 2009; Chen et al., 2011). Moreover, taurine treatment reduces hepatic lipids in chronically ethanol-treated rats and increases SIRT-1 level in zebra fish and also has a preventive function against atherosclerotic diseases, hyperlipidemia, and hepatic necrosis (Kokame et al., 1996; Corain et al., 1996; Kerai et al., 1999; Balkan et al., 2002).

Based on the above mentioned reports, it can be elucidated that Al induces oxidative stress and causes neurodegenerative diseases, but taurine has protective effect against neurotoxicity. However, the protective mechanism of taurine still remains controversial. Here, aluminum maltolate, $Al(mal)_3$, was used to induce neuronal cell apoptosis and the protective mechanism of taurine was investigated.

II. MATERIALS AND METHODS

1. Chemicals

DEME/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 pure-link™ RNA mini kit was purchased from Invitrogen (Carlsbsd, CA, USA). Taurine, aluminum chloride hexahydrate, maltol, hydrogen peroxide, 2,7-dichlorofluorescin diacetate (DCFDA), rhodamine 123, ribonclease A (RNase A) and propidium iodide (PI) were purchased from Sigma (St. Louis, MO, USA). A HiScrip I™ first-strand cDNA synthesis kit system was purchased from Bionovas Biotechnology (Toronto, Ontario, Canada). Annexin V-FITC was purchased from affymetrix eBioscience (Mountain View, CA, USA). Oligo (dT) 15 primer was purchased from Promega (Madison, WI, USA). The iQ SYBER 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 of cell culture grade and were purchased from common sources.

2. Preparation of Aluminum Maltolate

Aluminum maltolate, or $Al(mal)_3$, was prepared from aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) and maltol according to the method described by Bertholf et al. (1989). The stock solutions of $AlCl_3 \cdot 6H_2O$ (40.9 mM) and maltol (122.8 mM) were dissolved in distilled water and accompanied by mild heating to facilitate dissolution. Briefly, the pH was adjusted to 8.3 and the mixture was set at 65°C. After cooling, the off-white crystals obtained were filtered, washed several times by acetone, and dried overnight in an oven (Perl and Moalem, 2006).

3. Cell Culture and Experimental Setup

The human neuroblastoma SH-SY5Y cell line was obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA); the cll line was incubated with DMEM/F12 (1:1 ratio) medium supplemented 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. The experiment was assigned as four groups, including control group, taurine group, Al group, and taurine + Al group. Control group: The cells were cultured in the medium; Taurine group: The cells were cultured in the medium supplemented with 25 mM taurine; Al group: The cells were cultured in the medium supplemented with 600 μM Al $(mal)_3$; and taurine + Al group: The cells were cultured in the medium supplemented with 25 mM taurine and 600 μM Al $(mal)_3$.

4. Determination of Cell Viability

Cell viability was examined using Annexin V-FITC agent via flow cytometry ((Phillips et al., 1991). A total of 8×10^5 cells were seeded into a 6-well plate. The next day, the medium was removed and fresh medium with or without 600 μM $Al(mal)_3$ and 25 mM taurine was added and maintained for 24 h. After the incubation period, 5 μl Annexin V-FITC solution was added to the wells containing 100 μL medium, and the plate was incubated at 37°C for 30 min. After the incubation period, the cell pellet was washed with PBS and analyzed on a Becton-Dickinson FACScan using the CellQuest software. The quantitative results of fluorescence analysis were presented as mean value of three independent experiments.

5. Determination of Cell Cycle

Cell cycle was examined by PI staining (Rizvi et al., 2014). A total of 8×10^5 cells were seeded into a 6-well plate. On the next day, the medium was removed and fresh medium was added with or without 25 mM taurine and 600 μM $Al(mal)_3$ and further maintained for 24 h. After the incubation period, cells were fixed in 70% alcohol at -20°C overnight. Cells were stained with 500 μLPI solution (50 μg/mL PI, 0.1 mg/mL RNase A, 0.05% Triton X-100) for 40 min to facilitate selection of the viable cell population at 37°C. After the incubation period, the cell pellet was washed with PBS and analyzed on a Becton-Dickinson FACScan flow cytometer, which employed the CellQuest software.

6. Determination of Intracellular ROS

Intracellular oxidative stress was estimated with 2,7-dichlorofluorescin diacetate fluorescence using inverted fluorescence microscopy and flow cytometry. DCFDA was converted to fluorescent DCH by peroxides and thereby lost its cell-permeant ability and was retained inside cells (Phillips et al., 1991). A total of 8×10^5 cells were seeded into a 6-well plate. On the next day, the medium was removed and fresh medium was added with or without 25 mM taurine and 600 μM $Al(mal)_3$, which was further maintained for 24 h. Cells were incubated with 10 μM DCFDA in serum-free DMEM-F12 medium at 37°C for 30 min. After the staining period, medium was removed and cells were washed with ice-cold PBS. Sebsequently, cells were observed through inverted fluorescence microscope (Nikon, Japan) and analyzed on a Becton-Dickinson FACScan flow cytometer, which employed the CellQuest software.

7. Determination of MMP

MMP was determined using fluorescent rhodamine 123 (Rizvi et al., 2014). Briefly, a total of 8×10^5 cells were incubated with or without $Al(malt)_3$ and taurine overnight. After 24 h, the medium was removed and the cells were stained with 10 μM rhodamine 123 at 37°C for 30 min. Then, the cells were harvested with 0.25% trypsin and analyzed on a Becton-Dickinson FACScan flow cytometer, which employed the CellQuest software.

8. Quantitative Real-Time PCR

Gene expression was examined via quantitative real-time PCR (Phillips et al., 1991). Total RNA was extracted using the pure-link™ RNA mini kit, and 1 μg RNA was used for cDNA synthesis using the Hiscrip I™ first-strand cDNA synthesis kit system for quantitative real-time PCR (qPCR). The qPCR of NDRG-4, BDNF, SIRT-1 and β-2-microglobulin (B_2M) genes was carried out using the iQ SYBR Green Supermix system (Bio-Rad, USA). The gene expression results of NDRG-4, BDNF and SIRT-1 were normalized by B_2M levels. The sequences of primers are shown in Table 1.

Table 1. The sequences of the primers used in this study.

Primers	Sequences
NDRG-4-forward	5′-GGAGGTTGTCTCTTTGGTCAAGGT-3′
NDRG-4-reverse	5′-CTCATGACAGCAGCCACCAGAAT-3′
BDNF-forward	5′-AAACATCCGAGGACAAGGTG-3′
BDNF-reverse	5′-AGAAGAGGAGGCTCCAAAGG-3′
SIRT-1-forward	5′-TGCTGGCCTAATAGAGTGGCA-3′
SIRT-1-reverse	5′-CTCAGCGCCATGGAAAATGT-3′
B2M-forward	5′-GAGGTTTGAAGATGCCGCATT-3′
B2M-reverse	5′-TGTGGAGCAACCTGCTCAGATA-3′

9. Western Blotting

After the experimental period, cells were lysed in 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 protein extraction. The protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Pan et al., 2010). Subsequently, the membrane was blocked by its placement in Tris buffer saline (pH 7.5) containing 0.1% tween-20 (TBST) and 5% defatted milk powder for 2 h at room temperature. Then, the membrane was washed twice with TBST and incubated with a corresponding primary antibody (1:500) overnight and an HRP-conjugated secondary antibody (1:5,000) at room temperature for 2 h. Finally, the membrane was washed with TBST twice and visualized using an electrochemiluminescence kit in an imaging system. The band area is quantified by Quantity One software and considered as the amount of expressed Grp78. The quantitative results were presented as mean values of three independent experiments.

10. Statistical Analysis

The results were represented as mean ± SEM values of four independent experiments and statistically analyzed by ANOVA followed by the Tukey test. A P value of < 0.05 value was considered statistically significant.

III. RESULTS

1. Effect of Taurine on Cell Cytotoxicity Induced by $Al(mal)_3$ Treatment of SH-SY5Y Cells

Fig. 1 shows the effect of 600 μM $Al(mal)_3$ on cell viability

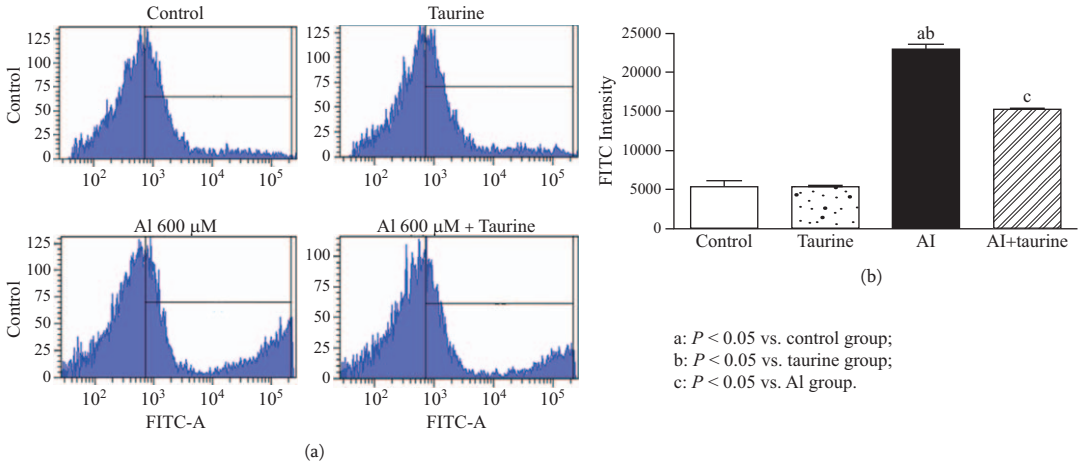


Fig. 1. Al-induced apoptosis and the protective effect of taurine on human neuroblastoma SH-SY5Y cells. Cells were treated with or without Al 600 μM Al and 25 mM taurine for 24 h. (a) Apoptosis is examined by flow cytometry. (b) The quantitative data are represented as the mean ± SEM values of four independent experiments.

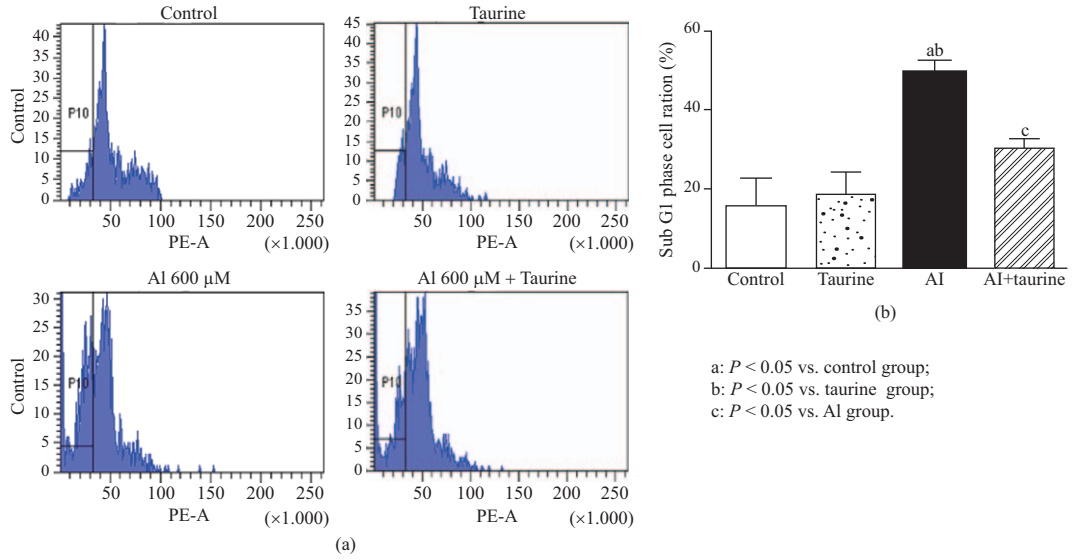


Fig. 2. Al-induced cell cycle arrest and the protective effect of taurine on human neuroblastoma SH-SY5Y cells. Cells were treated with or without 600 μM Al and 25 mM taurine for 24 h. (a) Cell cycle is examined by flow cytometry. (b) The quantitative data are represented as the mean ± SEMvalues of four independent experiments.

when cells were treated with or without 25 mM taurine. Both $Al(mal)_3$ and taurine concentrations were in accordance with the data reported by Chen et al. (2011) and Chou et al. (2013), respectively. The signal of cell apoptosis is presented as a fluorescent unit (FU) and is significantly increased in the Al group (23,119 FU) compared with the control group (5,056 FU), but the signal in the Al + taurine group was restored to 15,219 FU as opposed to that in the Al group (Fig. 1(b)). Additionally, taurine in the taurine group had no effect on the viability of SH-SY5Y cells in compared with the viability of cells in the control group.

2. Effect of Taurine on Cell Cycle Arrest Induced by Al(mal)3 Treatment Iof SH-SY5Y Cells

According to the cell viability experiment, it was investigated whether taurine prevented cell cycle arrest in SH-SY5Y cells on being subjected to treatment with $Al(malt)_3$ (Fig. 2(a)). The quantitative data show that the number of cells in the Al group significantly increased to 49.3% in the sub G1 phase, but that in the taurine + Al group decreased to 37.6 % (Fig. 2(b)). There is no difference in cell distribution between the taurine group and control group. These data demonstrated that $Al(mal)_3$ increased the number of sub G1 phase cells to induce cell death, but taurine prevented cell cycle arrest when combined with $Al(mal)_3$ treatment of SH-SY5Y cells. The respective cell ratio G1 phase cell ratios in the control and taurine groups were similar, but those in the Al and taurine + Al group were significantly different. This data indicated that taurine prevented cell cycle arrest in SH-SY5Y cells when used in combination with $Al(mal)_3$ treatment. How-

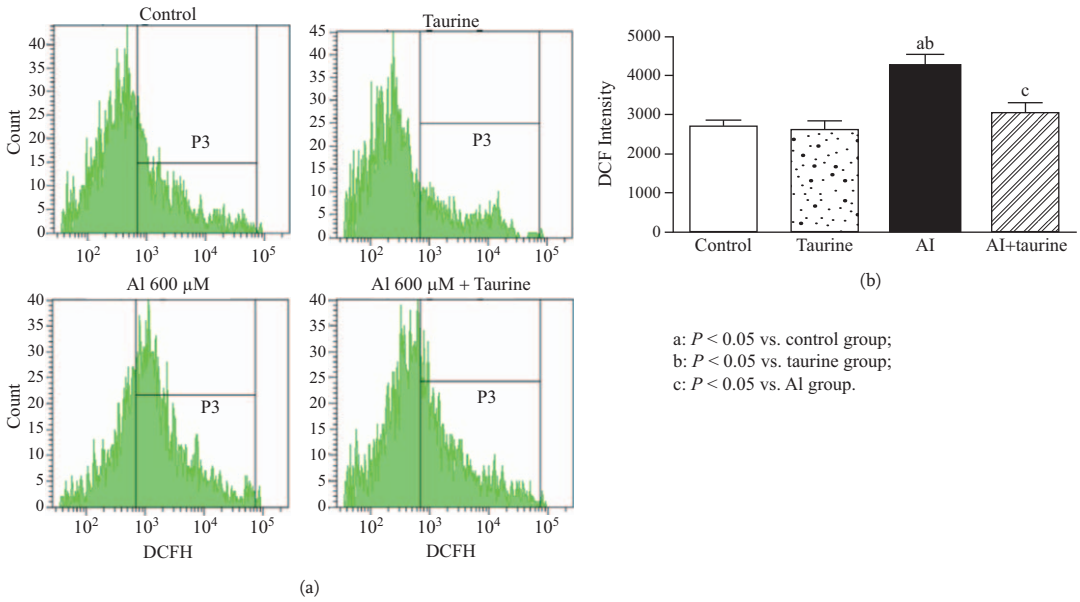


Fig. 3. Al-induced intracellular ROS levels and the protective effect of taurine on human neuroblastoma SH-SY5Y cells. Cells were treated with or without 600 μM Al and 25 mM taurine for 24 h. (a) Intracellular oxidative stress is examined by flow cytometry. (b) The quantitative data are represented as the mean ± SEM values of four independent experiments.

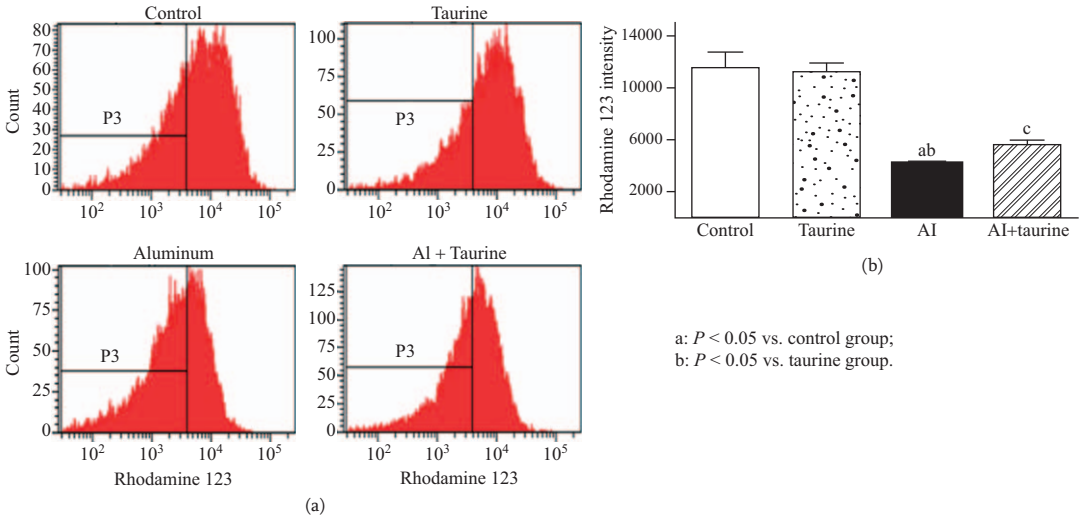


Fig. 4. Decrease in mitochondrial membrane potential caused by Al and the protective effect of taurine on human neuroblastoma SH-SY5Y cells. Cells were treated with or without 600 μM Al and 25 mM taurine for 24 h. (a) Mitochondrial membrane potential is examined by flow cytometry. (b) The quantitative data are represented as the mean ± SEM values of four independent experiments.

ever, the cell ratios in S phase and G2/M phases were similar for all the four groups.

3. Effect of Taurine on Intracellular ROS Produced after Al(mal)$_3$ Treatment of SH-SY5Y Cells

Using flow cytometry, the signals of intracellular ROS level were found to be 2,658 FU; 2507 FU; 4,128 FU; and 3,173 FU for the control group, taurine group, Al group, and taurine + Al group, respectively (Fig. 3). The intracellular ROS level in thee Al group was significantly increased by 55% compared with that in the control group at 24 h (Fig. 3(b)). However, the level in the taurine + Al group was significantly decreased by 28% compared with that in the Al group (Fig. 3(b)). The data revealed a significant inhibition of the intracellular ROS level brought about by taurine, indicating that taurine protected SH-SY5Y cell from damage caused by Al(mal)$_3$ induced oxidative stress.

4. Effect of Taurine on Mitochondria Apoptosis Induced by Al(mal)$_3$ Treatment of SH-SY5Y Cells

MMP examination revealed that the signal of MMP in SH-SY5Y cells was 10,718 FU; 10,678 FU; 4,298 FU; and 5,485 FU for the control group, taurine group, Al group, and taurine + Al

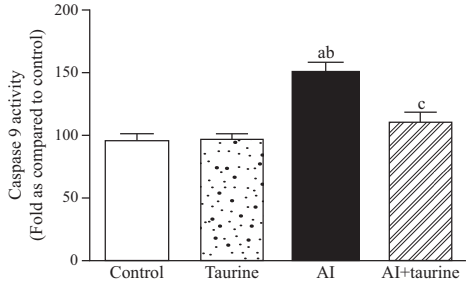
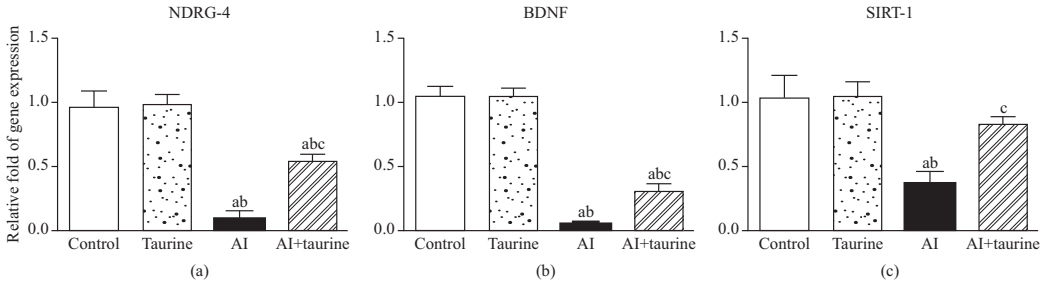


Fig. 5. Al-induced caspase 9 expression and the protective effect of taurine on human neuroblastoma SH-SY5Y cells. Cells were treated with or without 600 μM Al and 25 mM taurine for 24 h. The quantitative data are represented as the mean ± SEM values of four independent experiments. [a]*P* < 0.05 vs. control group; [b]*P* < 0.05 vs. taurine group; [c]*P* < 0.05 vs. Al group.



a: *P* < 0.05 vs. control group; b: *P* < 0.05 vs. taurine group; c: *P* < 0.05 vs. Al group.

Fig. 6. Al-reduced neurotrophic factor gene expression and the protective effect of taurine on human neuroblastoma SH-SY5Y cells. (a) NDRG-4 (b) BDNF, and (c) SIRT-1 genes were analyzed by quantitative real-time PCR. Cells were treated with or without 600 μM Al and 5 mM taurinefor 24 h. The quantitative data are represented as the mean ± SEM values of four independent experiments.

group, respectively (Fig. 4). The results revealed that MMP in SH-SY5Y cells significantly decreased by 60% in the Al group and by 48 % in the taurine + Al group compared with that in the control group.

The protease activity of caspase 9 was examined to detect mitochondria apoptosis (Fig. 5). The data showed that $Al(malt)_3$ induced caspase 9 activity in SH-SY5Y cells and increased it by 36% compared with the activity in the control group (Fig. 5). In contrast, a 26% reduction in caspase 9 expression was noted in the taurine + Al group (Fig. 5). These data suggest that taurine can prevent mitochondrial apoptosis in SH-SY5Y cells when combined with $Al(mal)_3$ treatment.

5. Effect of Taurine on Neurotrophic Gene Expression Induced by $Al(malt)_3$ Treatment of SH-SY5Y Cells

To investigate whether taurine regulates neurotrophic gene expression, the NDRG-4, BDNF and SIRT-1 gene expression were examined. The results showed that NDRG-4, BDNF and SIRT-1 gene expression levels were significantly reduced in SH-SY5Y cells after $Al(malt)_3$ treatment for 24 h (Fig. 6). However, taurine + Al group exhibited significant restoration of NDRG-4, BDNF and SIRT-1 gene expression levels compared with those in the Al group (Fig. 6). In addition, the expression levels of all genes were not different between the taurine group and control group.

6. Effect of Taurine on ER Stress Induced by $Al(malt)_3$ Treatment of SH-SY5Y Cells

Perturbation of ER homeostasis induces cell apoptosis through the activation of Grp78. The data demonstrated that $Al(malt)_3$ induced Grp78 expression in SH-SY5Y cells, but taurine reversed Grp78 expression in SH-SY5Y cells treated with $Al(malt)_3$ (Fig 7(a)). The data demonstrated that the Al group and taurine + Al group showed a significant 3.5-folds and 2.6-flods increase in Grp78 protein levels, respectively, compared with the Grp78 protein level in the control group (Fig. 7(b)). Moreover, no significant differences in the Grp78 protein level were noted between the taurine and control groups.

IV. DISCUSSION

Various metals, pesticides and phenols have been considered as environmental pollutants (Wasi et al., 2013). In this context, Al has been associated with global health risk in some diseases (Hammes et al., 2012). In this context, treatment of human neuroblastoma SH-SY5Y cells with $Al(malt)_3$ at a concentration of 600 μM for 24 h induced neuronal cell cycle arrest. Previous data revealed that $Al(malt)_3$ induced oxidative stress to cause mitochondrial and ER damage in SH-SY5Y cells. The intracellular ROS levels and MMP assays revealed that treatment of SH-SY5Y cells with $Al(mal)_3$ brought about an increase in cellular ROS levels, activation of the caspase 9 pathway, and decrease in mitochondrial membrane potential, thereby causing mitochondrial apoptosis. Moreover, $Al(mal)_3$ causes ER impairment via Grp78 pathway. In facts, Al plays a major role in the patho-

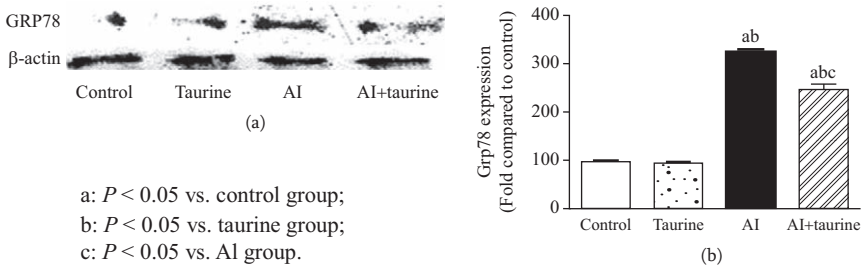


Fig. 7. The Al-induced Grp78 expression and the protect effect of taurine in SH-Y5Y cell. Cells were incubated with or without indicated concentration of Al 600 μM and taurine 25 mM for 24 hr. (a) Grp78 expression is examined by Western blotting. (b) The quantitative data are represented as the mean ± SEM from four independent experiments.

gentic mechanisms of neurological disease and other related disorders owing to its ability to cross-link hyperphosphorylated proteins (Marwarha et al., 2013).

Various studies have revealed that Al was detected in amyloid fibers in senile plaque core in the brain of AD patients and it was associated with cognitive deficiency. The generation of ROS has been implicated in the oxidative damage of mitochondrial and ER proteins as well as in the loss of the functions of both mitochondria and ER (Yamamot et al., 2011). A previously published report revealed that $Al(mal)_3$ can reduce the expression of BDNF-induced activity-regulated cytoskeleton-associated protein (Arc) by interrupting ERK signaling in SH-SY5Y cells to cause cognitive dysfunction (Bertholf et al., 1989). Many studies have suggested that the loss of NTFS including BDNF, SIRT-1 and NDRG-4 impairs the hippocampus and cortex to decrease learning capacity and memory (Bertholf et al., 1989; Broadbent et al., 2004; Wang et al., 2009). Indeed, NDRG-4 is required for the cell cycle progression and differentiation of neuronal cells (Prentice et al., 2017). With respect to energy metabolism, SIRT-1 participates for neuron survival and regulates energy metabolism by promoting mitochondrial biogenesis (Song et al., 2012). Thus, arsenite treatment reduced the expression of NDRG-4, BDNF and SIRT-1 in SH-SY5Y cells (Chen et al., 2011). Similarly, in our study, the treatment of SH-SY5Y cells with $Al(mal)_3$ decreased BDNF, SIRT-1 and NDRG-4 levels, indicating that Al is associated with neuronal cell cycle arrest and apoptosis. Previous studies have reported that ER is also the site for amyloid β-protein generation via calcium influx for worsening neurodegenerative diseases (Kerai et al., 1999). On the other hand, $Al(mal)_3$ reportedly induced oxidative stress which perturbed the ER, thereby causing the release of calcium and ER stress-related proteins such as CHOP/GADD153 and caspase 12 to cause neuronal cell apoptosis (Perl and Moalem, 2006). In this study, the expression of Grp78, an ER stress-related protein, increased after the treatment of SH-SY5Y cells with $Al(mal)_3$. The results supported the hypothesis that $Al(mal)_3$ promotes the generation of ROS to induce mitochondrial and ER stress, thereby activating the apoptotic pathway. The results of Al toxicity in this study are similar to those reported in the above mentioned studies.

Taurine, a nonprotein amino acid, is a natural antioxidant and an antiatherosclerotic agent (Balkan et al., 2002). Taurine protects granular cells of the cerebellar cortex against glutamate-induced cellular toxicity by modulating intracellular calcium homeostasis and energy metabolism (Han et al., 2013). Moreover, taurine prevents arsenite-induced cell cycle arrest in neuroblastoma cells by increasing the expression of BDNF, NDGR-4 and SIRT-1 (Chen et al., 2011). This report aroused our interest in examining the protective effects of taurine in SH-SY5Y cells that were subjected to $Al(mal)_3$ treatment.

V. CONCLUSION

This study proved that taurine prevented cell cycle arrest and reduced intracellular ROS levels to decrease mitochondria and ER stress. Taurine also restored the expression levels of NDGR-4, SIRT-1 and BDNF genes, which had been substantially decreased owing to $Al(mal)_3$ treatment. Hence, based on the results of the present study, it can be concluded that $Al(malt)_3$ enhanced mitochondrial and ER stress and inhibited neurotrophic gene expression, and that taurine plays a regulatory role in $Al(mal)_3$-treated SH-SY5Y cells via NTFS

DECLARATIONS

Author Contribution Statements

Bi-Yu Liu: Performed the experiments; analyzed and interpreted the data; and wrote the paper.

Yuh-Ju Lee, Hsian-Chin Jen: Contributed reagents, materials, analysis tools, or data.

Deng-Fwu Hwang: Conceived and designed the experiments, and performed the experiments.

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