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REGULAR ARTICLE

Gene expression in primary cultured astrocytes affected by aluminum: alteration of chaperons involved in protein folding

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Abstract
Objectives Aluminum is notorious as a neurotoxic metal. The aim of our study was to determine whether endoplasmic reticulum (ER) stress is involved in aluminum-induced apoptosis in astrocytes.
Methods Mitochondrial RNA (mRNA) was analyzed by reverse transcription (RT)-PCR following pulse exposure of aluminum glycinate to primary cultured astrocytes. Tunicamycin was used as a positive control.
Results Gene expression analysis revealed that Ire1β was up-regulated in astrocytes exposed to aluminum while Ire1α was up-regulated by tunicamycin. Exposure to aluminum glycinate, in contrast to tunicamycin, seemed to down-regulate mRNA expression of many genes, including the ER resident molecular chaperone BiP/Grp78 and Ca^{2+}-binding chaperones (calnexin and calreticulin), as well as stanniocalcin 2 and OASIS. The down-regulation or non-activation of the molecular chaperons, whose expressions are known to be protective by increasing protein folding, may spell doom for the adaptive response. Exposure to aluminum did not have any significant effects on the expression of Bax and Bcl2 in astrocytes.
Conclusions The results of this study demonstrate that aluminum may induce apoptosis in astrocytes via ER stress by impairing the protein-folding machinery.

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Keywords Aluminum · Astrocyte · Unfolded protein response · ER stress · ER resident molecular chaperons

Abbreviations

ATF6	Activating transcription factor 6
Bbc3	Bcl2 binding component 3
Bcl2	B cell lymphoma 2
BiP	Immunoglobulin-binding protein
CHOP	C/EBP homology protein
GADD153	Growth arrest and DNA damage inducible gene 153
Grp78	Glucose-regulated protein 78
OASIS	Old astrocyte specifically induced substance
PERK	PKR-like endoplasmic reticulum kinase
PS2V	Aberrant splicing isoform of the presenilin-2
PUMA	p53-Up-regulated modulator of apoptosis
RT-PCR	Reverse transcription-polymerase chain reaction
UPR	Unfolded protein response

Introduction

The physiological roles of endoplasmic reticulum (ER) include the regulation of protein synthesis, folding and targeting, and maintenance of cellular calcium homeostasis. The ER is often perturbed when cells are deprived of essential nutrients and/or exposed to toxins or consequent upon mutations in the synthesized proteins themselves [1], resulting in the accumulation of misfolded or unfolded protein in the ER, the so-called ER stress [2]. Unfolded or misfolded proteins are harmful to cells in that cell survival itself can be threatened as a consequence [3]. In order to

overcome ER stress, this organelle responds via activation of a specific signaling pathway generally referred to as unfolded protein response (UPR). The UPR are molecular chaperon-dependent adaptive signaling pathways that are programmed to ensure quality control within the ER by enhancing the protein folding capability and misfolded protein degradation and by limiting the load of new proteins on the ER folding apparatus. The ER relies on numerous resident chaperone proteins, a high level of calcium, and an oxidative environment to carry out these functions efficiently [4].

The aggregate effect of UPR is containment and reversal of ER stress. The UPR thereby constitutes a classic homeostatic feedback loop that adjusts the ER protein folding capacity according to need [5]. However, when misfolded proteins accumulate in excessive amounts, they may overwhelm the 'quality control' system designed to promote correct folding and eliminate faulty proteins. In the event that ER stress is not contained during a finite time window, the mammalian UPR directs the cell to an apoptotic pathway [6–10]. Apoptosis is a major form of cell death, characterized by a series of district morphological and biochemical alterations [11, 12] brought about by various physiological and pathological conditions as well as chemical agents [11, 13].

Aluminum is notorious as a neurotoxic chemical and has been linked to a number of neurodegenerative diseases, although such findings have been received with some controversy [14–18]. Nevertheless, several recent findings have implicated astrocytes as the principal target of the toxic action of aluminum [19–22] and that aluminum can cause astrocyte death via apoptosis [23–25]. However, these reports only showed morphological evidence of apoptosis, and the molecular mechanisms by which aluminum causes astrocyte death are yet to be unraveled. Detailed knowledge of this molecular mechanism would likely provide insight into how such cell death could be prevented. This is very important given that recent studies have demonstrated that astrocytes play active roles in neuronal regulation and modulation [26–28]. It has also been suggested that the loss of astrocyte functions may precede neurodegeneration and that aluminum could be a contributing factor [29, 30]. For example, astrocytes have been shown to undergo an active process of apoptosis in Alzheimer's disease (AD) [31] and that this process may precede the death of neurons [32]. Moreover, chronic exposure to aluminum has been reported to accelerate the production of the aberrant splicing isoform of presenilin-2 (PS2V), a diagnostic feature of sporadic AD [33]. PS2V protein has also been found to affect the UPR and cause an increase in the production of both amyloid beta (Aβ)-40 and Aβ-42 [34]. Furthermore, mutations in the presenilin gene have been reported to cause the inhibition of the ER stress transducers Ire1, activating transcription factor (ATF)6, and PKR-like endoplasmic reticulum kinase (PERK), resulting in the decreased expression of ER chaperons [35].

The aim of the study reported here was to determine how aluminum exposure affects the expression of a number of important ER stress-related genes, given the likely link between aluminum and ER stress and the observed apoptosis in astrocytes reported in earlier studies [23, 25, 30]. The B cell lymphoma 2 (Bcl2) family of proteins was also studied to clarify the contributions of ER and mitochondria in the pathways involved in aluminum-induced apoptosis in cultured astrocytes.

Materials and methods

Primary culture

Primary cultures of cortical astrocytes were prepared from 5- to 7-day-old mice as previously described [24]. Briefly, the cells were grown in D-MEM/F12 or D-MEM during the experimental period (cat. no. 11330 or 11885; GIBCO BRL, Life Technologies, Grand Island, NY) containing 10% fetal bovine serum (GIBCO BRL) and 0.05 mg/ml gentamicin (Sigma, St. Louis, MO), incubated at 37°C in humidified atmosphere of 5% CO_2, and used for experiments at near confluence after second passages by trypsinization. In all experiments, the cells were stressed with 0.1 mM aluminum glycinate (Tokyo Kasei Kogyo, Japan) for 48 h or with a 6- to 12-h pulse exposure followed by continued culture in normal medium for about 7 days. In some experiments, concentrations lower or higher than 0.1 mM were employed to assess concentration-dependent effects. Previous morphological studies on apoptosis did not find any adverse effects on cell viability/proliferation among cells exposed to test compounds at concentrations similar to those employed in our study [24].

Western blot analysis

Cells were lysed on ice for 10 min with lysis buffer containing 10 mM HEPES-KOH (pH 7.8), 10 mM KCl, 1 mM EDTA (pH 8.0), 0.05% NP-40, 1 mM DTT plus protease inhibitor cocktail (Roche, Mannheim, Germany) followed by brief homogenization with sonication. Aliquots of cell lysates (20 μg/ml) were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 10–12% polyacrylamide gels and transferred onto a 0.2-μm Immun-blot PVDF membrane for protein blotting (Bio-Rad, Hercules, CA). The membranes were incubated overnight in 5 or 10% (w/v) skim milk in 0.1% TBS-T, then stained with the respective monoclonal primary antibodies followed by horseradish peroxidase-conjugated second antibody. The

Table 1 Primers of target genes

Target gene	Forward primer	Reverse primer	Product size (bp)
Ire1α	CCCAAATGTGATCCGCTACT	TTGAGAGAATGCAGGTGTGC	183
Ire1β	ACTGTGGATCCAGGAAGTGG	TGGCTGTGTCTTGGTAGCTG	193
ATF6α	GGCCAGACTGTTTTGCTCTC	CCCATACTTCTGGTGGCACT	215
ATF6β	GGGTCCATCCCCTGATAGTT	CACCTTGGATGAGGACCACT	185
PERK/Eif2ak3	CGGATTCATTGAAAGCACCT	ACGCGATGGGAGTACAAAAC	194
Bbc3/PUMA	GCCCAGCAGCACTTAGAGTC	TGTCGATGCTGCTCTTCTTG	191
Bip/Grp78	TGGAGTTCCCCAGATTGAAG	GCGCTCTTTGAGCTTTTTGT	199
CHOP/GADD153	CCTAGCTTGGCTGACAGAGG	CTGCTCCTTCTCCTTCATGC	196
Stanniocalcin 2	TCCAGCAATTAGGGAAATGG	CCCAGCTCTGTTCACACTGA	230
Calnexin	GGCTAGACGACGAACCTGAG	AGGCTTCCATTTGCCCTTAT	188
Calreticulin	AGGCTCCTTGGAGGATGATT	TCCCACTCTCCATCCATCTC	207
OASIS	ACCTGGACCACTTTGTGGAG	TGGTGTCCTCCATCTTGACA	210

See text and abbreviation list for the full names of the target genes

luminescence reaction was elicited using an ECL kit (Amersham, Piscataway, NJ) and the protein bands were visualized using a computer-aided FUJIFILM Luminescent Image Analyzer (LAS-1000plus; Fuji Photo Film, Tokyo). The same membrane was stripped for all subsequent analyses. The antibodies used were: mouse monoclonal anti-B cell lymphoma (Bcl-2; C-2), anti-Bcl-2-associated X (Bax; B-9) (Santa Cruz Biotechnology, Santa Cruz, CA), and anti-α-tubulin (Ab-1) (Oncogene, San Diego, CA).

Reverse transcription-PCR analysis

Validation of the effect of aluminum on the expression of selected genes was performed by reverse transcription (RT)-PCR analysis (BioRad) using apoptosis PCR bax/bcl2 multiplex primer sets (APO-PCR; Sigma–Aldrich, Saint Louis, MO). Unique oligonucleotide primer pairs of genes that have been implicated in ER stress-induced apoptosis were designed (Table 1) using PRIMER3 software (http://www.genome.wi.mit.edu/cgi-bin/primer3_www.cgi) [36]. Total RNA was purified from aluminum-treated and untreated control cells using the Mini RNeasy kit (QIAGEN, Valencia, CA). The ER stress inducer tunicamycin, a protein *N*-glycosylation inhibitor, was employed as a positive control. The purified RNA was subjected to RT-PCR in a two-step protocol using M-MLV reverse transcriptase (Invitrogen, Grand Island, NY) and Taq polymerase (Applied Biosystems, Foster City, CA). The number of cycles and annealing temperature was according to the manufacturer's protocol for APO-PCR. The bands in the photographs were quantified by Image J 1.43u software. The animal experiments were approved by the committee on animal research of Tottori University, and the investigation conforms to the guiding principles for the care and use of laboratory animals in toxicology.

Results

The anti-apoptotic Bcl2 and pro-apoptotic Bax are well-studied members of the Bcl2 family of proteins that respectively inhibit or enhance cytochrome *c* release under mitochondria-controlled apoptosis. Hence, the expressions of these two proteins were studied by Western blotting analysis following exposure of primary cultured astrocytes to aluminum as described in the Materials and methods. Aluminum glycinate did not affect the expression of these two proteins under different exposure regimens and various doses, including pulse exposure followed by continued culture in normal medium for 7 days (Fig. 1a). Tunicamycin was employed as the positive control in subsequent experiments. Total RNA was extracted from the cells, and the mRNAs of the Bcl2 and Bax genes were observed using RT-PCR. Exposure to either tunicamycin or aluminum glycinate did not evoke any observable change in the expression of these genes (Fig. 1b). Cells exposed to tunicamycin, however, did show an up-regulation of the expression of the pro-apoptotic gene, Bcl2 binding component 3/p53-up-regulated modulator of apoptosis (Bbc3/PUMA), another member of the Bcl-2 family [37], while aluminum had no apparent effect effect on its expression under different exposure regimens (Fig. 2).

Ire1α is the most studied of the two isoforms of the Ire1 gene; consequently, we first analyzed primary cultured astrocytes exposed to aluminum glycinate or tunicamycin for 6, 12, or 24 h for changes in the expression of the Ire1 gene (Fig. 3a). The results showed that aluminum seemed to have only a minor effect on the expression of Ire1α, similar to that seen in the control experiment, and that this effect was time dependent. In contrast, tunicamycin up-regulated the expression of Ire1α relative to the control at the 12- and 24-h timepoints (Fig. 3b). We then investigated Ire1β gene

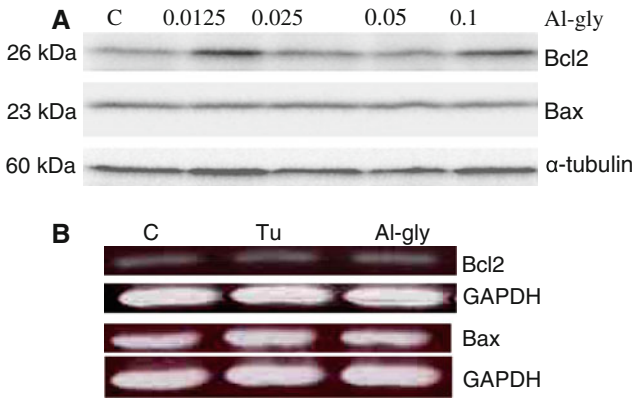

Fig. 1 The expression of the B cell lymphoma 2 (*Bcl2*) family of proteins in primary cultured astrocytes in the presence of aluminum. **a** Western blot analysis of Bcl-2-associated X (*Bax*) and Bcl2 protein expression in primary astrocytes cultured in the absence (*C* control) or presence of graded doses of aluminum glycinate (*Al-gly*; 0.0125–0.1 mM) for 24 h. The membrane was stained with Bcl2 monoclonal primary antibodies followed by horseradish peroxidase-conjugated second antibody. The same membrane was subsequently stripped for Bax and α-tubulin analyses. The results are representative of about six blots obtained from three different exposure regimens. **b** Bcl2 and Bax mRNA expressions in primary astrocytes cultured for 24 h in the absence (*C* control) or presence of tunicamycin (*Tu*, 5 μg/ml) and Al-gly (0.1 mM). Total RNA was analyzed by reverse transcription (RT)-PCR for the expression of Bcl2 and Bax. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was analyzed as internal standard. The results are representative of three independent analyses from two replicate cultures

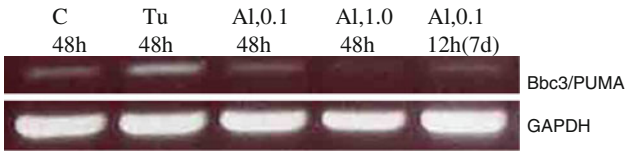

Fig. 2 Semiquantitative RT-PCR analysis of the expression of Bcl2 binding component 3/p53-up-regulated modulator of apoptosis (*Bbc3/PUMA*) in the presence of aluminum. Total RNA was analyzed by RT-PCR for the mRNA expressions of Bbc3/PUMA in primary cultured astrocytes. Cells were treated with Tu (5 μg/ml) for 48 h or Al-gly (*Al*; 0.1 or 1.0 mM) for 48 or for 12 h followed by a 7-day culture in normal medium. GAPDH mRNA was analyzed as the internal control. The results are representative of three independent analyses from two replicate cultures

expression using both Ire1β and Ire1α primers. The cells were exposed to aluminum or tunicamycin for 48 h or aluminum for 12 h followed by culture in normal medium for 7 days. Under this exposure regimen, in contrast to Ire1α, the expression of Ire1β was not detectable in either the control or tunicamycin-treated cells, and only aluminum glycinate up-regulated Ire1β in cells that had been exposed to pulses of aluminum followed by culture in normal medium for 7 days (Fig. 4a, b). The up-regulation of Ire1α by tunicamycin was more than twice that observed in the control cultures at the 48-h timepoint (Fig. 4b) and was similar to that observed 12

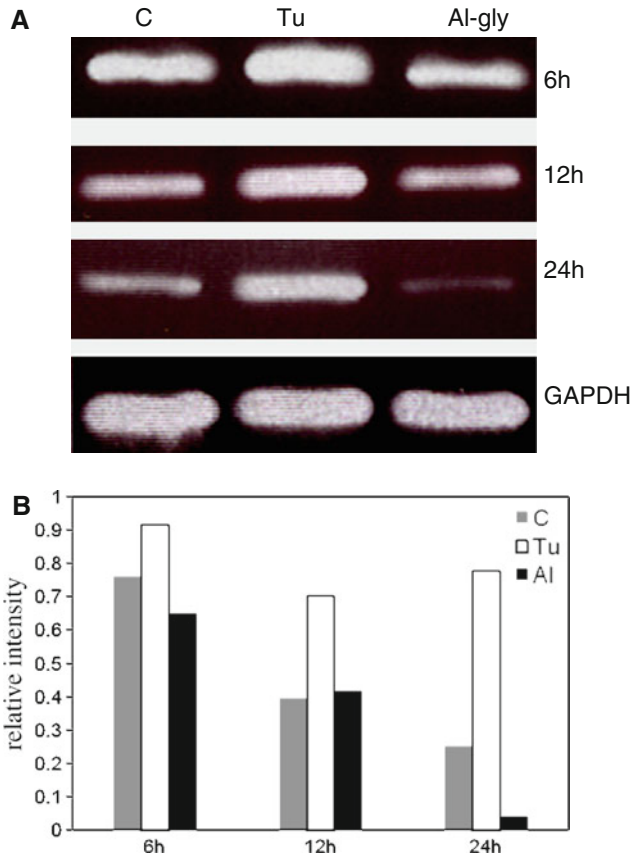

Fig. 3 **a** Semiquantitative RT-PCR analysis of Ire1α genes in primary cultured astrocytes following time-course exposure to aluminum. Total RNA was analyzed by RT-PCR for the kinetics of Ire1α mRNA expression in primary cultured astrocytes for the indicated times in the absence (*C* control) or presence of Tu (5 μg/ml) or Al-gly (0.1 mM). GAPDH mRNA was also analyzed as an internal standard. The results are representative of three independent analyses from two replicate cultures. **b** Relative intensities of the bands

and 24 h of exposure (Fig. 3b). Other principal components of the UPR pathway, including PERK, ATF6α and ATF6β, were were studied (Fig. 5). PERK was not detectable in either the control or tunicamycin- and aluminum-treated cells. ATF6α was up-regulated in tunicamycin-treated cells only, while neither ATF6α nor ATF6β was up-regulated in aluminum-treated cells.

Expression of the Ca^{2+} homeostasis related genes, including stanniocalcin 2 (STC2), calreticulin, and calnexin, were examined in cells exposed to aluminum or tunicamycin for 24 h. STC2 was not detectable in the control cells and aluminum-treated cells, but this gene was up-regulated in tunicamycin-treated cells. Tunicamycin also slightly up-regulated calreticulin and calnexin, while aluminum appeared to to have no effect on the expression of these genes (Fig. 6).

Primary cultured astrocytes were exposed to aluminum glycinate for various lengths of time, and tunicamycin was employed as the positive control. While tunicamycin

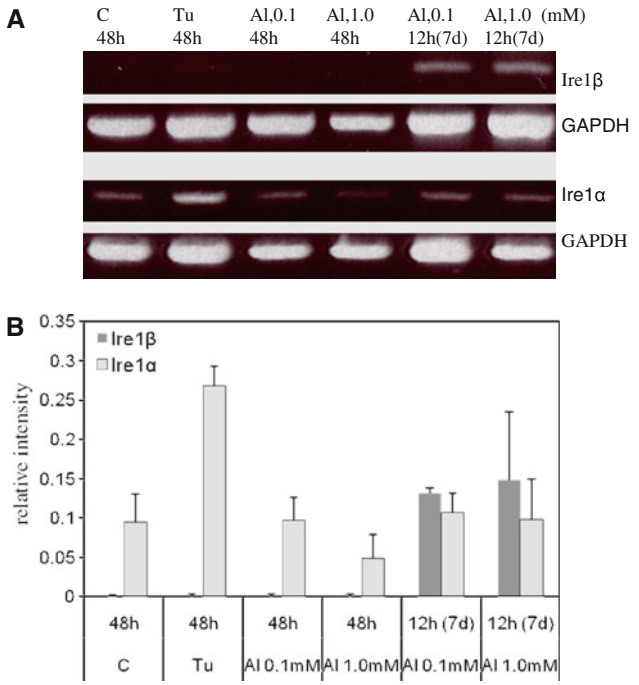

Fig. 4 **a** Semiquantitative RT-PCR analysis of Ire1β And Ire1α genes in primary cultured astrocytes following exposure to aluminum and Tu for the indicated time. Pulse exposure to aluminum followed by continued culture for 7 days in normal medium resulted in up-regulation of the expression of Ire1β in primary cultured astrocytes. Total RNA was analyzed by RT-PCR for Ire1α and Ire1β mRNA expression in primary cultured astrocytes. Cells were treated with Tu (5 μg/ml) for 48 h or Al-gly (*Al*; 0.1 or 1.0 mM) for 48 or for 12 h followed by 7 days of culture in normal medium. GAPDH mRNA was also analyzed as the internal standard. The results are representative of three independent analyses from two replicate cultures. **b** Relative intensities of the bands (mean ± standard deviation, $n = 3$)

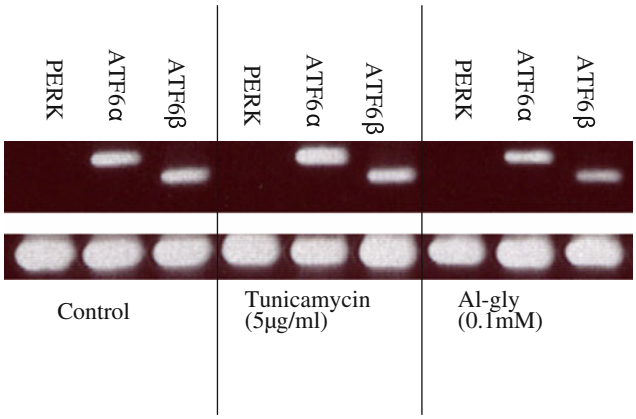

Fig. 5 Semiquantitative RT-PCR analysis of selected genes in primary cultured astrocyte following 12 h of exposure to aluminum and some known apoptotic agents. Aluminum did not affect the expression of activating transcription factors (*ATF*) 6α and β. Total RNA was analyzed by RT-PCR for the mRNA expressions of PKR-like endoplasmic reticulum kinase (*PERK*), ATF6α and β and GAPDH in primary cultured astrocytes. Cells were treated with tunicamycin (5 μg/ml) or Al-gly (0.1 mM) for 12 h. The results are representative of three independent analyses from two replicate cultures

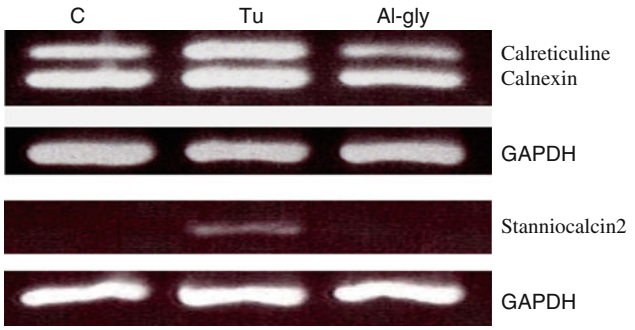

Fig. 6 Semiquantitative RT-PCR analysis of selected genes in primary cultured astrocytes following 24 h of exposure to aluminum (*Al-gly*; 0.1 mM) and Tu (5 μg/ml). Tu, but not Al-gly, up-regulated the expression of calreticuline, calnexin, and stanniocalcin 2. Total RNA was analyzed by multiplex RT-PCR for the mRNA expressions of calreticuline, calnexin, stanniocalcin 2, and GAPDH. The results are representative of three independent analyses from two replicate cultures

up-regulated the expression of immunoglobulin-binding protein/glucose-regulated protein 78 (BiP/Grp78) gene, aluminum modestly down-regulated the expression of this gene (Fig. 7). The expressions of transcription factors, CEBP homologous protein (CHOP/GADD153)-coding gene and old astrocyte specifically induced substance (OASIS), an ER stress transducer in astrocytes, were also examined following exposure to aluminum or tunicamycin. Tunicamycin up-regulated the expression of these genes but aluminum did not affect their expression after 24 h exposure (Fig. 8).

Discussion

Executioners of apoptosis are known to function in different cellular compartments and act on distinct substrates, but there is only a limited body of knowledge on the contribution of organelles, such as the ER, to the apoptotic process [38]. There is, however, increasing evidence that the ER can play pivotal roles in regulating cell survival and apoptosis in a variety of cell types, including neurons and astrocytes. The results of our study demonstrate that ER stress may be involved in aluminum-induced apoptosis in astrocytes, although we found no evidence of the participation of the mitochondrial-associated pathway in this process. There was no up-regulation of either the protein or mRNA expression of the anti-apoptotic Bcl2 and pro-apoptotic Bax. Bcl2 and Bax are known to inhibit and enhance, respectively, cytochrome *c* release in the mitochondria apoptotic pathway [39, 40]. The release of cytochrome *c* from the intermembrane space into the cytoplasm usually results in the activation of caspase 3, which in turn

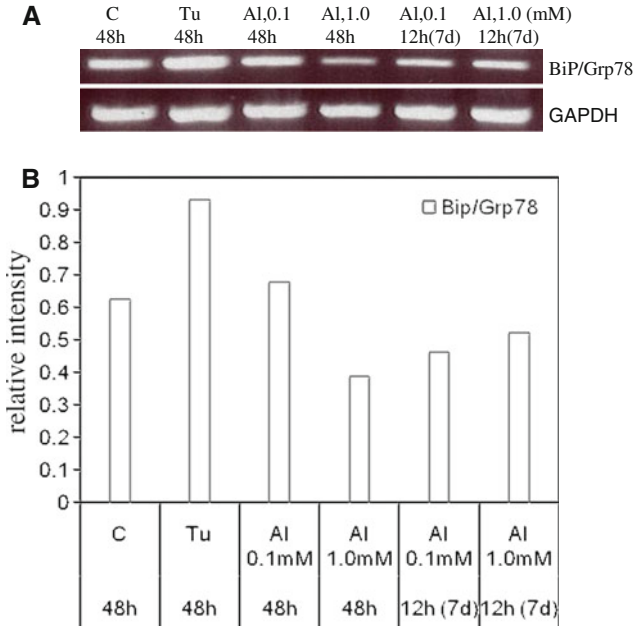

Fig. 7 **a** Semiquantitative RT-PCR analysis of the immunoglobulin-binding protein/glucose-regulated protein 78 (*BiP/Grp78*) gene in primary cultured astrocytes following exposure to aluminum (Al-gly; *Al*) and Tu for the indicated time periods. Total RNA was analyzed by RT-PCR for the mRNA expression of Bip/Grp78. Al-gly did not up-regulate the expression of Bip/Grp78. Cells were treated with Tu (5 μg/ml) for 48 h or with Al-gly for 48 or for 12 h followed by 7 days of culture in normal medium. The results are representative of three independent analyses from two replicate cultures. **b** Relative intensities of the bands

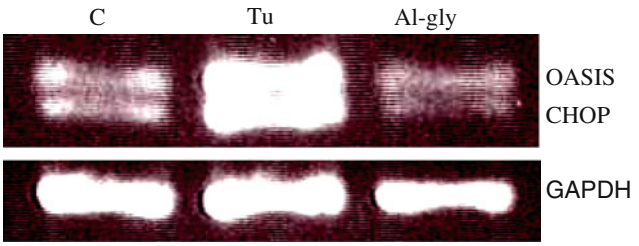

Fig. 8 Semiquantitative RT-PCR analysis of selected genes from primary cultured astrocyte following 24 h of exposure to aluminum and Tu. The expressions of C/EBP homology protein (*CHOP*) and old astrocyte specifically induced substance (*OASIS*) was not stimulated by exposure to Al-gly. Total RNA was analyzed by multiplex RT-PCR for the mRNA expressions of CHOP, OASIS, and GAPDH in primary cultured astrocytes. Cells were treated with Tu (5 μg/ml) or Al-gly (0.1 mM) for 24 h. The results are representative of three independent analyses from two replicate cultures

activates the downstream execution phase of apoptosis. Interestingly, tunicamycin, but not aluminum, up-regulated mRNA of Bbc3/PUMA, a pro-apoptotic member of the Bcl-2 family that mediates p53-dependent and -independent apoptosis [41].

Experiments with yeast have revealed that Ire1 is the most proximal component of the UPR pathway [42]. The two isoforms identified in the mammalian genome are Ire1α and Ire1β [43, 44]. Other known proximal sensors of the UPR in metazoan species are PERK and ATF6 [45]. Together with Ire1, PERK and ATF6 regulate components that act to suppress the initiation of protein synthesis, up-regulate the capacity of the ER to fold newly synthesized proteins, and degrade misfolded/unfolded protein [4]. Under non-stressed conditions, Bip (Grp78), an ATP-dependent ER chaperone, binds to the three ER resident transmembrane proteins (i.e., Ire1, PERK, and ATF6) which negatively regulate the UPR by keeping these proteins in an inactivated state [46]. When misfolded proteins accumulate in the ER, BiP/GRP78 binds instead to misfolded proteins [47–49] and allows activation of these UPR sensors, leading to decreased protein synthesis and the increased production of ER chaperones [46, 50]. Our results show that of these three proximal sensors, aluminum exposure only up-regulated Ire1β, while BiP/GRP78 (an ER resident chaperone) was down-regulated. Tunicamycin, on the other hand, up-regulated both Ire1α and BiP/GRP78 under similar conditions. The differential effects of tunicamycin and aluminum on Ire1α and Ire1β may indicate differences in their UPR mechanism.

Both Ire1α and Ire1β possess kinase activity that is required for activating downstream signaling [43], but it is not yet understood how aluminum and tunicamycin differentially regulate their expression in astrocytes. Ire1α is known to be constitutively expressed in all cells and tissues, whereas Ire1β expression is known to be restricted to gut epithelial cells [45]. Although Ire1β was not detectable in unstressed primary astrocytes, aluminum-stressed astrocytes were able to up-regulate this factor to the level approximating that of Ire1α (Fig. 4b). To the best of our knowledge, this is the first report to show the significant expression of Ire1β in the nervous tissue, but the significance of this finding remains to be elucidated. Nevertheless, over-expression of Ire1α and Ire1β can activate a reporter gene that harbors an ER stress-response element (ERSE) in a manner that requires the endoribonuclease activity of Ire1 [43]. Thus, the up-regulation of Ire1β by aluminum glycinate in our study under an exposure regimen similar to that previously reported in a study reporting morphological evidence of apoptosis [24] demonstrates the involvement of ER stress. Therefore, Ire1α may not be essential for the transcriptional induction of several well-characterized UPR target genes [45], and Ire1α and Ire1β may be differentially induced, depending on the stimulus and/or tissue. Further studies may clarify this hypothesis.

Aluminum glycinate, in contrast to tunicamycin, seemed to down-regulate—or have no effect at all—the expression of many genes, including the ER resident molecular chaperon BiP/GRP78 and Ca^{2+} binding chaperones (calnexin and calreticulin) as well as STC2, and transcription factors, such as the CEBP homologous protein

(CHOP/GADD153)-coding gene and OASIS. Calnexin, a type I integral membrane protein of the ER, exhibits a high degree of amino acid sequence and structural similarity (identity) to its soluble homolog, calreticulin [51], and appears to perform similar functions. Both proteins are chaperones whose functions include Ca^{2+} binding, lectin-like activity, and the recognition of misfolded proteins [52]. Both proteins are important for Ca^{2+} homeostasis and directly affect the folding and post-translational modification of virtually all glycosylated, secreted, or integral membrane proteins that pass through the ER [53–55]. The majority of cell-surface receptors, channels, and transporters as well as proteins that reside in intracellular organelles, including the ER, are glycosylated. BiP/GRP78, another ER resident chaperone, appears to play an important role in the folding and post-translational modification of non-glycosylated proteins [52, 56]. Thus, non-induction or down-regulation of these chaperones may affect diverse of cellular functions in addition to protein folding [57].

STC2 is a novel target of the mammalian UPR that shares limited sequence similarity with an anti-hypercalcemic hormone first discovered in fish [58]. Two widely expressed STC-related proteins, STC1 and STC2, have been identified in mammals where they have been implicated in mineral metabolism [59, 60]. STC2 expression has been shown recently to be induced in cultured cells by ER stress agents, and attenuation of its expression during the UPR renders mouse N2a neuroblastoma cells and HeLa cells vulnerable to cell death elicited by thapsigargin, whereas over-expression of STC2 protects cells against thapsigargin-induced apoptotic cell death [61]. In our study, only tunicamycin up-regulated STC2, while its expression was not detectable in both control and aluminum-stressed astrocytes. Another significant protein studied is OASIS, an ER stress transducer specific to astrocytes. OASIS is a membrane-bound transcription factor that activates genes in the ER stress response [62]. Over-expression of OASIS has been reported to result in the induction of BiP/GRP78 and suppression of ER-stress-induced cell death, whereas its knockdown partially reduced BiP/GRP78 levels and led to ER stress in susceptible astrocytes [63]. Our results show that OASIS, which provides astrocytes with resistance to ER stress, was not activated in the presence of aluminum when compared to tunicamycin and that this may render astrocytes susceptible to death. Thus, the down-regulation or non-activation of the molecular chaperones and transcription factors whose expressions are known to be protective by increasing protein folding and limiting further accumulation of misfolded proteins may spell doom for the adaptive response that promotes cell survival.

Conditions that impair protein folding in the ER and thus promote the accumulation of mutant proteins have a significant adverse effect on cellular survival. A number of diseases have been linked to the toxic effects of mutant proteins that accumulate in the ER [64]. For example, the misfolding of secreted Aβ 39–43 residues in terms of length is linked by a plethora of evidence to the pathology of AD [65–67]. Moreover, mutations in the presenilin gene have been reported to cause the inhibition of ER stress transducers, Ire1, ATF6, and PERK, resulting in the decreased expression of ER chaperons [35]. The aberrant splicing isoform (PS2V) generated by skipping exon5 of the presenilin-2 (PS2) gene is a diagnostic feature of sporadic AD (SAD). It has been demonstrated that chronic exposure to aluminum accelerates PS2V production induced by hypoxia in human neuroblastoma cells [33]. PS2V has also been reported to cause significant increases in the production of both Aβ 40 and Aβ 42 [34]. The inhibition of ER chaperones, such as BiP/GRP78 and calnexin, has also been linked to the increased production of the level of Aβ 40 and Aβ 42 in conditioned medium of cells that produce mutant types of APP [68]. Thus, further studies are needed to clarify the links between aluminum, PS2V, ER chaperones, and the accumulation of unfolded proteins and neurodegenerative disorders. In fact, neurodegenerative disorders such as AD, Parkinson disease (PD), Huntingtons disease, amyotrophic lateral sclerosis or Lou Gehrig's disease, and prion protein diseases as well as their transgenic animal models all feature misfolded proteins and the aggregation of misfolded proteins [21, 69–72]. Although the specific role played by misfolded proteins in disease pathogenesis remains elusive [46], several authors have reported that neuronal death occurring in AD, PD, and cerebral ischemia has its origin in the ER [35]. ER stress pathways and the subsequent cellular responses may provide a mechanistic connection between misfolded proteins and disease [48].

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