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Journal of Inorganic Biochemistry 99 (2005) 1895–1898
JOURNAL OF Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

Nanomolar aluminum induces pro-inflammatory and pro-apoptotic gene expression in human brain cells in primary culture

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Received 23 March 2005; received in revised form 19 April 2005; accepted 20 April 2005
Available online 14 June 2005

Abstract

Aluminum, the most abundant neurotoxic metal in our biosphere, has been implicated in the etiology of several neurodegenerative disorders including Alzheimer's disease (AD). To further understand aluminum's influence on gene expression, we examined total messenger RNA levels in untransformed human neural cells exposed to 100 nanomolar aluminum sulfate using high density DNA microarrays that interrogate the expression of every human gene. Preliminary data indicate that of the most altered gene expression levels, 17/24 (70.8%) of aluminum-affected genes, and 7/8 (87.5%) of aluminum-induced genes exhibit expression patterns similar to those observed in AD. The seven genes found to be significantly up-regulated by aluminum encode pro-inflammatory or pro-apoptotic signaling elements, including NF-κB subunits, interleukin-1β precursor, cytosolic phospholipase A_2, cyclooxygenase-2, beta-amyloid precursor protein and DAXX, a regulatory protein known to induce apoptosis and repress transcription. The promoters of genes up-regulated by aluminum are enriched in binding sites for the stress-inducible transcription factors HIF-1 and NF-κB, suggesting a role for aluminum, HIF-1 and NF-κB in driving atypical, pro-inflammatory and pro-apoptotic gene expression. The effect of aluminum on specific stress-related gene expression patterns in human brain cells clearly warrant further investigation.

Keywords: Aluminum; βAPP; COX-2; $cPLA_2$; DAXX; DNA array; HIF-1; Human neural cells; Inflammation; IL-1β, NFκB; Nuclear pore complex; Promoter

The harmful effects of aluminum salts on normal physiological and biochemical systems are many, and over the last 30 years, a considerable body of evidence has emerged concerning the specific actions of aluminum on the structural and functional integrity of the genetic apparatus [1–3]. Multiple adverse biological effects of aluminum on nucleic acids have been repeatedly demonstrated, and instances of highly specialized interactions with nuclear genomic structures illustrate specific aluminum targeting within the neural (neuronal and glial) cells of the central nervous system [1,2]. To further investigate the nature of these interactions, in this study we examined the effects of aluminum sulfate on full-spectrum gene expression in human neural (HN) cells in primary culture using DNA arrays that profile the expression of every human gene. Treatment of 2.5 week old HN cells with 100 nM aluminum sulfate for 3.5 days was found to emulate many of the stress-related gene expression changes as previously reported in human brain tissues affected by late stage Alzheimer's disease (AD) [3–7]. These studies suggest the involvement of soluble aluminum compounds with human brain gene

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doi:10.1016/j.jinorgbio.2005.04.021

expression, and in particular, the molecular processes involved in brain-specific gene transcription.

Previous reports examining the effects of aluminum on run-on gene transcription, based on the incorporation of [α-^{32}P]-uridine triphosphate into newly synthesized messenger RNA, showed that in isolated human brain cell nuclei, 100 nM ambient aluminum had potent modulatory effects on RNA polymerase II-directed processes [1,3]. Therefore, in the experiments described here, the effects of 100 nM aluminum on total gene expression patterns were examined in greater detail. Human neural (HN) cells, a primary, untransformed co-culture of neurons and glia (Clonetics CC-2599; Bio-Whittaker/Cambrex Cell Biology, Walkersville MD) were grown for 3 weeks as previously described [4,5]. At 3 weeks of development this cell culture contains approximately equal populations of differentiated human neurons and glia [4]. During HN cell growth, cell culture maintenance medium was changed every 3.5 days; during the last 3.5 days of culture, cells were incubated in serum-free medium that contained either 100 nM magnesium sulfate (control; $MgSO_4$) or 100 nM aluminum sulfate (treated; $Al_2(SO_4)_3$) from freshly prepared stock solutions [3]. After three full weeks of culture, four control plates and four treated plates of HN cells were subjected to total RNA and protein isolation using Trizol reagent (Invitrogen, Carlsbad CA), as previously described [4,6]. Total RNA concentration and spectral purity were determined using RNA Nano Labchip analysis and a 2100 Spectral Bioanalyzer (Caliper Technologies, Mountainview, CA; Agilent Technologies, Palo Alto, CA) [5,6]. 28S/18S ratios consistently exceeded 1.4 and the A_{260}/A_{280} of total RNA (based on peak area) was typically $\geqslant 1.8$. Poly A+ messenger RNA was found to range in size from 0.2 to 8 kb [7]. No significant differences in the spectral purity, molecular size or yield of total RNA between control or treated HN cells were noted. The SuperScript Choice System (Invitrogen) was used for RNA probe synthesis [4,6,7]. Briefly, eight Test3 DNA arrays (900341; Affymetrix) and eight human genome U133 plus 2.0 analytical Genechips (900466; Affymetrix; containing 33,000 gene targets) were used in total genome expression analysis. DNA arrays were scanned, pixel intensities and gene expression signals were quantified, and features were extracted using Microarray Suite ver 5.0, (Affymetrix) and Genespring ver 7.2 algorithms (Silicon Genetics, Redwood City, CA) [5–7]. Statistical significance of treated gene expression levels over controls was analyzed using a two-way factorial analysis of variance (p, ANOVA; Statistical Analysis System; SAS Institute, Cary, NC). Gene promoter maps identifying potential DNA binding sites in specific pro-inflammatory and pro-apoptotic gene promoters were created and displayed using DNASIS MAX algorithms (Mirai-Bio Inc, Hitachi Genetic Systems, Alameda, CA).

Data analysis for gene expression in HN cells in the presence of aluminum is compared in Table 1 against 24 of the most significant gene expression levels found to be altered by a factor of $\geqslant$3-fold ($p < 0.05$) in AD brain [5–7]. The results indicate that in AD, of the 24 gene expression levels found to be altered by a factor of $\geqslant$3-fold ($p < 0.05$), 16 of 24 (66.7%) were found to be down-regulated. Of this group, 11/16 (68.8%) were also found to be down-regulated, at the level of gene expression, in aluminum sulfate-treated HN cells when compared to controls. Interestingly, of the up-regulated genes in AD, 7/8 (87.5%) exhibit similar expression patterns to those observed in aluminum-treated HN cells. Because gene expression patterns are a reflection of the physiological status of the cell, these data suggest that pathological processes directed by these atypical gene expression profiles, such as inflammation and apoptosis, are common to both AD and aluminum-treated HN cells. To further investigate the possibility of coordinate up-regulation of these pro-inflammatory and pro-apoptotic genes, the immediate promoters of 5/7 up-regulated genes were screened, analyzed and mapped for TF-DNA binding sites and the analysis is shown in Fig. 1. Gene promoters were analyzed using DNASIS DNA Sequence analysis software capable of recognizing ~360 consensus and relaxed TF-DNA recognition motifs. Preliminary analysis shows that these up-regulated genes contain multiple binding sites for the relatively rare, oxidative-stress sensitive TFs HIF-1 (5′-R*CGTG*-3′) [8,9] and NF-κB (5′-GG*GACT*TT-3′) [8,10] in their immediate promoters (core recognition sequences italicized). The correlation of an occurrence of an HIF-1- or NF-κB-DNA binding site in each pro-inflammatory or pro-apoptotic gene promoter with the abundance of that specific RNA signal intensity was found to be highly significant ($r^2 = 0.88$; $p < 0.05$).

The vast majority of all cases of AD are sporadic and of unknown origin, and environmental toxins injurious to normal brain function have been strongly implicated [1–3,12–14]. Notably, expression of the familial Alzheimer genes encoding presenilin-1, presenilin-2 and the apolipoprotein E4 isoform were found not to be overtly affected by aluminum in these experiments. The major risk factor for the development of AD is aging, suggesting an accumulation of biological insults over decades of life. Specific compartments of the brain appear to accumulate aluminum from the environment as a function of aging [1–3]. This is the first study describing the effects of aluminum sulfate, at 100 nM concentrations, on total gene expression patterns in untransformed human brain cells in primary culture using DNA arrays. The molecular biology, transport and in vivo speciation of aluminum in genetic systems is not well understood, however, as little as 50 nM ambient aluminum has been shown to dramatically perturb RNA polymerase II-direct gene transcription in isolated mammalian brain cell