

Aluminum in Neurological Disease - a 36 Year Multicenter Study

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Abstract

Aluminum is a ubiquitous neurotoxin highly enriched in our biosphere, and has been implicated in the etiology and pathology of multiple neurological diseases that involve neural degeneration, behavioral impairment and cognitive decline. Over the last 36 years our group has analyzed the aluminum content of the temporal lobe neocortex of 511 high quality human brain samples from 18 diverse neurological and neurodegenerative disorders, including 2 groups of age-matched controls. Brodmann anatomical areas including the inferior, medial and superior temporal gyrus (A20-A22) were selected for analysis: (i) because of their essential functions in massive neural information processing operations including cognition and memory formation; and (ii) because subareas of these anatomical regions are unique to humans and are amongst the earliest areas affected by progressive neurodegenerative disorders such as Alzheimer's disease (AD). We utilized the analytical technique of (i) Zeeman-type electrothermal atomic absorption spectrophotometry (ETAAS) combined with (ii) an experimental multi-elemental analysis using the advanced photon source (APS) ultra-bright storage ring-generated hard X-ray beam (7 GeV) and fluorescence raster scanning (XRFR) spectroscopy device at the Argonne National Laboratory, US Department of Energy, University of Chicago IL, USA. These data represent the largest study of aluminum concentration in the brains of human neurological and neurodegenerative disease ever undertaken. Neurological diseases examined were AD (N=186), ataxia Friedreich's type (AFT; N=6), amyotrophic lateral sclerosis (ALS; N=16), autism spectrum disorder (ASD; N=26), dialysis dementia syndrome (DDS; N=27), Down's syndrome (DS; trisomy21; N=24), Huntington's chorea (HC; N=15), multiple infarct dementia (MID; N=19), multiple sclerosis (MS; N=23), Parkinson's disease (PD; N=27), prion disease (PrD; N=11) including bovine spongiform encephalopathy (BSE; 'mad cow disease'), Creutzfeldt-Jakob disease (CJD) and Gerstmann-Straussler-Sheinker syndrome (GSS), progressive multifocal leukoencephalopathy (PML; N=11), progressive supranuclear palsy (PSP; N=24), schizophrenia (SCZ; N=21), a young control group (YCG; N=22) and an aged control group (ACG; N=53). Amongst these 18 common neurological conditions and controls we report a statistically significant trend for aluminum to be increased only in AD, DS and DDS compared to age- and gender-matched brains from the same anatomical region. The results continue to suggest that aluminum's association with AD, DDS and DS brain tissues may contribute to the neuropathology of these neurological diseases but appear not to be a significant factor in other common disorders of the human central nervous system (CNS).

Keywords: Aluminum; Alzheimer's disease (AD); Dialysis dementia syndrome (DDS); Down syndrome (DS; Trisomy 21); Electrothermal atomic absorption spectrophotometry (ETAAS); neuropathology; X-ray fluorescence raster (XRFR) scanning spectroscopy

Introduction

Aluminum is an environmentally abundant and proinflammatory, trivalent metal neurotoxin that has been implicated in the onset, development and propagation of neurodegeneration and cognitive decline in several human neurological disorders including AD, DDS and DS (trisomy 21). As such, aluminum accumulation within the central nervous system (CNS) over the course of aging appears to reach a critical threshold in which sufficient amounts of this neurotoxin accumulates to induce proinflammatory signaling, dysregulation of gene expression (particularly in neurons), irreversible brain cell damage, and functional decline resulting in deficits in cognition, memory and behavior [1-15]. More specifically, aluminum, an extremely high charge density cation ($Z^2/r=18$), appears to induce a general neurotoxicity towards both intracellular and extracellular signaling functions in the CNS wherever phosphate or polyphosphate is encountered, such as in the phosphate-

rich genetic material that includes RNA, DNA, free nucleotides such as adenosine triphosphate (ATP), phosphoproteins and single stranded nucleic acids [3,16-20]. More precisely, bioavailable aluminum appears to be responsible for a significant reactive oxygen species (ROS) mediated genotoxicity, that is, toxicity toward the genetic material of the cell and molecular-genetic operations that include transcription, intra

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and extranuclear genetic signaling, epigenetics and gene-expression that has been extensively described by various laboratories [3,7,9,10,21-35]. Much of this genotoxicity appears to be mediated through (i) upregulation of the heterodimeric, proinflammatory transcription factor NF- κ B (p50/p65) complex; (ii) significant increases in NF- κ B-sensitive microRNA (miRNA) and messenger RNA (mRNA) linked signaling circuits; and (iii) deficits in gene-expression. These have been shown to drive multiple aspects of inflammatory neurodegeneration including amyloidogenesis, altered innate-immune responses, deficits in neurotrophic signaling and synaptogenesis, and the inability to clear self-aggregating waste material from the brain cell cytoplasm and parenchyma [5,6,10,12,29-38].

As part of our major research interests on environmental biochemistry and neurotoxicity of biosphere abundant metallotoxins, aluminum is the 3rd most abundant element (after oxygen and silicon) and the most abundant metal making up about ~8.1% (w/v) of the entire earth's crust the determination of neurotoxic metals including aluminum in human tissues has been an ongoing operation in our laboratories for almost four decades. *It is important to point out that even though aluminum may be more abundant in one neurological disease or tissue fraction than another, it is further important to demonstrate that aluminum at physiologically realistic concentrations is capable of contributing to an aluminum-driven neuropathology that is relevant to that particular CNS disease* [6,14,22,39,40-42]. For example, ambient aluminum sulfate at low nanomolar concentrations, as might be encountered within the genetic material of the aging CNS, has the ability to emulate the upregulated levels of the same microRNAs (miRNAs) as are apparent in AD, DS, some aged human brain tissue samples and in several transgenic murine models of these neurodegenerative diseases [30,31,35,42,43]. Interestingly, the synergism of aluminum with other potentially neurotoxic metals, such as environmental iron and mercury, in mediating and enhancing incapacitating neurotoxic effects within the human CNS are just beginning to become understood [27,41].

Methods

At autopsy, human brain temporal lobe (Brodmann areas A20-A22) tissues were collected from deceased patients with a postmortem interval (PMI; death-to-brain-freezing at 81°C) ranging from 1.2 to 5.0

hr. Patient clinical, medial and familial history, physical examination, clinical dementia rating (CDR), pneumoencephalogram and/or other neuropathological determinations were compatible with the diagnosis of each respective neurological disorder. To avoid gender-based neurochemical or neurophysiological bias only female brains were utilized in this study with the exception of the ASD group which consisted of 7 females and 19 males. Extensive post-mortem examination revealing brain weight, brain atrophy, neurofibrillary degeneration, senile plaques, evidence of microbleeds and stroke, hippocampal pyramidal degeneration and related clinical parameters were consistent with a diagnosis of each neurological disorder. Because of a massive data loss 23-31 August 2005 due to hurricane Katrina only the mean and standard deviation for the age of each neurological group studied was available. Great care was taken in the removal and processing of tissues and workers in autopsy suites were supervised to minimize possible aluminum contamination. For aluminum determinations brain tissues were typically handled and processed in a negative pressure clean room area used for the manipulation of extremely labile single-stranded RNA molecules. Further details of the analytical methods for aluminum samples are given in (Supplementary file 1).

Results

Using ETAAS as little as 0.1 ug/gm (dry weight) aluminum can be reliably detected, however, the ETAAS method measures the total amount of aluminum, and the possibility that this element is bound in a nontoxic or non-specific form or in a physiological situation that has no neurotoxic effect cannot be totally excluded at this time. Although a considerable amount of investigational work has been done by this laboratory on the genotoxic effects of aluminum on gene expression, it will be necessary to further identify and characterize the tissue binding sites in AD, DDS and DS to further establish the role of aluminum in the pathogenesis of each disease. For example, it has been shown that in AD, aluminum may target certain 'open' or 'euchromatic' regions of the brain's genetic material and hence be compartmentalized to specific chromatin structures such as the inter-nucleosomal 'histone H1' linker region, i.e. in highly compartmentalized fractions of the nuclei of entire brain cells [1,2,7,9,40,44]. In addition, not all of the pathological changes in AD, DDS, DS and the other neurological conditions studied

Abbreviation	Neurological disease	Al, ug/g dry weight				p,ANOVA	NS or S	Mean age (yr)+/- S.D.
		N	Mean	S.D.	Range			
AD	Alzheimers disease	186	8.08	2.91	1.9-16.8	<0.0001	S	73.1 +/- 15.6
AFT	ataxia-Friedreichs type	6	1.15	0.18	0.9-1.4	1	NS	69.3 +/- 11.5
ALS	amyotrophic lateral sclerosis	16	1.22	0.14	0.95-1.4	1	NS	67.5 +/- 10.1
ASD	autism spectrum disorder	26	1.22	0.2	0.9-1.6	1	NS	11.1 +/- 6.4
DDS	dialysis dementia syndrome	27	3.69	1.14	1.2-6.2	<0.0001	S	72.4 +/- 13.2
DS	Down's syndrome (trisomy 21)	24	4.53	1.18	2.0-7.1	<0.0001	S	75.3 +/- 9.3
HC	Huntington's chorea	15	1.69	0.9	0.3-3.1	1	NS	70.4 +/- 14.3
MID	multiple infarct dementia	19	1.35	0.31	1.0-2.1	1	NS	70.4 +/- 10.3
MS	multiple sclerosis	23	1.37	0.43	0.7-2.1	1	NS	71.5 +/- 9.3
PD	Parkinson's disease	27	1.77	0.76	0.4-3.2	0.9998	NS	72.5 +/- 10.1
PrD	prion disease (BSE, CJD, GSS)	11	1.31	0.34	0.9-2.1	1	NS	74.2 +/- 9.8
PML	progressive multifocal leukoencephalopathy	11	1.5	0.56	0.7-2.3	1	NS	73.1 +/- 16.3
PSP	progressive supranuclear palsy	24	1.45	0.4	0.5-2.3	1	NS	71.9 +/- 14.4
SCZ	schizophrenia	21	1.74	0.56	0.7-2.7	1	NS	69.3 +/- 11.6
YCG	young control group	22	1.2	1.19	0.9-1.5			10.2 +/- 6.1
ACG	aged control group	53	1.34	0.28	0.16-1.8			71.4 +/- 9.3

N=number of individual brains examined; SD: Standard Deviation; range=lowest to highest aluminum signal quantified; S: significant; NS: Not Significant.

Table 1: Statistical analysis of neurological diseases.

here can be readily explained by these findings and our study does not exclude the possibility that other etiological factors including other neurotoxic processes, molecules or environmental metals may additionally contribute to the pathogenic mechanism of progressive inflammatory neurodegeneration (see also Discussion section, below).

Results for the aluminum content of temporal lobe tissues from 16 neurological disorders, a young control group (YCG) and an aged control group (ACG) are presented in (Table 1); raw data for each neurological disorder is presented in (Supplementary file 2). The neurological disorders AFT, ALS, ASD, HC, MID, MS, PD, PrD, PML, PSP and SCZ, while all representing neurological disorders with a neuroinflammatory and/or neurodegenerative component that include progressive memory, behavioral or cognitive deficits did not show any significant increase in aluminum in the medial superior temporal lobes (Brodmann A20-A22) when compared to age-matched controls (Table 1). We note that the only neurological conditions exhibiting increased aluminum versus age- and gender-matched healthy, neurologically normal controls was AD to a mean of ~8.08-fold over these controls (N=186; range 1.9-16.8 ug aluminum/gm tissue); DS (trisomy 21) to a mean of ~4.53-fold over age- and gender-matched controls (N=24; 2.0-7.1 ug aluminum/gm tissue); and DDS to a mean of ~3.69-fold over age and gender-matched controls (N=27; range 1.2-6.2 ug aluminum/gm tissue). These values are within the range of previously determined tissue aluminum studies using ETAAS [25,45]. Of all brains studied the highest aluminum concentrations were consistently found in AD tissues. Interestingly all DS patients are known to expire from AD-type pathological change in their brains and at post-mortem examination all DS patients exhibit some form of AD-type alterations in the brain parenchyma and/or the extraneuronal or extracellular space. These include, prominently, the appearance of highly proinflammatory lesions including amyloid beta (A β) peptide enriched senile plaque deposits and neurofibrillary tangles [15,45-47]. The loss of normal kidney function in aged, long-term DDS patients exposes the entire circulatory system, including the cerebrovascular circulation, and potentially CNS compartments, to very large amounts of aluminum, usually in the form of aluminum containing compounds in the dialysis fluids resulting in aluminum intoxication, marked by significant motor, speech and cognitive disturbances, seizures and progressive dementia [18,19,25,48,49].

The latest US federal government compiled medical and scientific data, including updated disease information with demographic statistics on facts concerning AD, AFT, ALS, ASD, DDS, DS, HC, MID, PD, PML, PSP, SCZ and PrP [that includes BSE (mad cow disease), CJD and GSS], provided by the National Institutes of Health, Bethesda MD, USA and other resources and constantly updated are provided in (Supplementary file 3).

Results employing other novel and experimental analytical techniques for aluminum quantitation and other trace metal analysis using X-ray fluorescence raster scanning (XRFR) spectroscopy to advance and improve the analysis and resolution of aluminum abundance in neurobiological tissues is presented in (Supplementary file 4) [50,unpublished data]. While still in development, using the APS XRFR spectroscopy device, data were acquired using an energy-dispersive germanium detector capable of detecting Al, P, S, Cl, K, Ca, Fe and Zn nondestructively in complex biological samples. We have been able to detect a significantly larger signal for aluminum abundance in AD temporal lobe neocortex ranging from 6-9-fold over age and gender-matched healthy controls and the results are highly significant ($p < 0.0001$, ANOVA). In this ongoing project our long term goal is to advance our ability to analyze and quantify aluminum and

other metal abundance, speciation and complexity in extremely small samples of pathological neurobiological tissues compared to age- and gender-matched controls (Supplementary file 4).

Discussion

The molecular, cellular, nuclear, genetic, epigenetic and systemic mechanisms by which aluminum exerts selective neurotoxicity and genotoxicity remains incompletely understood. Many different pathogenic signaling pathways mediated by aluminum toxicity have been described by our group and others [3,5,10,11,19,27,31,38,47]. One major aluminum-induced pathogenic signaling pathway driving aluminum genotoxicity with relevance to many different human diseases has been discovered in which: (i) aluminum crosses aging, diseased or dysfunctional biophysical barriers including the gastrointestinal (GI) and blood-brain barriers (BBB); (ii) accesses aluminum-sensitive compartments within the CNS and supports the generation of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS); (iii) these highly reactive species in turn strongly induces phosphorylation of the NF- κ B inhibitor (I κ B) thereby leading to the activation of the heterotypic NF- κ B (p50/p65) dimer; (iv) NF- κ B-sensitive microRNAs (such as miRNA-34a and miRNA-146a) are significantly induced (due to the presence of multiple NF- κ B-DNA-binding sites in the miRNA-34a and miRNA-146a gene promoters); and (v) these NF- κ B-sensitive miRNAs next interact with the 3'-untranslated region (3'-UTR) of target messenger RNAs (mRNAs) thereby leading to their degradation and decreasing the expression of that target mRNA [2,13,15,30-32,36,51].

Hence, a highly select and pathogenic group of upregulated, NF- κ B-sensitive, miRNAs ultimately causes, for example, the down-regulation of several key brain essential mRNAs including those involved in synaptogenesis, in the regulation of innate immunity, inflammatory and neurotrophic signaling and in amyloidogenesis [15,42,43]. Interestingly, it has recently been shown that human microbiome-derived lipopolysaccharide (LPS), and more specifically the LPS of the Gram negative obligate anaerobe *Bacteroides fragilis* (BFLPS), strongly adsorbs aluminum, and is highly capable of inducing both ROS, RNS and NF- κ B and in triggering inflammatory neurodegeneration in human brain cells in primary culture. This therefore establishes for the first time a link between potent aluminum containing, proinflammatory neurotoxins actively secreted by the GI tract microbiome and inflammatory signaling within human brain cells [52,53, unpublished observations].

In addition to ETAAS and XRFR, other experimental, investigative and analytical methodologies for quantifying the abundance of aluminum in CNS tissues include high-field 19.6T 27 Al solid-state MAS NMR technologies which are designed to improve the detection and localization of aluminum in neurobiological tissue samples [54]. These tissues include those of the normally aging human CNS, those with the neurological diseases referred to in this report, transgenic murine models of these same neurological disorders, and the use of primary human neuronal-glia (HNG) cell models co-cultured *in vitro* [27,52,54, unpublished 2018]. Related to aluminum localization and quantitative analytical work, other techniques such as '*molecular shuttle chelation*', involving the use of ascorbate, desferrioxamine and Feralex-G in combination to remove nuclear-bound aluminum have been advanced, although once bound, aluminum is particularly refractory to chelation-based removal, especially in the phosphate-enriched environment of euchromatin and the transcriptionally active microcompartments of human brain cell nuclei [12,14,17,35]. Interestingly, the only clinical trial specifically designed to remove

aluminum from the brains of live control and AD patients (N=48) using the trivalent metal chelator desferrioxamine (DF) resulted in a halving of the rate of neurodegeneration and cognitive decline in the DF-treated group [55].

Lastly, these like all similar analytical studies on metallotoxins in neurological disease have limitations. We focused on aluminum content in females largely due to brain tissue sample availability, but preliminary data for example in AD, show that males with AD exhibit the same trend for aluminum accumulation in the temporal lobe region of the brain versus controls with no neurological disease. Small sampling sizes from extremely rare neurological disorders such as prion disease (BSE; 'mad cow disease', CJD and GSS) and ataxia Friedreichs type (AFT) and others are problematic as there is variability in neurotoxic metal concentrations amongst individuals, and small sample size and skewed power analysis could induce statistically nonsignificant results. We would like to reiterate that if aluminum, or in fact any metal, is enriched in tissues diagnosed with a certain disease, while certainly suggestive, should not be enough evidence to link that metal to any disease. Rigorous and reproducible experimentation with appropriate controls are required to show that aluminum, or in fact any other neurotoxic metal, at the concentrations found under physiological conditions *in vivo* are capable of causing a focused disruption of a neurological process, and preferably some disturbance that has a direct relevance to the neurological disease process itself. For example, it has been shown that the extremely high positive charge density aluminum may specifically target certain accessible, polyphosphate-rich and highly electronegative euchromatic regions of brain cell nuclei to disrupt gene expression. Because aluminum appears to be compartmentalized to specific 'open' and 'accessible' chromatin structures its concentration may be actually highly localized, and at a higher concentration, than those found on average and at random determinations throughout the entire tissue sample under study [31,32,40].

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Massachusetts, Worcester MA, USA; University of Pennsylvania School of Medicine, Philadelphia PA, USA, and the University of Toronto Brain Bank, Toronto ON, Canada. All authors agree on the content of this publication. Research on metal neurotoxicity, human and murine microRNAs, small noncoding RNA (sncRNA), proinflammatory and pathogenic signaling in the Lukiw laboratory involving the innate-immune response, neuroinflammation and amyloidogenesis in AD, ASD, PrD and in other human neurological disorders was supported through an unrestricted grant to the LSU Eye Center from Research to Prevent Blindness (RPB); the Louisiana Biotechnology Research Network (LBRN), the Alzheimer Association and NIH grants NEI EY006311, NIAAG18031 and NIAAG038834 (WJL).

Ethics Statement

All acquisition, handling, experimental and analytical procedures involving postmortem human brain tissues were carried out in an ethical manner in strict accordance with the ethics review board policies at brain and tissue donor institutions and at the Louisiana State University (LSU) Health Sciences Center. Informed consent from next of kin was obtained at brain and tissue donor institutions for all tissue samples prior to autopsy and donation; coded postmortem brain tissue samples (containing no personal identifying information of the donors) were obtained from the 18 brain and tissue banks listed in the Acknowledgements section above. The ethical use of postmortem human brain tissues and their analyses were also carried out in strict accordance with the Institutional Biosafety Committee and the Institutional Review Board Committee (IBC/IRBC) ethical guidelines IBC#18059 and IRBC#6774 at the LSU Health Sciences Center, New Orleans LA 70112 USA.

Conflict of Interest Statement

Declaration of interest for all authors including financial and personal relationships with other people or organizations: none. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. The experimental work in this paper was funded by the LSU Eye Center from Research to Prevent Blindness (RPB), the Louisiana Biotechnology Research Network (LBRN), the National Institutes of Health (NIH), Bethesda MD, USA and the Alzheimer Association Chicago IL, USA, and was not supported by any pro- or anti-aluminum lobby or private foundation.

Authors Contributions

DRCM, PNA, WW, AIP, MEP, TPAK, YZ, NS, VJ and WJL aided in the acquisition of all brain tissues and performed all analytical experiments involving ETAAS and XRF; DRCM, PNA, ZF, WW, AIP, YZ and WJL collected, organized and tabulated all data and performed statistical analysis; DRCM generated many preliminary observations and notes on all aluminum data and their statistics; AIP and WJL wrote the paper.

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