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Aluminium in brain tissue in autism

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ABSTRACT

Autism spectrum disorder is a neurodevelopmental disorder of unknown aetiology. It is suggested to involve both genetic susceptibility and environmental factors including in the latter environmental toxins. Human exposure to the environmental toxin aluminium has been linked, if tentatively, to autism spectrum disorder. Herein we have used transversely heated graphite furnace atomic absorption spectrometry to measure, for the first time, the aluminium content of brain tissue from donors with a diagnosis of autism. We have also used an aluminium-selective fluor to identify aluminium in brain tissue using fluorescence microscopy. The aluminium content of brain tissue in autism was consistently high. The mean (standard deviation) aluminium content across all 5 individuals for each lobe were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes respectively. These are some of the highest values for aluminium in human brain tissue yet recorded and one has to question why, for example, the aluminium content of the occipital lobe of a 15 year old boy would be 8.74 (11.59) μg/g dry wt.? Aluminium-selective fluorescence microscopy was used to identify aluminium in brain tissue in 10 donors. While aluminium was imaged associated with neurones it appeared to be present intracellularly in microglia-like cells and other inflammatory non-neuronal cells in the meninges, vasculature, grey and white matter. The pre-eminence of intracellular aluminium associated with non-neuronal cells was a standout observation in autism brain tissue and may offer clues as to both the origin of the brain aluminium as well as a putative role in autism spectrum disorder.

1. Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental conditions of unknown cause. It is highly likely that both genetic [1] and environmental [2] factors are associated with the onset and progress of ASD while the mechanisms underlying its aetiology are expected to be multifactorial [3–6]. Human exposure to aluminium has been implicated in ASD with conclusions being equivocal [7–10]. To-date the majority of studies have used hair as their indicator of human exposure to aluminium while aluminium in blood and urine have also been used to a much more limited extent. Paediatric vaccines that include an aluminium adjuvant are an indirect measure of infant exposure to aluminium and their burgeoning use has been directly correlated with increasing prevalence of ASD [11]. Animal models of ASD continue to support a connection with aluminium and to aluminium adjuvants used in human vaccinations in particular [12]. Hitherto there are no previous reports of aluminium in brain tissue from donors who died with a diagnosis of ASD. We have measured aluminium in brain tissue in autism and identified the location of aluminium in these tissues.

2. Materials and methods

2.1. Measurement of aluminium in brain tissues

Ethical approval was obtained along with tissues from the Oxford Brain Bank (15/SC/0639). Samples of cortex of approximately 1 g frozen weight from temporal, frontal, parietal and occipital lobes and hippocampus (0.3 g only) were obtained from 5 individuals with ADI-R-confirmed (Autism Diagnostic Interview-Revised) ASD, 4 males and 1 female, aged 15–50 years old (Table 1).

The aluminium content of these tissues was measured by an established and fully validated method [13] that herein is described only briefly. Thawed tissues were cut using a stainless steel blade to give individual samples of *ca* 0.3 g (3 sample replicates for each lobe except for hippocampus where the tissue was used as supplied) wet weight and dried to a constant weight at 37 °C. Dried and weighed tissues were digested in a microwave (MARS Xpress CEM Microwave Technology Ltd.) in a mixture of 1 mL 15.8 M HNO_3 (Fisher Analytical Grade) and 1 mL 30% *w/v* H_2O_2 (BDH Aristar). Digests were clear with no fatty residues and, upon cooling, were made up to 5 mL volume using

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Table 1
Aluminium content of occipital (O), frontal (F), temporal (T) and parietal (P) lobes and hippocampus (H) of brain tissue from 5 donors with a diagnosis of autism spectrum disorder.

Donor ID	Gender	Age	Lobe	Replicate	[Al] μg/g
A1	F	44	O	1	0.49
				2	4.26
				3	0.33
				Mean (SD)	1.69 (2.22)
			F	1	0.98
				2	1.10
				3	0.95
				Mean (SD)	1.01 (0.08)
			T	1	1.13
				2	1.16
				3	1.12
				Mean (SD)	1.14 (0.02)
			P	1	0.54
				2	1.18
				3	NA
				Mean (SD)	0.86 (0.45)
			All	Mean (SD)	1.20 (1.06)
A2	M	50	O	1	3.73
				2	7.87
				3	3.49
				Mean (SD)	5.03 (2.46)
			F	1	0.86
				2	0.88
				3	1.65
				Mean (SD)	1.13 (0.45)
			T	1	1.31
				2	1.02
				3	2.73
				Mean (SD)	1.69 (0.92)
			P	1	18.57
				2	0.01
				3	0.64
				Mean (SD)	6.41 (10.54)
			Hip.	1	1.42
			All	Mean (SD)	3.40 (5.00)
A3	M	22	O	1	0.64
				2	2.01
				3	0.66
				Mean (SD)	1.10 (0.79)
			F	1	1.72
				2	4.14
				3	2.73
				Mean (SD)	2.86 (1.22)
			T	1	1.62
				2	4.25
				3	2.57
				Mean (SD)	2.81 (1.33)
			P	1	0.13
				2	3.12
				3	5.18
				Mean (SD)	2.82 (1.81)
			All	Mean (SD)	2.40 (1.58)
A4	M	15	O	1	2.44
				2	1.66
				3	22.11
				Mean (SD)	8.74 (11.59)
			F	1	1.11
				2	3.23
				3	1.66
				Mean (SD)	2.00 (1.10)
			T	1	1.10
				2	1.83
				3	1.54
				Mean (SD)	1.49 (0.37)
			P	1	1.38
				2	6.71
				3	NA
				Mean (SD)	4.05 (3.77)
			Hip.	1	0.02
			All	Mean (SD)	3.73 (6.02)
A5	M	33	O	1	3.13
				2	2.78
				3	1.71
				Mean (SD)	2.54 (0.74)
			F	1	2.97
				2	8.27
				3	NA
				Mean (SD)	5.62 (3.75)
			T	1	1.71
				2	1.64
				3	17.10
				Mean (SD)	6.82 (8.91)
			P	1	5.53
				2	2.89
				3	NA
				Mean (SD)	4.21 (1.87)
			All	Mean (SD)	4.77 (4.79)

ultrapure water (cond. < 0.067 μS/cm). Total aluminium was measured in each sample by transversely heated graphite furnace atomic absorption spectrometry (TH GFAAS) using matrix-matched standards and an established analytical programme alongside previously validated quality assurance data [13].

2.2. Fluorescence microscopy

All chemicals were from Sigma Aldrich (UK) unless otherwise stated. Where available frontal, parietal, occipital, temporal and hippocampal tissue from 10 donors (3 females and 7 males) with a diagnosis of ASD was supplied by the Oxford Brain Bank as three 5 μm thick serial paraffin-embedded brain tissue sections per lobe for each donor (Table S1). Tissue sections mounted on glass slides were placed in a slide rack and de-waxed and rehydrated via transfer through 250 mL of the following reagents: 3 min in Histo-Clear (National Diagnostics, US), 1 min in fresh Histo-Clear, 2 min in 100% *v/v* ethanol (HPLC grade) and 1 min in 95, 70, 50 & 30% *v/v* ethanol followed by rehydration in ultrapure water (cond. < 0.067 μS/cm) for 35 s. Slides were agitated every 20 s in each solvent and blotted on tissue paper between transfers to minimise solvent carry-over. Rehydrated brain tissue sections were carefully outlined with a PAP pen for staining, in order to form a hydrophobic barrier around the periphery of tissue sections. In between staining, tissue sections were kept hydrated with ultrapure water and stored in moisture chambers, to prevent sections from drying out. Staining was staggered to allow for accurate incubation times of brain tissue sections. We have developed and optimised the fluor lumogallion as a selective stain for aluminium in cells [14] and human tissues [15]. Lumogallion (4-chloro-3-(2,4-dihydroxyphenylazo)-2-hydroxybenzene-1-sulphonic acid, TCI Europe N.V. Belgium) was prepared at *ca* 1 mM via dilution in a 50 mM PIPES (1,4-Piperazinediethanesulphonic acid) buffer, adjusted to pH 7.4 with NaOH. Lumogallion staining was performed via the addition of 200 μL of the staining solution to rehydrated brain tissue sections that were subsequently incubated at ambient temperature away from light for 45 min. Sections for autofluorescence analyses were incubated for 45 min in 200 μL 50 mM PIPES buffer only, pH 7.4. Following staining, glass slides containing tissue sections were washed six times with 200 μL aliquots of 50 mM PIPES buffer, pH 7.4, prior to rinsing for 30 s in ultrapure water. Serial sections numbered 1 and 2 for each lobe were incubated in 50 mM PIPES buffer, pH 7.4 or stained with 1 mM lumogallion in the same buffer, respectively, to ensure consistency across donor tissues. All tissue sections were subsequently mounted under glass coverslips using the aqueous mounting medium, Fluoromount™. Slides were stored horizontally for 24 h at 4 °C away from light, prior to analysis via fluorescence microscopy.

Stained and mounted human brain tissue sections were analysed via