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Hippocampal tin, aluminum and zinc in Alzheimer's disease

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The use of inductively coupled plasma source mass spectrometry (ICP-MS) for multi-element analysis has led to the observation, in two separate studies, of increased blood tin in Alzheimer's disease (AD). We have therefore applied the technique of ICP-MS to hippocampal tissues obtained post-mortem from patients with AD and from controls. There was no significant difference in tin concentrations in AD. There were increased concentrations of aluminum and silicon, and reduced concentrations of zinc and selenium. It is postulated that displacement of hippocampal zinc by heavy metals may be important in producing clinical memory disturbance. However, analysis of the CA1 region, rather than of the dentate gyrus, would have been preferable.

Keywords: aluminum, Alzheimer's, dementia, tin, zinc

Introduction

Following the first report of increased aluminum concentrations in brain in Alzheimer's disease (AD) (Crapper *et al.* 1973), there were confirmatory reports (Trapp *et al.* 1978, Candy *et al.* 1986, Ward & Mason 1987), using different techniques. Studies which did not find increased aluminum in brain in AD (McDermott *et al.* 1979, Markesbery *et al.* 1981), were probably discrepant, according to Krishnan *et al.* (1988), because of the use of large samples for atomic absorption spectrometry in one case and from phosphorus interference with the aluminum signals on neutron activation analysis (NAA) in the other. However, three studies, one with energy dispersive X-ray microanalysis (Jacobs *et al.* 1989), one with nuclear microscopy and a range of analytical techniques including particle induced X-ray emissions (Landsberg *et al.* 1992), and one with both electron probe microanalysis and analytical ion microscopy (Chafi *et al.* 1991) have failed to find any evidence of aluminum in plaques or tangles in AD.

These results would suggest that contamination of tissues by aluminosilicates may account for some of the discrepant findings. As we treat samples from patients and from controls in an identical way, there would have to be preferential contamination of the AD samples to give higher blood aluminum in AD in three separate series of patients (Van Rhijn *et al.* 1989, Corrigan *et al.* 1991b, 1993). Also, in a previous study of bulk brain tissue using neutron activation analysis we reported increased aluminum concentrations in frontal and temporal cortex, caudate nucleus, and putamen (Corrigan *et al.* 1991a). The newer technique of inductively coupled plasma mass spectrometry (ICP-MS) gives lower values for aluminum in blood and the application of this technique has resulted in a focus on tin as another possible toxic factor in AD, tin concentrations in blood in AD being elevated in two separate studies (Corrigan *et al.* 1991b, 1992). We have now applied ICP-MS to hippocampal tissues in AD to determine whether tin concentrations are altered. When using NAA we presented dry weight results but, due to different preparation, results consistent with previous studies only emerged with ICP-MS when wet weight data were considered. Wet weight data are therefore used throughout this paper. We think it important to report this further study of bulk tissue as previously these have not been carried out with ICP-MS. If it is confirmed that aluminum is not deposited in plaques and tangles, it is more likely that the excessive aluminum in bulk tissue in AD is a result of secondary deposition rather than contamination. Alternatively, it may be that aluminum

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accumulation produces changes in gene expression (Muma *et al.* 1988) which, in susceptible individuals, lead to the formation of neurofibrillary tangles. Aluminum could be involved in the pathogenesis of tangles without being deposited at their centre. Neuroblastoma cells maintained in medium containing aluminum react with an antibody to phosphorylated tau which reacts specifically with AD neurofibrillary tangles (Guy *et al.* 1991).

In patients with renal failure, high serum aluminum concentrations in life were associated with high brain aluminum post-mortem, but there was no clear association between aluminum accumulation in brain and the formation of plaques and tangles (Candy *et al.* 1992). The neuropathogenetic cascade (Hardy 1992) may not even involve aluminum, but the epidemiological reports (Flaten 1987, Martyn *et al.* 1989) would support the view that aluminum may be one factor in the development of a multi-factorial disease.

Materials and methods

Hippocampal tissue was obtained from the dentate gyrus from 12 patients with AD (79.5 ± 9.2 years; range: 65–93 years; 10 females and two males) and from 12 controls (78.5 ± 9.0 years; range: 63–89 years; four females and eight males). There was no significant difference in post-mortem delay (29.5 ± 22.2 versus 31.5 ± 13.8 h). AD diagnosis was confirmed neuropathologically. Instruments used to collect tissue and utensils used for transport were of the same material for all cases and controls, and care was taken to avoid contamination of samples during collection and transport.

Concentrations of aluminum, zinc, tin, selenium, silicon, calcium, bromine, rubidium, titanium, iron, vanadium, strontium, barium, copper, molybdenum and manganese were obtained by ICP-MS (Ward *et al.* 1991).

Results

As in our previous NAA study, there were higher aluminum and silicon concentrations in AD, and lower zinc and selenium concentrations (Table 1). The main disparate finding was for calcium which was lower in the AD tissues.

The lower concentration of iron is of interest as there is a strong negative correlation of iron with aluminum in the AD tissues (Table 2). There was a positive correlation of aluminum and tin in the AD tissues despite there being no increase in tin concentrations. The negative correlation of aluminum with selenium is more marked in the control tissues.

In the total group of 24 subjects, lithium was the only element which showed an association with age (RS 0.46, *P* 0.02). This association was more prominent in the control group (RS 0.68, *P* 0.01) than in the AD patient group (RS 0.19, *P* NS). With small numbers it is difficult to adjust for the difference in sex distribution: in the control group the four female subjects had lower levels of iron (42.75 verus 53.33, *P* 0.02), zinc (10.1 versus 14.1, *P* 0.045) and iodine (8.6 versus 13.6, *P* 0.04). Some of the differences might therefore relate to the uneven

Table 1. Concentrations of elements in hippocampal tissue obtained post-mortem from individuals with AD and from controls

	AD (n = 12)	Control (n = 12)	Mann–Whitney U-test P
Al $(\mu g\,g)^{-1}$	0.30 ± 0.08	0.12 ± 0.07	0.0001
Zn $(\mu g\,g)^{-1}$	8.13 ± 2.90	12.76 ± 3.35	0.002
Sn $(ng\,g)^{-1}$	28.80 ± 13.42	20.71 ± 11.01	NS
Se $(\mu g\,g)^{-1}$	0.06 ± 0.02	0.08 ± 0.02	0.05
Si $(\mu g\,g)^{-1}$	23.13 ± 5.84	16.53 ± 3.74	0.004
Ca $(\mu g\,g)^{-1}$	104.74 ± 23.34	143.98 ± 33.53	0.005
Br $(\mu g\,g)^{-1}$	0.51 ± 0.14	0.57 ± 0.13	NS
Rb $(\mu g\,g)^{-1}$	0.70 ± 0.15	0.69 ± 0.21	NS
Ti $(ng\,g)^{-1}$	3.71 ± 1.42	4.91 ± 1.64	0.04
Fe $(\mu g\,g)^{-1}$	39.85 ± 11.92	49.80 ± 7.82	0.05
V $(ng\,g)^{-1}$	0.29 ± 0.12	0.33 ± 0.08	NS
Sr $(ng\,g)^{-1}$	6.95 ± 1.12	5.92 ± 1.80	NS
Ba $(ng\,g)^{-1}$	2.83 ± 0.88	3.48 ± 0.92	<0.1
Cu $(\mu g\,g)^{-1}$	4.46 ± 2.19	5.11 ± 1.14	NS
Mo $(ng\,g)^{-1}$	6.74 ± 1.10	6.57 ± 1.44	NS
Mn $(ng\,g)^{-1}$	1.05 ± 0.21	1.09 ± 0.37	NS

Table 2. Correlations of aluminum and tin with the other elements (where one is significant at P 0.005) in hippocampal tissue for 12 patients with AD, 12 controls and in the two groups considered together

	Total ($n = 24$)	AD ($n = 12$)	Control ($n = 12$)
Al/Zn	−0.89, 0.0000001	−0.92, 0.00002	−0.64, 0.03
Al/Sn	0.68, 0.0002	0.84, 0.0006	0.62, 0.03
Al/Se	−0.73, 0.00005	−0.44, NS	−0.85, 0.0005
Al/Si	0.75, 0.00002	0.39, NS	0.62, 0.03
Al/Ca	−0.61, 0.002	0.34, NS	−0.65, 0.02
Al/Fe	−0.72, 0.00006	−0.78, 0.003	−0.55, < 0.1
Sn/Fe	−0.66, 0.0005	−0.72, 0.008	−0.50, < 0.1
Sn/Mo	0.57, 0.004	0.51, < 0.1	0.37, NS

Spearman's correlation coefficients with two-tailed probability are used.

sex distribution but we would note that in the study of Ward & Mason (1987) there were more males than females in the AD groups yet lower concentrations of zinc were observed in AD.

Discussion

Crawford & Connor (1972) suggested that zinc was involved in the maturation and function of the mossy fibers, and zinc deficiency was reported to alter the function of the mossy fibers (Hesse 1979). Subsequently it was demonstrated that electrical stimulation of hippocampal slices facilitated uptake and release of zinc from the mossy fibers (Howell *et al.* 1984). While zinc may be associated with the cholinergic system through its association with a nerve growth factor (Stewart *et al.* 1984), most of the recent work has focused on other neurotransmitters. In cortical neurons, zinc may reduce NMDA-mediated excitation while having the opposite effect on quisqualate-mediated excitation and no effect on kainate excitation (Peters *et al.* 1987). In cultured hippocampal neurons, zinc antagonizes NMDA-mediated and GABA-mediated responses (Westbrook & Mayer 1987), and a physiological role at the presynaptic and postsynaptic GABA B receptors has been proposed (Xie & Smart 1991). Zinc has been suggested as an important component of the long-term potentiation mediated by the NMDA receptors in the CA1 region of the hippocampus (Weiss *et al.* 1989). Zinc reduces the mean open times of single NMDA channels, perhaps by allosteric inhibition (Legendre & Westbrook 1990) and probably by inhibiting glycine binding (Yeh *et al.* 1990), supporting the possibility that zinc modifies the glycine binding site in such a way that glycine and zinc modulate NMDA responses (Forsythe *et al.* 1988).

There seems little doubt that decreased zinc concentrations in brain should have functional consequences. Unfortunately, however, we do not know how much of the zinc is bound to proteins which would modulate its availability (Ebadi & Hama 1986). It is also important to have more detailed dissection of the hippocampus in AD. Most of the zinc is in the CA3 region where it also appears to be most active (Anikstejn *et al.* 1987). The glutamate receptors in the CA3 region, however, are not of the NMDA type, and the process of long-term potentiation thought to be involved in memory formation requires the NMDA channels of the CA1 region. Rats on a low zinc diet accumulate more aluminum in the hippocampal regions of their brain (Wenk & Stemmer 1983). Constantinidis (1991) has proposed that disruption of the blood–brain barrier by amyloid deposition permits the entry of toxic metals to the cerebral cortex but that it is resultant displacement of zinc and loss of activity of zinc-dependent enzymes, which leads to the formation of neurofibrillary tangles, neuronal death and clinically evident dementia. Wenstrup *et al.* (1990) reported increased mercury concentrations in brain tissue in AD and an increased Hg:Zn ratio. It may be the displacement of hippocampal zinc, whether by tin, aluminium, mercury or lead (Petit & Alfano 1983), which results in memory disturbance (Van Rhijn *et al.* 1989).

The reduction in selenium concentrations in hippocampus in AD confirms the previous findings of Ward & Mason (1987) and Corrigan *et al.* (1991a) and may represent altered antioxidant protection of the polyunsaturated fatty acids of the brain tissue. Treatment directed towards this may be beneficial in preventing or slowing the deterioration in cognitive function which occurs in AD (Van Rhijn *et al.* 1990).

Ferrier *et al.* (1990), reviewing findings on calcium

in dementia discuss reduced activity of calcium-dependent enzymes in brain in AD, reduced calcium-binding proteins in brain in AD, reduced calcium uptake by skin fibroblasts and by lymphocytes in AD, and reduced plasma calcium with reduced platelet calcium uptake into platelets in Down's Syndrome. Garruto *et al.* (1985) reported increased intraneuronal deposition of both calcium and aluminum in amyotrophic lateral sclerosis of Guam. However, the reduced gastrointestinal absorption of calcium in dementia of Alzheimer's type (Ferrier *et al.* 1990) would suggest that the low brain calcium demonstrated in AD with ICP-MS is more accurate than the high brain calcium we found using NAA.

While there was no difference in tin concentrations in the hippocampus in AD, it remains possible that organo-tin compounds are important neurotoxins in AD but that they are not deposited in the brain after they have damaged neurons. Lack of information about the particular form of the tin and its binding is a considerable handicap in the interpretation of these results. Trimethyl tin is the species used in an animal model of dementia (Earley *et al.* 1989).

The negative correlation of aluminum with iron, which was more marked in the Alzheimer's group than in the control group, and the lower concentration of iron in the AD tissues may support the proposal of Cannata *et al.* (1991) that brain aluminum is significantly increased in iron-depleted animals. The aluminum–transferrin complex is the physiologically relevant form of aluminum with respect to cellular uptake (McGregor *et al.* 1991) and aluminum, when bound to transferrin, may inhibit iron uptake partly by down-regulating transferrin receptor expression and partly by interfering with the intracellular release of iron from transferrin (McGregor *et al.* 1990).

Increased aluminum concentrations may give reduced iron availability or deposition in brain. Conversely, however, low iron, like low zinc, concentrations may be relevant in promoting the increased aluminum accumulation.

The concentrations of aluminum reported in this study are lower even than those of Trapp *et al.* (1978). The other studies of post-mortem brain tissue in AD using atomic absorption spectrometry and NAA have reported higher aluminum concentrations whether or not they found a difference in AD. Van Rhijn *et al.* (1989) have discussed the use of ICP-MS rather than NAA for analysis of aluminum in serum. They considered that ICP-MS provided lower values but more accurate ones because of a lack of interference from other elements such as phosphorous. We think that there is a strong case for arguing that the analysis of bulk brain tissue by ICP-MS is now giving the most accurate results for aluminum concentrations. It is unfortunate that other studies have tended to focus only on aluminum: it is to be hoped that ICP-MS will generate a new series of studies of brain tissue in AD and other diseases.

These results give further support to the idea that it may be possible to alter the availability of aluminum and therefore to prevent some of its neurotoxicity by maintaining optimal concentrations of other elements. In the absence of any effective treatment for AD, prophylaxis or, at the least, slowing of deterioration are goals worth pursuing.

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