Calcium, Metals and Nervous System in the Elderly

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Biologic processes in the aging of the brain and its pathologic alterations have been precisely investigated. Dementia, for example, is a most common symptom of the age-related central nervous system (CNS) function, and is the main symptom found in senile dementia and Alzheimer disease (AD). Although AD has long been considered as a case with an onset before age 65, recent clinicopathological and biochemical studies indicated that AD and senile dementia are a single process, with a general agreement between them.

Morphological changes found in these are not specific and include decreased brain weight, neuronal loss, degenerative changes in the dendrite of pyramidal cells, increase of lipofuscin *etc.* Some characteristics of AD, although being less marked in the elderly brain, are Alzheimer neurofibrillary tangle (NFT), senile plaque, granulovacuolar degeneration and Hirano body, which are also occasionally observed in amyotrophic lateral sclerosis (ALS) and Parkinson disease (PD).

Since the incidence of these degenerative diseases of the CNS tissue increases with age, aging factor(s) may be closely related to the degenerative process. Through the serial studies done on CNS degenerative diseases, the role of metals has been suggested as a pathogenetic participation of the degenerative process.

In this paper, the comparative study of metal behavior between these conditions is reported.

Methods

Metal content in the CNS tissue was determined by the elemental analytical methods, including neutron activation analysis (NAA), X-ray microanalysis (XMA) and particle induced X-ray emission (PIXE) methods, in cases with degenerative CNS diseases and controls. For the structural analysis, X-ray powder diffraction (XPD) and infra-

red absorption spectrum (IRA) methods were used.

Content of various metals, which include calcium (Ca), manganese (Mn), aluminum (Al), copper (Cu) and phosphorus (P) was mainly analyzed by NAA with or without simultaneous and non-destructive methods. Morphological distribution of these metals in the CNS tissue was investigated by XMA. For the screening search of metals to be detected, PIXE was used. In the identification of the chemical structure of these metals, XPD and IRA were adopted.

Results

1) *Ca content by NAA.* The average content of Ca was 252 ± 86 ppm (ranging 407 to 105 ppm) in controls, ranging in age from 38 to 54, 381 ± 176 ppm in controls 80 years of age (ranging 723 to 87 ppm), and 405 ± 105 ppm (ranging 729 to 236 ppm) in ALS cases, ranging in age from 28 to 40 years. The Ca content in the aged control is much closer to that of ALS cases, although the range of its content varies remarkably from 87 to 723 ppm in different localizations (Table 1).

2) *Simultaneous determination of Ca, Mn and Al by NAA.* In aged control, not only Ca but also Mn and Al contents are higher than normal control, and close to those of ALS cases.

	C1	C2	C3
Ca	231 ± 77	295 ± 190	222 ± 47
Mn	1.4 ± 0.5	1.4 ± 0.4	1.7 ± 0.2
Al	11.0 ± 3.3	23.8 ± 4.9	16.4 ± 9.1

	CA	ALS
Ca	466 ± 179	434 ± 124 (ppm)
Mn	2.4 ± 0.5	1.3 ± 0.2
Al	33.2 ± 16.2	33.7 ± 18.5

In any of the ALS cases, there is the positive correlation in the content between Ca and Al, or between Ca and Mn. No correlation, however,

Table 1. Calcium content of central nervous system tissue in amyotrophic lateral sclerosis and in normal and aged control cases.

	Amyotrophic lateral sclerosis (ppm)	Normal control (ppm)	Aged control (ppm)
Precentral	371	216	518
Postcentral	236	236	410
Frontal	452	406	—
Parietal	416	407	377
Insula	379	249	—
Temporal	412	395	275
Occipital	387	343	447
Hippocampus	506	233	—
Thalamus	373	176	354
Caudate nucleus	405	189	628
Capsula interna	342	152	723
Globus pallidus	366	201	172
Putamen	289	191	87
Crus cerebri	501	244	165
Substantia nigra	352	105	342
Red nucleus	469	200	284
Pons	319	288	—
Medulla	538	247	284
Spinal cord	729	314	612
Cerebellum, gray	379	332	422
Cerebellum, white	293	170	—
Mean	405	252	381
Standard deviation	105	86	176

was seen in any control case between the ages of 28 to 40. But in the aged control group of 80 years, a positive correlation between Ca and Al was indicated.

3) *Al content in AD case by NAA.* Average content of Al in the CNS tissue in AD cases showed 33.5 ± 6.3 ppm, while controls 18.8 ± 0.9 ppm. Average Ca content in the CNS tissue of AD cases was 837 ± 420 ppm and in controls 131 ± 47 ppm.

There is no significant difference in Al content in the CNS tissue between AD and aged controls, although Ca content is much higher in the AD cases.

	AD1	AD2	AD3
Ca	787 ± 212	$1,350 \pm 439$	$1,013 \pm 202$
Al	24.8 ± 4.5	42.5 ± 25.6	32.2 ± 8.7

	AD4	C1	C2
Ca	197 ± 43	124 ± 37	138 ± 34 (ppm)
Al	34.2 ± 13.2	19.6 ± 10.2	17.7 ± 8.6

4) *Metal analysis by PIXE.* Trace elements detected by alpha particle excited X-ray fluorescence analysis of the cervical spinal cord from cases with ALS and controls were Al, Si, P, S, Cl, K, Ca, Ti, V, Mn and Fe. The content of Al, Si, P, Ca, Ti, V, Mn and Fe was higher in cases with ALS than in controls.

The aged, however, showed a high Ca content, close to that of the cases with ALS, but other metals, usually found in high concentrations in cases with ALS, were not detected, as seen in Fig. 1.

5) *Ca-Hydroxyapatite (Ca-Hap) by XPD and IRA.* Metal studies conducted by XPD (Fig. 2)

Fig. 1. He⁺ elicited X-ray analysis (spinal cord).

Fig. 2. Comparison of X-ray diffraction patterns of ashed cervical cord specimens of ALS and control cases, and calcium-hydroxyapatite.

and IRA (Fig. 3) suggest that Ca substances found in ALS cases exist as a form of Ca-Hap, substituted by other metallic ions such as Al, Mn, etc.

Ca-Hap content in the brain and spinal cord in the ALS case (63 years, female) showed 317 ppm in the brain and 685 ppm in the spinal cord, compared with 33 ppm in the brain and 102 ppm in the spinal cord of the control case (79 years, female). Content of Ca-Hap in the AD case also

Fig. 3. Comparison of infra-red absorption spectra of ashed cervical cord specimens of ALS and control cases, and calcium-hydroxyapatite.

showed less than 100 ppm in the brain.

Discussion

Apart from the neuropathological changes found in the aging process, metal behavior in the CNS tissue of the aged has not been well demonstrated.

Metals, especially toxic elements such as mercury, cadmium, lead, etc., usually accumulate in the body with age. This depositing tendency is also observed in the CNS tissue, which manifests various clinicopathological changes. There is, however, a wide variation in the content of different metal elements; for example, there is less Cu and Zn content in the CNS tissue, but more content of Fe and Mg with age.

In our serial studies of metal analysis, the content of Ca, Mn and Al was higher in the aged CNS tissue than in normal controls. Ca content in the normal CNS tissue is usually 200 to 250 ppm, when determined by NAA, and increases to over 400 ppm in the aged control group, as 80 years old. In the ALS case, Ca content corresponds to that of the aged, with a positive correlation between Ca and Mn or between Ca and Al. There

was, however, no correlation in any normal control case. This was found only in the aged.

Increased Al content in the brain tissue in certain conditions have been reported by several investigators; in AD by Crapper *et al.* (1), in dialysis encephalopathy by Alfrey *et al.* (2) and in the senile brain by McDermott *et al.* (3). We also reported increased content of Al with a positive correlation to Ca in the brain tissue of degenerative CNS disease cases, including ALS, Parkinson disease, Guam PD and AD (4). In the AD case, there was a high content of Al in the CNS tissue, with a positive correlation to Ca. This increased Al is diffusely distributed in gray and white matter, and basal ganglia.

The neurotoxicity of Al in animals has been clearly demonstrated; Al-induced neurofibrillary degeneration (NFD) was found using intracerebral (Klatzo *et al.* (5)), intraventricular (Yates *et al.* (6)) and subcutaneous (DeBoni *et al.* (7)) injection of Al salts, although the toxic mechanism is still obscure.

Al accumulation in the nuclear region and perikaryal cytoplasm of NFT-bearing hippocampal neurons was reported in ALS and PD cases in Guam (Perl *et al.* (8)), suggesting the association between intraneuronal Al and NFT formation.

A possible relationship between Al and NFD formation is indicated, but NFD, found as the component of the human NFT, is the paired helical filaments (PHFs), which are not the same as Al-induced NFD (Terry *et al.* (9)). Interestingly, there is a possible environmental reflection by Al exposure in ALS/PD changes with NFT formation in Guam cases, in which a high content of Al in the CNS tissue was found. A striking positive linear correlation between the number of NFT-bearing hippocampal neurons and brain Al content, was observed in those Guam PD cases. These findings, together with the formation of Al-induced NFT in susceptible animals, stimulate further interest (10).

The question of how these metals deposit is also interesting. Simultaneous determination by PIXE detected high content of Al, Si, P, Ca, Fe, etc. in the cervical spinal cord and frontal lobe samples of ALS cases. Coexistence of Ca and other metals, with their positive correlation in tissues and Ca/P atomic ratio of 1.6 (11), strongly

suggests the formation of a certain chemical compound of calcified substance, *i.e.*, of Ca-Hap, because Ca/P atomic ratio of Ca-Hap is 1.67. Related to Hap formation, Mg in Mg ATP, which inhibits the apatite production, may be substituted by Al, developing into Al-ATP and participating in apatite formation as an accelerator.

It is being confirmed by the structural analyses that the calcified substances in the CNS tissue of ALS cases are Ca-Hap, substituted by Al, Mn and other metallic ions. The content of Ca-Hap in brain samples of ALS case showed over 100 ppm, but only 3 ppm in the control. In the spinal cord, ALS cases showed over 200 ppm, but control was 500 ppm.

It is, therefore, assumed that, although the aged shows a high Ca content in the CNS tissue, this deposit of Ca differs from those in ALS cases which may exist as a form of Ca-Hap.

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