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Histochemical and X-ray microanalytical localization of aluminum in amyotrophic lateral sclerosis and parkinsonism-dementia of Guam

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Summary. Histochemical staining for aluminum, using Solochrome azurine or Morin, provided a rapid, simple and reliable means of identifying areas and structures of the brain of interest for closer scrutiny by X-ray microanalysis in patients with amyotrophic lateral sclerosis and parkinsonism-dementia of Guam. Neuronal perikarya, dendritic processes, and the walls of some cerebral vessels were aluminum positive by Solochrome azurine staining. In some cases, the deposition of aluminum was rather diffuse, particularly in the white matter. Fluorescent localization of aluminum using Morin was equally sensitive and specific, but provided less morphological detail than Solochrome azurine. Confirmation of histochemical detection of aluminum was achieved by examining adjacent tissue sections using wavelength-dispersive spectrometry coupled to a computer-controlled electron beam X-ray microprobe. Although the minimum detectable limits for aluminum by these histochemical procedures are unknown, the lower detection limit of our X-ray microanalytical technique is 10–100 ppm dry weight. Solochrome and Morin staining, as verified by X-ray microanalysis, afford a useful and reliable means of surveying multiple anatomical regions for aluminum deposition in naturally occurring and experimentally induced neurodegenerative disorders.

Key words: Solochrome – Aluminum – X-ray microanalysis – Guam

Aluminum toxicity has been implicated in infants, children and adults receiving total parenteral nutrition [7, 8, 16] and in various neurodegenerative diseases [1–5, 12, 13, 15], including Alzheimer disease, amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia (PD) of Guam and dialysis dementia. Deposits of aluminum and other trace and essential elements have been detected in neural and nonneural tissues, such as blood and bone. Although humans are exposed daily to large quantities of aluminum, only trace amounts of this environmentally abundant element are normally detected in healthy tissues. In healthy individuals most of the ingested aluminum is excreted in the feces, and the small amount that is absorbed is probably conjugated with transferrin and excreted in the urine [15].

Previous studies, using energy-dispersive spectrometry [13], secondary ion mass spectrometry [9], laser microprobe mass analysis [14] and wavelength-dispersive spectrometry coupled to a computer-controlled electron beam X-ray microprobe [4, 5], have established the co-deposition of aluminum with calcium and/or silicon in the perikarya and dendritic processes of neurofibrillary tangle (NFT)-bearing hippocampal neurons in Guamanian patients with ALS and PD. While these highly sophisticated, novel techniques afford a high degree of sensitivity and specificity, the difficulty of tissue preparation, the lengthy processing time required to generate data, the inability to survey large areas of any given tissue at one time, and the equipment "down-time" make them unsuitable as survey techniques. We, therefore, investigated the suitability of well-established but seldom used histochemical staining for the localization of aluminum prior to elemental imaging.

Materials and methods

Cryostat-cut, 10-μm-thick serial sections of formalin-fixed hippocampus and spinal cord tissues from three Guamanian patients with ALS (ages 55, 63, 71 years), from five with PD (ages 53, 57, 64, 66, 74 years), and from five neurologically and neuropathologically normal Guamanian (ages 43, 48, 52 years) and Caucasian (ages 18 and 73 years) controls were stained for aluminum using Solochrome azurine and/or Morin. Solochrome

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Table 1. Histochemical localization and X-ray microanalytical imaging of aluminum in hippocampus of patients with amyotrophic lateral sclerosis and parkinsonism-dementia of Guam

Disease category	Age, sex	Duration of illness in years	NFT by Bodian silver stain	Congo red	Solochrome azurine and/or Morin	X-ray microprobe analysis
Amyotrophic lateral sclerosis						
1	63 M	25	+	+	+	+
2[a]	55 M	2	+	+	+	+
3	71 M	15	+	−	−	−
Parkinsonism-dementia						
4[a]	64 M	7	+	+	+	+
5[a]	66 F	4	+	+	+	+
6	57 F	9	+	+	−	−
7[b]	53 F	9	+	+	+	+
8	74 F	6	+	+	−	
Control cases						
9	52 M	N/A	−	−	−	−
10	48 M	N/A	−	−	−	−
11	43 M	N/A	−	−	−	−
12[c]	73 M	N/A	−	−	−	−
13	18 M	N/A	−	−	−	−

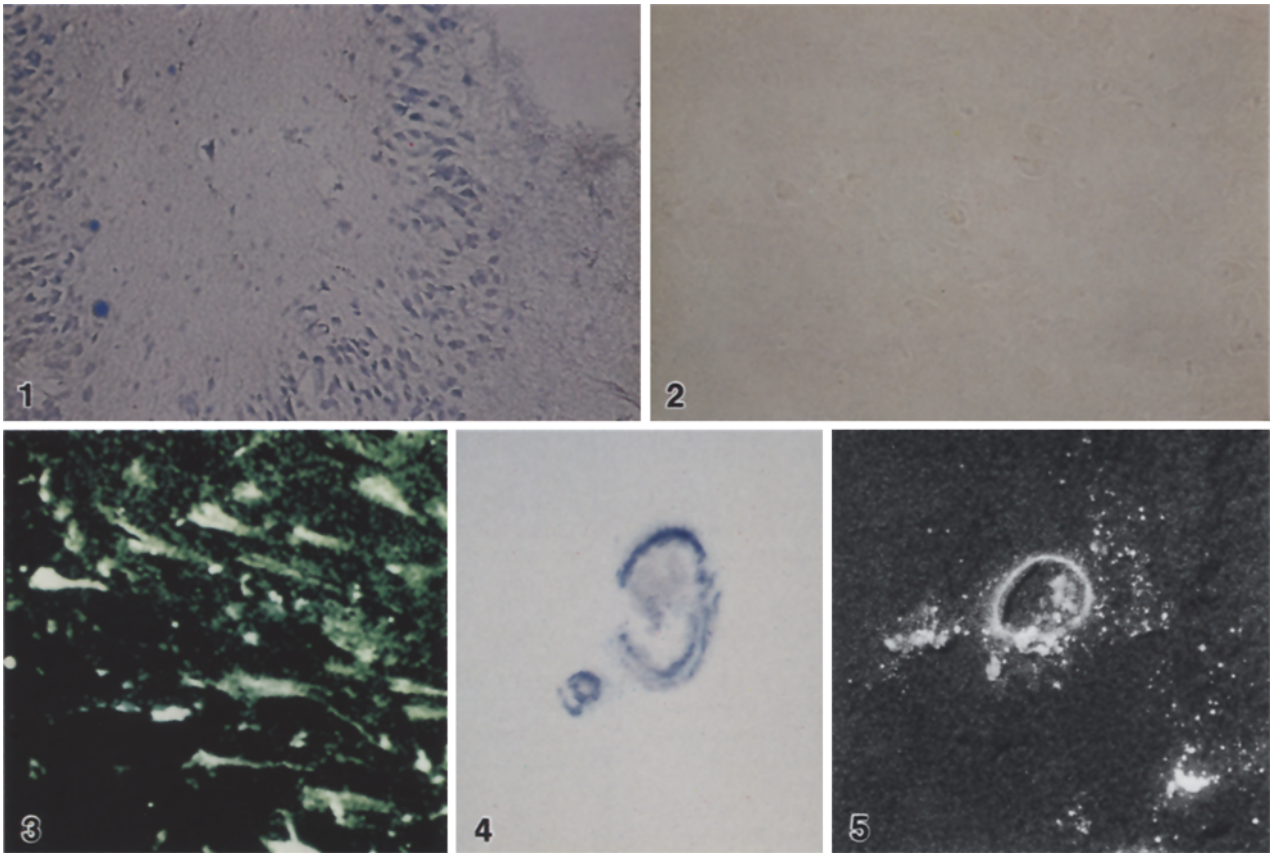
[a] Diffuse aluminum deposition was observed in the white matter of a section of spinal cord
[b] Great variations in aluminum deposition were observed in different sections of hippocampus. Aluminum was also detected in the wall of cerebral vessels and in one neuron in serial sections of the frontal cortex
[c] Intracytoplasmic red-green birefringent material was observed in two neurons after Congo red staining of serial sections. No aluminum deposition was detected
N/A, not applicable; NFT, neurofibrillary tangles

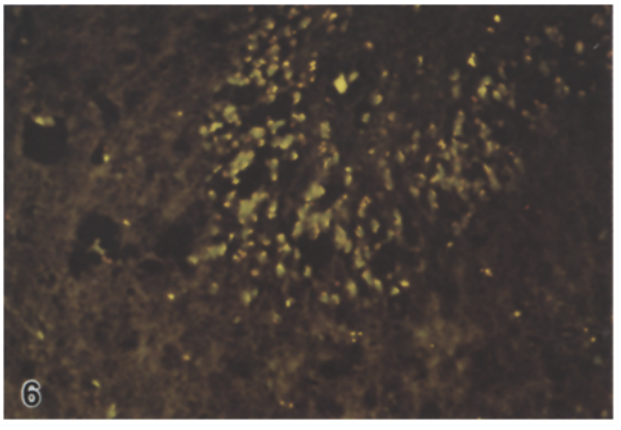
azurine (Gurr, BDH Chemicals Ltd., Poole, England) was used as a 0.2% aqueous solution [10]. Tissue sections on gelatinized slides were stained for 30–40 min, then washed, dehydrated, cleared in xylene, and mounted in Permount. Morin, a fluorescent stain, was used as a 0.2% solution in 85% ethanol containing 0.5% acetic acid. Sections for Morin staining were pretreated for 10 min in 1% hydrochloric acid, washed with distilled water, then immersed for 10 min in Morin solution [11]. The slides were then washed, dehydrated, cleared, and mounted using a low-fluorescent medium (Depex Mounting Medium, Atomergic Chemicals Corp, Plainview, New York). Deionized distilled water was used to prepare all fixatives, buffers, and staining solutions. Only teflon or polyethylene containers and stainless steel instruments were used to guard against elemental contamination. Adjacent, unstained 20-μm-thick sections were mounted onto spectroscopically pure carbon discs for X-ray microanalysis of aluminum to verify the sensitivity and specificity of the histochemical stains (Fig. 1) [4]. An additional adjacent section was stained for amyloid using Congo red.

Results and discussion

Aluminum was detected by histochemical staining in selected areas within the hippocampus, spinal cord and frontal cortex of patients with ALS and PD (Table 1). With Solochrome azurine, neurons of ALS and PD patients stained differently in different regions, while no staining occurred in control patients without NFT formation (Figs. 1 and 2). In some cases diffuse staining was observed in the cytoplasm, while in others rod-shaped structures were evident. Nucleoli were sometimes observed as a dark-blue dot as described more than 20 years ago by Klatzo et al. [6]. Variable degrees of staining were also observed in the neuropile and white matter. The intraneuronal staining and diffuse extracellular staining observed in some tissues from patients with ALS and PD was confirmed by X-ray microanalysis, ruling out nonspecific background staining in the latter (Fig. 3). However, we cannot exclude that some extracellular diffusion of aluminum may have occurred during fixation. In many cases, glial cell nuclei were stained. Endothelial cells and the walls of some cerebral vessels were also stained with Solochrome azurine and confirmed by X-ray microanalysis (Figs. 4 and 5). Morin fluorescent staining was confirmatory, but less morphological information was obtained in some cases (Fig. 6). However, good histochemical localization of aluminum using Morin has been reported by Wen and Wisniewski in rabbits with experimental aluminum encephalopathy [17].

Congo red staining for amyloid on adjacent sections showed variable amounts of congophilic, green birefringent material under polarized light in the cytoplasm of NFT-bearing neurons, which were also observed in nonadjacent sections stained by Bodian silver impregnation. NFT-bearing neurons, identified by Congo red staining, were found mainly in aluminum-positive areas, but in other cases there was a poorer





correlation between aluminum deposition and NFT formation.

The overall usefulness of Solochrome azurine and Morin as a histochemical survey tool for elemental aluminum prior to the use of high technology elemental imaging instrumentation and techniques is considerable. Although it is impossible to determine the lowest detectable limit for aluminum using Solochrome azurine or Morin, the lower detection limits for aluminum using X-ray microanalysis and wavelength-dispersive spectrometry is between 10 and 100 ppm dry weight tissue. We recommend Solochrome azurine for routine histochemical use in dis-

Fig. 1. Solochrome azurine staining for aluminum in a section of the hippocampus of a patient with parkinsonism-dementia of Guam. The positive blue staining indicates the intraneuronal presence of aluminum and its diffuse distribution in the white matter. × 84

Fig. 2. Solochrome azurine staining for aluminum in a section of the hippocampus of a neurologically normal Guamanian control patient free of neurofibrillary tangle formation. Positive blue staining does not occur either intra- or extracellularly. × 175

Fig. 3. Elemental image of aluminum using wavelength dispersive spectrometry and computer-controlled electron beam X-ray microanalysis. The aluminum image is of a formalin-fixed cryostat-cut section of the hippocampus of a patient with parkinsonism-dementia of Guam. Semiquantitative estimates for aluminum are 500 ppm dry weight for the brightest neurons in the field. Some aluminum is also found extracellularly. Details of the procedure can be found in [4]. The field size is 200 μm × 200 μm

Fig. 4. Solochrome azurine staining of the wall of two cerebral blood vessels in a patient with parkinsonism-dementia of Guam. × 42

Fig. 5. Elemental image of aluminum of a nearby cerebral blood vessel to the ones stained with Solochrome azurine in Fig. 4. The image was generated using wavelength-dispersive spectrometry and computer-controlled electron beam X-ray microanalysis described in Fig. 3. The field size is 200 μm × 200 μm

Fig. 6. Green fluorescent Morin staining of hippocampal neurons in a patient with parkinsonism-dementia of Guam. Staining is confined mostly to the neurons in this patient without significant extracellular fluorescence. × 154

orders in which aluminum toxicity is suspected. Further confirmation by X-ray microanalysis or other elemental detection systems using larger series of cases and controls will provide more information on the correlation between these techniques and help to establish the lower limit of sensitivity of Solochrome and Morin.

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