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Research Article

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Intranuclear Inclusions in Cerebellar Golgi Cells of Patients with Cerebellar Tumors

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Abstract

Fibrillar Intranuclear inclusions are described in cerebellar Golgi cells of three patients with cerebellar tumors. Cortical biopsies taken during neurosurgical treatment were immediately processed for transmission electron microscopy. The intranuclear inclusion appears as a straight rodlet up to 3 um in length and from 0.4 um in width, immersed in the nucleoplasm and without topographic relationship with the nucleolus. This rodletshows a periodic or crystalloid structure formed by dense bands 9.2 nm thick, separated by clear spaces of 5.4 nm in width (Fig. 2), and in some regions displays a lattice or crystalloid appearance produced by oblique superposition of the dense bands. The findings are discussed in relationship with intranuclear inclusion found in viral and central neurodegeneraive diseases.

Keywords: Golgi cells, Intranuclear Inclusions, Viral Diseases, Brain Tumors, Electron Microscopy.

Introduction

Intranuclear filamentous and cristalline inclusión have been described in a large variety of nerve cells in normal and pathological conditions [1-16]. The significance of these substructures remains to be elucidated. In the present study we describe the presence of intranuclear inclusions in three patients with astrocytoma, angioma and meningioma, We have examined in cortical biopsies these substructures by means of transmission electron microscopy. The findings are discussed in relationship with intranuclear inclusion previously reported in viral and central neurodegenerative diseases.

Material and Methods

Cortical biopsies of three patients with clinical diagnosisof astrocytoma, angioma and meningioma were examined with transmission electron microscope. Table 1 contains the clinical data and lists the cerebellar regions from which each cerebellar biopsy was taken during neurosurgical treatment. The neurosurgical study was performed and the cerebellar biopsies were taken according to the basic principles of Helsinki declaration.

Two to five mm thick cerebellar biopsies were immediately fixed in the surgical room in 4% glutaraldehyde - 0.1 M phosphate or cacodylate buffer, pH 7.4, at 4 °C. After 2 h glutaraldehyde-fixation period, the cortical biopsies were trimmed into approximately 1 mm fragments and observed under a stereoscopic microscope to check the quality of fixation of the sample, glutaraldehyde diffusion rate and the brownish coloration of the surface and deeper cortical regions, indicative of good glutaraldehyde fixation by immersion technique. The cortical slabs were also per-formed to assure optimal diffusion rate of glutaraldehyde and osmium tetroxide fixatives. Immersion in fresh glutaraldehyde solution of 1 mm slices was done for 2 h. Secondary fixation in 1% osmium tetroxide - 0.1 M phosphate buffer, pH 7.4, was carried out for 1-2 h at 4°C. Black staining of the cortical slices was also observed under a stereoscopic microscope to check osmium tetroxide diffusion rate and quality of secondary fixation. Slices were then rinsed for 5 to 10 min in phosphate or cacodylate buffer of similar composition to that used in the fixative solution, dehydrated in increasing concentrations of ethanol, and embedded in Araldite or Epon. For proper orientation during the electron microscope study and observation of cerebellar layers, approximately 0.1 to 1 um thick sections were stained with toluidine blue and examined with a Zeiss pho-to microscope. Ultrathin sections, obtained with Porter-Blum and LKB ultramicrotomes were stained with uranylacetate and lead citrate and observed in a JEOL 100B transmission electron microscope (TEM) at magnifications ranging from 30,000 to 60,000X. For each case, approximately 100 electron micrographs were studied.

Table I: Neurosurgical Study

Sample identification	Age and sex	Clinical Data	Diagnosis
1.MRR	45 y, F	Tremor in upper and lower extremities, incoherent speech, difficulty in walking, headache, visual hallucinations, cloudy sensorium and stupor	Cerebellar meningioma Right Cerebellar hemisphere
2. ARM	30y, M	Headache, dismmetry, gait disturbance, tremor in lower extremities	Cerebellar astrocytoma Left cerebellar hemisphere
3.MIJ	50y,F	Headache, dysmetria, tremor.	Cerebellar angioma. Right Cerebella hemisphere

Results

In longitudinal sections (Figure 1), the intranuclear inclusion appears as a straight rodlet up to 3 um in length and from 0.4 um in width, immersed in the nucleoplasm and without topographic relationship with the nucleolus. This rodlet shows a periodic or crystalloid structure formed by dense bands 9.2 nm thick, separated by clear spaces of 5.4 nm in width (Figure 2), and in some regions displays a lattice or crystalloid appearance produced by oblique superposition of the dense bands.

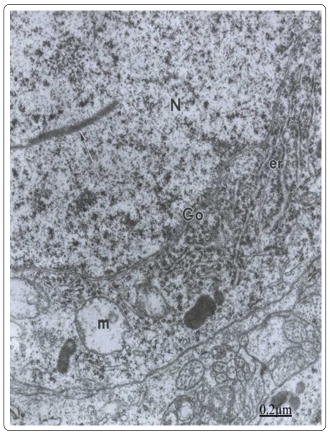

Figure 1: Human cerebellar granular layer. Edematous human cerebellar Golgi cell (Go) showing the intranuclear (N) inclusion (arrow). Note the notably swollen mitochondria (m) and the well developed and distended endoplasmic reticulum (er). X 24,000.

At higher magnification the fibrillar inclusion show a cristaline-like arrangement 8 Figure 2).

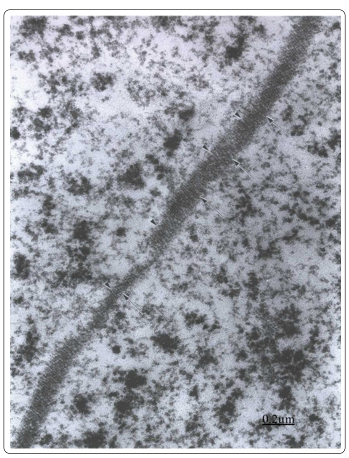

Figure 2: Higher magnification of the intranuclear inclusion illustrated in the previous figure showing the periodical substructure formed by dark dense lines (arrows) 9.2 nm thick in parallel arrangement, separated by clear intervals (arrowheads) of 5.4 nm. X 60,000.

Discussion

These inclusions do not show structural similarity with other types of intranuclear inclusions previously described in nerve cells, such as bundles of fine filaments, fibrillar lattice, microtubular bundles and microtubular crystalloids [1-7].

Dhib-Jalbut and Liwnicz, demonstrated intranuclear (Cowdry type A) and intracytoplasmic inclusions [17]. Immunocytochemical stain with the complement-fixing measles antibody was positive for intranuclear and intracytoplasmic inclusions in a case of subacute sclerosing panencephalitis. Tanaka et al, reported numerous viral intranuclear inclusions, in the neurons and glial cells in Adult-onset subacute sclerosing panencephalitis [18]. These inclusions were made of tubular nucleocapsids of paramyxovirus.

The functional significance of thisintranuclearrodlet is unknown. Al-Maghrabiet al. reported intranuclear inclusions, which expressed ubiquitin in Alzheimer disease and adult-onset dementia [14]. Tabriziet al. reported intranuclear inclusions in the spiny neurons of caudate nucleus and related them with excitotoxicity and damage of mitochondrial respiratory chain [19]. Grunewald and Beal, described also ubiquitin-positive neuronal intranuclear inclusions in Huntington's disease [12]. Ho et al., have associated the intranuclear inclusions with excitotoxicity, oxidative stress, impaired energy metabolism, abnormal protein interactions and apoptosis. Our study support Ho et al., findings, since the intranuclear inclusions were observed in edematous human cerebellar cortex associated to intracranial tumors, where the above mentioned conditions indicated by Ho et al., are present [20]. In addition, the observation of swollen mitochondria, as illustrated in Fig. 1, suggests that we are dealing mainly with impaired energy metabolism.

According to the immunocytochemical study of Woulfe and Muñoz, they are composed, as least in part, of tubulin [16]. Fujigasakiet al., related the intranuclear inclusions with stressful conditions on neuronal cells, such as aging and polyglutamine neurotoxicity [21]. Schmidt et al., Becker et al., Lieberman et al. and Evert et al. [15] related the intranuclear inclusions with abnormal protein aggregates in neurodegenerative polyglutamine Quan et al found in methamphetamine abuse patienintranuclear inclusion-type Ubiquitin (Ub)-positivity at the level of nigral neurons, and the granular 'dot-like' Ub-immunoreactivity area in the crus cerebri (cortico-spinal tracts) [11,13,15,22,23].

Neuronal intranuclear inclusion disease (NIID), a slowly progressive neurodegenerative disease is characterized by eosinophilic hyaline intranuclear inclusions in the central and peripheral nervous system, and also in the visceral organs et al. [24].

Cha et al, described intranuclear filaments, as rod- or needle-like shapes in a 16-year-old boy16-year-old boywith long-term epilepsy-associated tumor in the amygdale [25]. Ultrastructural analysis revealed thin filamentous intranuclear structures in tumor cells. The clinicopathological implications of the intranuclear inclusions remain unknown.

Josephs et al. reported neuronal intranuclear inclusions and fine neurites of the CA1 region of the hippocampus in patients with primary age-related tauopathy and hippocampal sclerosis [26].

Huntington's disease (HD) is a monogenic neurodegenerative disorder caused by a trinucleotide CAG repeat expansion in the huntingtin gene resulting in the formation of intranuclear inclusions of mutated huntingtin. The accumulation of mutated huntingtin leads to loss of GABAergic medium spiny neurons (MSNs); subsequently resulting in the development of chorea, cognitive dysfunction and psychiatric symptoms.

Intranuclear inclusions and cytoplasmic and perinuclear inclusions were predominantly found in cortices (frontal, temporal and motor), spinal cord and hippocampal dentate gyrus of patients with frontotemporal dementia and amyotrophic lateral sclerosis [27,28].

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