degeneration of the transverse myelopathy, or the subacute myelo-optico-neuropathy (SMON), an intoxication of clioquinol (hydoxyquinoline), which primarily involves the spinal ganglia and the axons [Tateishi J et al. 1972, 1973] (Fig, 11B). On the pathomechanism of this finding, Ikuta et al. (1982) reported a retrograde trans-synaptic degeneration of the afferent fibers to the degenerated Clarke's column, based on the findings of marked loss of neurons in the Clarke's column at the severely degenerated side of the posterior funiculus (Fig. 11A).

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THE MYSTERIOUS DISEASES OF GUAM

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To all patients, past and present, afflicted with Lytico and Bodig and their families who had courageously participated in the research.

Chapter 9.

New Finding in Neuropath

Kiyomitsu Oyanagi

The Nature of Neuropathological Findings of PDC and ALS of Guam

Fig. 62. Kiyomitsu Oyanagi 2001

Parkinsonism-dementia Complex (PDC)

Macroscopic Features: The PDC brains shows frontal and temporal lobe atrophy which is quantitatively similar to that in Alzheimer's disease, but the atrophy in the basal ganglia and brain stem of PDC is morphometrically the same as that in progressive supranuclear palsy (PSP) [1].

Microscopic features. Characteristics of PDC are widespread neurofibrillary tangles (NFTs) with a small number or virtual absence of senile plaques, accentuated in the temporal lobe and brain stem, and neuronal loss, which is severe in the Ammon's horn and substantia nigra, and almost coincident with the distribution of NFTs. The NFTs are tau- and ubiquitin-immunopositive [2,3,4], and composed of mainly paired helical filaments (PHF), and partly straight tubules in the cerebrum, but mainly straight tubules in the spinal cord [5]. The NFTs are predominantly distributed in the superficial layers in the cerebral cortex [6]. Neuropil threads (curly fibers) and astrocytic gliosis are relatively sparse [4,7]. The large neurons in the neostriatum, which are considered to be cholinergic interneurons, decrease to 40% of control level, correlatively to the loss of cholinergic large neurons in the basal nucleus of Meynert, while the loss is marked to 10% in the nucleus accumbens [8]. Alpha-synuclein inclusions are observed in the neurons in the amygdala of 38% of the patients with PDC and many of these inclusions coexisted with tau-positive pretangles or NFTs [9], as observed in familial Alzheimer's disease [10]. The substantia nigra represents severe loss of neurons, not only pigmented (dopaminergic) neurons but also nonpigmented (GABAergic) neurons [11]. Lewy bodies are rarely seen in the substantia nigra. Identical neuropathologic features were documented in Filipino patients who lived on Guam [12].

Glial inclusions. Tau-immunopositive and Gallyas-positive astrocytic granular hazy inclusions (AGHI) are observed in PDC. Astrocytes in amygdala, motor cortex, and inferior olivary nucleus show the inclusions. Crescent shaped or coiled inclusions are present in the oligodendroglia of the anterior nucleus of the thalamus, motor cortex, midbrain tegmentum, and medullary pyramids[13].

Tau-positive fine granules (TFGs) in the cerebral white matter. TFGs are globe-shaped, 3-6 μm in size, and predominantly observed in the frontal white matter in 30 out of 35 PDC patients. However, no TFGs are found in the brains of PSP, MID, Pick's disease, AD, or CBD. Thus, TFGs exclusively found in PDC brains are a novel finding in the human brain, and serve as a specific neuropathological marker of PDC[14].

Differential diagnosis. Disorders of the elderly exhibiting dementia and movement disorders with widespread NFTs and glial inclusions composed of abnormally phosphorylated tau proteins are in the differential diagnosis given the clinical history. Guam PDC has not been described in Western societies, but cases of PDC on Kii peninsula in Japan have many similarities[15]. The major differences include PSP, corticobasal degeneration (CBD), post-encephalitic parkinsonism (PEP), and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). The predominance of NFTs with relatively few neuropil threads and glial tangles in Guam PDC are different from the widespread occurrence of numerous threads and glial tangles in PSP, CBD and FTDP-17. The minimal neuronal loss in the subthalamic nucleus, absence of grumose degeneration in the dentate nucleus and rare tuft-shaped astrocytes help to differentiate PDC from PSP. The absence of astrocytic plaques and ballooned neurons, and the relatively small number of pretangles and foamy axonal spheroids help differentiate PDC from CBD. Thorn-shaped tau-positive astrocytes have been reported to be restricted to within the third ventricle wall and around the cerebral aqueduct in PEP. AGHI (astrocytic granular hazy inclusion) and TFGs (tau-positive fine granules) have been exclusively reported in Guam PDC.

Amyotrophic Lateral Sclerosis (ALS) of Guam

It had been proposed that ALS of Guam and Guam PDC were a single disease entity , and that Guam ALS was a disease different from typical sporadic ALS[16,17]. Guam ALS was considered distinct because: (1) the topographic distribution of NFTs and neuronal loss in ALS was similar to Guam PDC; (2) patients with combined PDC and ALS (PDC-ALS) were recognized; and (3) ALS as well as PDC patients were sometimes admixed within a kindred. Recently, however, it has become clear that NFTs are prevalent in the normal population of Guam [18] and that NFTs in the setting of Guam ALS are merely a background phenomenon (Guam ALS - NFTs = Classic ALS) [5,19]. The current evidence suggests that the basic mechanism of motor neuron degeneration in Guam ALS is similar to classic ALS [20].

Conclusion.

PDC is a distinct disease entity (NFT with extensive neuronal loss accentuated in Ammon's horn and substantia nigra plus AGHI and TFG).

Guam ALS is equivalent to Classic ALS plus NFTs.

Mixed or overlapped case exists with both PDC and ALS.

Biochemistry of NFTs

Abnormally phosphorylated tau protein of NFTs in Guam PDC is composed of a major tau triplet, with molecular weights of 68, 64, and 55 kDa consistent with a mixture of 3 repeat (3R) and 4R tau. This is the same pattern as in Alzheimer's disease and is different from the 4R in PSP [21,22].

Pathogenesis and trace metals

Early in 1977, Ikuta and Makifuchi discovered aluminium (Al) in the AHCs of spinal cord in Japanese ALS patients [23]. Perl et al. confirmed intraneuronal Al accumulation in NFT-bearing neurons in the hippocampus of Guam ALS-PDC in 1982 [24], as observed in Alzheimer's disease [25]. Yase's colleague reported the presence of calcium (Ca) and hydroxyapatite in Guamanian brain with ALS-PDC [26].

Garruto et al. succeeded in imaging of Ca and Al in NFT-bearing neurons in PDC [27]. (see Chapter on trace metal).

Experimental models

Based on the possible pathogenesis proposed, experimental studies focusing on low magnesium (Mg) and Ca and high Al and on plant neurotoxins have been explored; however, no animal model completely recapitulates Guam PDC or ALS. Repeated oral administration of beta-methylamino-2-aminopropionic acid (BMAA), the proposed toxic factor within cycad flour, to macaques produces chromatolysis of Betz cells, simple atrophy of anterior horn cells in the spinal cord and neuritic swelling in the substantia nigra [28]. A low-Ca, high-Al diet in monkeys induces neurofibrillary pathology characterised by accumulation of phosphorylated neurofilaments in the anterior horn cells [29]. The authors revealed exclusive loss of dopaminergic neurons in the substantia nigra

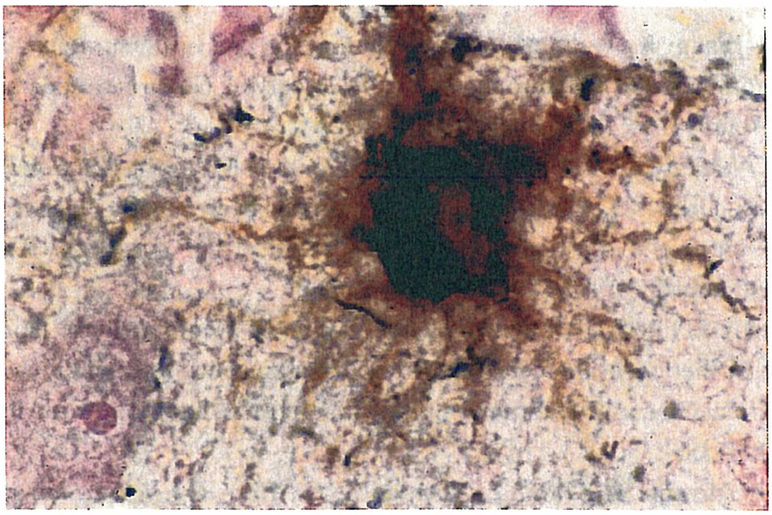

Fig. 63. Astrocytic granular hazy inclusion (AGHI). Motor cortex of a PDC patient. Double staining involving Gallyas preparation (black) and glial fibrillary acidic protein immunostaining (brown).

in rats with long duration exposure of low Mg intake over two generations [30].

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Ultrastructural Temporal Profile of the Dying Neuron and Surrounding Astrocytes in the Ischemic Penumbra: Apoptosis or Necrosis?

U. Ito, T. Kuroiwa, S. Hanyu, Y. Hakamata, E. Kawakami, I. Nakano, and K. Oyanagi

Summary. We investigated the temporal profile of isolated dying neurons (disseminated selective neuronal necrosis: DSNN) and the behaviors of astrocyte surrounding these dying neurons, in the ischemic penumbra of the cerebral cortex. In the ischemic penumbra, DSNN progressed slowly until 3 weeks after the ischemic insult. Cell bodies, cell processes, and end-feet of living astrocytes became swollen, with an increase in the number and in the volume of the mitochondria and accumulation of glycogen granules. The DSNN started 15 min after the ischemic insult, and progressed with increasing numbers of dark neurons having various degrees of electron density during 5 to 24 h. The isolated dark neurons showed homogeneous condensation of their cytosol, organelles, and nucleus, in which small loosely aggregated chromatin condensates were observed in the nuclear matrix and along the margin of the nuclear membrane. These chromatin condensations were positive for TUNEL staining. The swollen astrocytic cell processes surrounded the dark neurons. Astrocytic swelling was most prominent near the dendritic synapses. Finally, the isolated dark neurons became completely shrunken with very high electron density of the entire cell containing degenerated mitochondria having swollen matrices with occasional woolly densities. The shrunken neuron was fragmented into electron-dense debris by invading astrocytic cell processes. Some of the debris was phagocytized by astrocytes, and others moved into the extracellular space and were phagocytized by the perivascular microglia. Macrophages and other inflammatory cell were not observed in the penumbra. The ultrastructural characteristics of DSNN, in the present study, suggested necrotic neuronal death instead of apoptosis. Condensation of the isolated neuron was induced by swelling of astrocytic cell processes surrounding the dark neuron.

Key words. Apoptosis vs. necrosis – astrocytic swelling – disseminated selective neuronal necrosis – ischemic penumbra – maturation phenomenon of ischemic injuries – neuronal death

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Maturation Phenomenon in Cerebral Ischemia V
A.M. Buchan et al. (Eds.)

Introduction

Recently, the topic of apoptosis vs. necrosis of dying neurons after ischemic insult has been a matter of controversy [1, 4, 23]. We report our findings herein as well as discuss apoptosis vs. necrosis as the cause of this death.

Cerebral infarction develops rapidly after a large ischemic insult has occurred. We developed a model to induce a large ischemic penumbra around a small focal infarction in the cerebral cortex of Mongolian gerbils [7, 9] by giving a threshold amount of ischemic insult to induce cerebral infarction. The histopathology of this model revealed disseminated eosinophilic ischemic neurons (disseminated selective neuronal necrosis: DSNN) that increased in number in a large area of the cerebral cortex after revascularization, and a focal infarction developed only in the frontal lobe by 24 h after the start of recirculation [10]. Electron-microscopically, these disseminated eosinophilic ischemic neurons were observed as dark neurons with increased cytosolic electron-density. These dark neurons increased in numbers until day 4, and new one were still appearing 3 weeks after the start of recirculation. This observation corresponds to the maturation phenomenon of ischemic injuries [11], the original concept of the delayed neuronal death described in CA1 neurons [17].

Using this model, in this present study, we examined the ultrastructural temporal profile of these dying dark neurons in the ischemic penumbra of the parietal cortex with special attention given to the behavior of the astrocytes surrounding the dying neurons.

Materials and Methods

Under 2% halothane, 70% nitrous oxide, and 30% oxygen anesthesia, the left carotid artery of adult Mongolian gerbils was twice occluded for 10 min each time, with a 5 h interval between the 2 occlusions [8]. After each cervical surgery, animals soon recovered from the anesthesia and moved spontaneously. Ischemia-positive animals were selected based on the stroke index score determined after the first occlusion [26].

The gerbils were sacrificed at various times, i.e., at 15 min, at 5, 12, 24 h, at 4 days, and at 1, 2, 3 weeks following the second ischemic insult by intracardiac perfusion with glutaraldehyde fixative for electron microscopy and phosphate-buffered formaldehyde fixative for light microscopy.

Ultrathin sections including the 3rd~5th cortical layers were prepared from the parietal lobe of the left ischemic cerebral hemisphere at the mid-point between the interhemispheric and rhinal fissures as coronal sections at the level of the infundibulum. Alternative sections were double stained by uranyl acetate and lead solution, and observed with a Hitachi electron microscope. Paraffin sections were separately stained with hematoxylin-eosin (HE), periodic acid fuchsin Schiff (PAS), and TUNEL reagents (ApopTag: Intergen).

Results

Swelling of astrocytic cell processes, perivascular end-feet, and cell body had already begun 15 min after the ischemic insult, without accumulation of glycogen granules [12]. At this stage, isolated dark neurons with diffuse, increased electron density were found disseminated among the almost normal-looking neurons. Some of the normal-appearing neurons showed disaggregated ribosomes and slight swelling of the rough endoplasmic reticulum (rER) and Golgi apparatus. The swollen astrocytic cell processes surrounded these dark neurons. Small dots of chromatin condensation were observed scattered in the nuclear matrix as well as along the nuclear membrane of the dark neurons. However, no swelling was observed in mitochondria and the other cytosolic organelles. These dark neurons were never found in the control animals after the same procedures for fixation and preparation as used for the postischemic animals.

From 5 to 24 h, isolated dark neurons with different grades of high electron density increased in number among the almost normal looking neurons (Fig. 1 d), some of which showed disaggregation of ribosomes and slight swelling of their rER and Golgi apparatuses as seen at 15 min. These dark neurons still newly appeared even 3 weeks after the start of recirculation. In the swollen astrocytic cell processes, enlarged mitochondria having slightly swollen cristae and boosted electron density of the matrices had increased in number, together with increased accumulation of glycogen granules [12, 13]. These dark neurons were compatible with ischemic neuronal change seen by HE staining. These ischemic neurons were scattered among the normal-looking neurons in the ischemic penumbra of the cerebral cortex, and had a homogeneous eosinophilic cytosol containing a pycnotic and/or karyorrhectic nucleus (Fig. 2 a) [10]. In this stage, TUNEL-positive nuclei showing strong positively on the dotted chromatin condensations of the neuron were also observed scattered in the ischemic penumbra of the cerebral cortex (Fig. 2 b). The dark neurons were surrounded by remarkably swollen astrocytic cell processes, especially prominent nearby the dendritic synapses of the dark neurons [31] (Fig. 1 c), and showed darkened nuclei in which dots of chromatin condensates were observed (Fig. 1 b). Later than 12 h after the ischemic insult, some mitochondria of these dark neurons showed partial swelling of their matrices and disintegration of cristae with woolly densities (Fig. 1 a). Among these dark neurons, completely shrunken neurons surrounded by remarkably swollen astrocytic cell processes (Fig. 1 c) were increased in number from 12 to 24 h after the ischemic insult. These shrunken neurons often contained mitochondria with swollen matrices having woolly densities.

By 4 days after the ischemic insult, these shrunken neurons were fragmented by astrocytic cell processes that had invaded and separated the shrunken neuronal cytosol and nucleus. Up to this stage, no inflammatory cells and macrophages appeared in the ischemic penumbra. However, phagocytic activity of the perivascular microglia was observed.

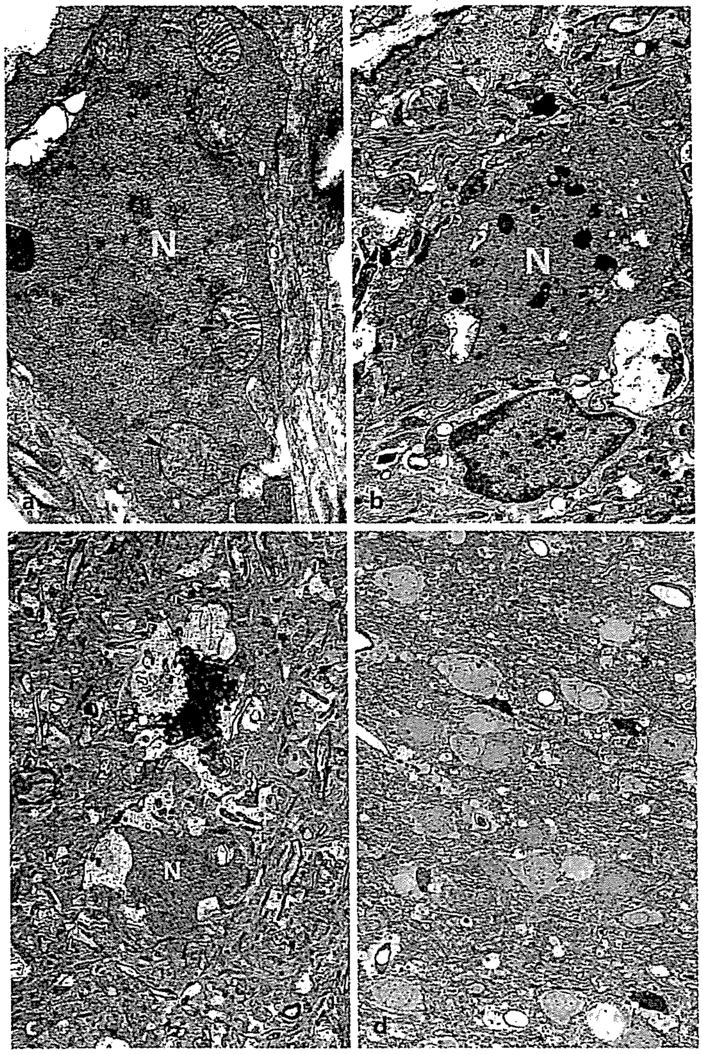

Fig. 1 a–d. Electron microscopy of electron dense dying neurons: **a** Later than 12 h after the ischemic insult, some mitochondria of these dark neurons shows partial swelling of their matrices and disintegration of cristae with woolly densities (arrows) (×6000); **b** the dark neuron shows darkened nucleus (N) in which dots of chromatin condensates are observed. Nuclear membrane is swollen and disintegrated (×4000); **c** two dark neurons with different grade of electron density are surrounded by remarkably swollen astrocytic cell processes, especially prominent nearby the dendritic synapses of the dark neurons. N: nucleus (×1500); **d** twelve hours after ischemic insult, isolated dark neurons with different grades of high electron density increased in number among the almost normal looking neurons (×500)