

CHAPTER 1**Mitochondrial and Golgi Apparatus' Alterations in Alzheimer's Disease: A Study of the Cerebellar Cortex Based on Silver Impregnation Technique and Electron Microscopy****Stavros J. Baloyannis****Research Institute for Alzheimer's disease, Iraklion Langada*

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder of the brain, inducing progressive severe presenile and senile cognitive decline, resulting in vegetative stage eventually. From the etiological point of view the main causative factor, remains unknown, in spite of the steady augmentation of the research efforts. Golgi staining revealed the substantial alterations of the dendritic branches and the tremendous loss of spines even in the initial stages of the disease. Electron microscopy reveals morphological changes of the mitochondria in neurons and astrocytes associated with fragmentation of cisternae of Golgi complex and pathological alteration of the dendritic spines, even in areas of the brain which demonstrate minimal tau pathology and few amyloid β deposits. We attempted to describe the ultrastructural alterations of the cerebellar cortex in early cases of AD, focusing our study mostly on mitochondria, Golgi apparatus, dendritic branches, dendritic spines and synapses in the cerebellar hemispheres and the vermis. Mitochondria demonstrated an impressive polymorphism in the soma, the axonal and dendritic profiles of Purkinje cells, the climbing fibers, the mossy fibers and the synapses. Electron microscopy revealed also dilatation of the cisternae of the smooth endoplasmic reticulum and marked fragmentation of cisternae of Golgi complex in large number of Purkinje cells, granule and stellate cells in the vermis and the cerebellar hemispheres. The fragmentation of the Golgi complex and the poverty in vesicles in cis- and trans-Golgi network in the soma of Purkinje cells in Alzheimer's brains coincide with the synaptic loss, the shortage of the dendritic arborization and the pathological alterations of the spines, the dendritic spines of the Purkinje cells were also reduced in number. Numerous spines included large multivesicular bodies, altered spine apparatus, and unusual mitochondria. Giant elongated spines were seen in a substantial number of Purkinje cells of the vermis and the cerebellar hemispheres. In many presynaptic terminals of parallel and mossy fibres, electron microscopy revealed a dramatic loss of the synaptic vesicles associated with marked polymorphism. On the basis of the mitochondrial and Golgi complex pathology, new therapeutic strategies protecting those organelles might be proposed for the treatment of early cases of AD.

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INTRODUCTION

Alzheimer's disease (AD) is the most tragic expression of presenile and senile dementia, based on a complex etiopathogenetic background involving numerous cellular interactions and neurochemical mechanisms, which is clinically characterized by profound memory loss, learning inability, language disturbances, visuo-spatial disorientation, mood and behavioral changes [1], and autonomic disorders. All these diseases increase as the disease advances, resulting in a vegetative state eventually [2].

The disease affects millions of elderly people, as the most frequent cause of AD is dementia, which provokes serious clinical, ethical, social and economic problems worldwide.

Neuropathologically, AD in both sporadic and familial forms is characterized by loss of neurons, in vulnerable areas of the brain, synaptic changes, mitochondrial abnormalities, cytoskeletal alterations, neuritic dystrophy, neuropil threads, capillary changes, blood brain barrier disruption, inflammatory responses. In addition, Hirano bodies and granulovacuolar degeneration are not very common phenomena, which sometimes seem to plot the morphological profile of the disease. However, the most characteristic morphological findings of precise and definite diagnostic value are (a) the tau pathology in the form of neurofibrillary tangles and (b) the deposits in the neuropil space of amyloid β , as neuritic plaques [3], which compose the principal diagnostic criteria of AD. In addition, the pathological presentation of Alzheimer's disease (AD) also involves tremendous synaptic loss which plays an important role in the gradual decline of the mental capacities, resulting in dementia.

Approaching the etiology of the disease, the main causative factor remains unknown, although the research on the field has been greatly intensified in the last thirty years. It might be obvious that the complex genetic predisposition in cases of familial AD[4], may support the heterogeneity of the disease, emphasizing the

concept that the clinical manifestations and the course of the disease are the eventual consequences of various protein interactions, functional and morphological changes, associated with underline genetic factors [5,6]. In addition, environmental factors and the aging process may also plot the etiological profile of the disease. The risk of Alzheimer's disease, when a maternal relative suffers from the disease, advocates, on the other hand in favor of the implication of the mitochondrial DNA (mtDNA) [7], which is a maternally derived factor.

The implication of amyloid- β , A β PP and tau protein may be crucial in the pathogenesis of the disease, without enlightening sufficiently the innermost pathological procedures. However, according to "amyloid cascade" hypothesis, amyloid- β (A β), which is a cleavage product of the β -amyloid protein precursor (A β PP) [8, 9] is the most possible causative factor in both familial and sporadic types of Alzheimer's Disease (AD) [10, 11], in view that elevated intra- or extracellular levels of A β oligomers protofibrils are believed to be of considerable pathogenic significance, since they are characterized by excessive neuronal and synaptic toxicity [12, 13,14]. It is hypothesized that a chronic disequilibrium and instability between the production and clearance of amyloid- β (A β) and its molecular misfolding may lead to synaptic alterations and glial activation step by step [15].

Morphological alterations of the neuronal organelles, concerning mainly microtubules, mitochondria, and Golgi apparatus, have been described by histochemical techniques as well as electron microscopy. Mitochondrial alterations were particularly prominent in neurons, which showed loss of dendritic spines and decrease of the surface of dendritic arbor. Many morphological alterations of AD, associated mostly with oxidative damage [15] could be attributed to mitochondria alterations, since the decrease of energy production in mitochondria may affect amyloid β -protein precursor metabolism, and increase the formation of amyloid- β [16]. Mitochondrial alterations, such as disruptions of mitochondrial function and mitochondrial dynamics, inducing considerable dysfunction of mitochondrial electron transport proteins, may be related to metabolic and energy deficiency in neurons in Alzheimer's disease and other disorders, which have been reported by many authors in several neurodegenerative diseases[17,18,19, 20,21,22] aging [23], and vascular lesions [24].

The fact that mitochondrial abnormalities occur in neurons lacking neurofibrillary tangles pleads in favor of the hypothesis that mitochondrial degeneration is one of the earliest signs of Alzheimer pathology, appearing before neurofibrillary tangles are evident [25]. Mitochondrial abnormalities might be considered as cause and effect of the oxidative stress and calcium deregulation [26, 27, 28] in AD and other age-related neurodegenerative disorders [29].

Morphological alterations of Golgi apparatus have been described in neurons, even in early cases of Alzheimer's disease, at a stage that the neurofibrillary tangles and the neuritic plaques are still rare, seen mostly in the hippocampus and in a limited number of neurons of the temporal, parietal and frontal cortices. Golgi apparatus changes may contribute substantially in the pathogenesis of AD [30, 31], since they are essential in protein trafficking, as most of the synthesized proteins are processed through the Golgi complex [32].

In the present study in order to figure out whether mitochondrial and Golgi abnormalities are very early cytopathological phenomena, occurring independently of the tau and amyloid- β pathology we attempted to describe the morphological changes of the mitochondria and the Golgi complex in early cases of Alzheimer's disease, in the cerebellar cortex, where amyloid- β deposits are minimal and neurofibrillary tangles are rarely observed.

MATERIAL AND METHODS

Patients

This morphological study is based on examination of twenty brains obtained at autopsy 2 to 6 hours after death at room temperature of 4° C. All the brains were obtained from patients with a definite history of dementia, aged 55-78 years, who fulfilled the psychological, psychiatric and neurological criteria of AD. The patients have had 16 years of education, and had fluency in their native language. Two of the patients were bilingual with equal fluency in both of the languages. The usual diagnostic procedures were applied, such as analysis of the medical history, physical examination, heart investigation, neurological examination, psychiatric assessment and neuropsychological testing. The cognition of the patients was evaluated by mini mental state examination (MMSE), dementia

rating scale (DRS) [33,32] and ADAS-COX test[35, 36]. All patients had an EEG, a carotid duplex Doppler, a computerized tomography (CT), a magnetic resonance imaging (MRI) of the brain and a single-photon emission computed tomography (SPECT), which were evocative for Alzheimer's disease. The death of the patients was due to heart arrest.

Ten additional brains macroscopically intact of apparently healthy individuals of the same age was used as normal controls with the patients, who died accidentally.

Electron Microscopy

Multiple samples from the vermis of the cerebellum and the cerebellar hemispheres, were selected bilaterally and immersed directly in Sotelo's fixative, composed of 1% paraformaldehyde, 2.5% glutaraldehyde in cacodylate buffer 0.1M, adjusted at pH 7.35. Then the specimens were further post fixed in 1% osmium tetroxide for 30 min. at a temperature of 18° C and dehydrated in graded alcohol solutions and propylene oxide. Then they were embedded in araldite and cut in ultrathin sections in a Reichert ultratome. The sections were contrasted by uranyl acetate and lead citrate and studied in a Zeiss 9aS electron microscope.

Morphometric studies were performed with an image analyzer. The mean surface area of the neurons, the mean nuclear area, the area of the Golgi apparatus, the number of the cisternae and the number of the vesicles were calculated. We counted and studied a hundred Purkinje cells of each brain and estimated the total percentage of neurons with fragmented Golgi apparatus and mitochondria alterations on a total number of 5,000 mitochondria, and 1,000 Golgi complexes on pictures in an electronical magnification of 56.000X. Circularity ratio (CR) was introduced to represent the shape of more than 20,000 mitochondria.

The statistical analysis of the data was evaluated by Student t tests.

Light Microscopy: in Golgi Staining

After the selection of the specimens for electron microscopy, the cerebellum was prepared for rapid Golgi silver techniques through a four-week fixation in formalin and then immersion of a number of selected specimens in a solution

composed of 7g potassium dichromate in 300 mL water for 10 days. Subsequently, the specimens were post immersed in a solution of 1% silver nitrate for 10 days. After a dehydration in alcohol solutions, the specimens were embedded in paraffin and cut at 100 μ and 25 μ , sections alternatively. Sections of 25 μ were post stained with methylene blue, according to Golgi-Nissl method. The sections were mounted between two cover slips and were examined in a Axiolab Photomicroscope (Carl Zeiss Group, Oberkochen, Germany).

The dendritic arbors of Purkinje cells, the morphology of the dendritic branches, the dendritic spines on sections stained with silver nitrate according to Golgi and Golgi-Nissl methods were studied [37].

RESULTS

Golgi Staining

The silver staining or *reazione nera* (black reaction) as it was called by Golgi [38] is a very useful, simple but delicate staining method for neurons, which clearly visualizes the three-dimensional morphology of the soma of neurons, the dendritic arbors, the dendritic spines, the axons, the axonic collaterals and the glial cells[39]in the structures of the central nervous system. Golgi technique has been applied extensively in the study of the morphological organization of the central nervous system by Santiago Ramón y Cajal [40] who worked particularly on the cerebellar cortex [41], defending successfully the “neuron doctrine” [42] and shared a Nobel Prize with Camillo Golgi in 1906. this technique after 140 years from its first application in neurohistology and neuropathology remains virtually unique for the demonstration of neurons and dendritic morphology in a biplanar or stereoscopic way [43], despite the delicate nature of the method and the unpredictability of the results. Thus, Golgi technique continues to remain instrumental for the morphological study and the morphometric assessment of neuronal circuits at the early stages of the neuronal degeneration and the histological profile of debilitating diseases of the central nervous system [43] as well as for accessing the staging of Alzheimer alterations [44].

Golgi staining revealed specimens neuronal loss and marked alterations of the dendritic branches of Purkinje cells in the vermis (Fig. 1) and the cerebellar

hemispheres (Fig. 2). Considerable decrease of the number of dendritic branches and marked loss of dendritic spines were noticed practically in large number of Purkinje cells of the cerebellar cortex (Fig. 3), in comparison with the controls specimens. Pathology of dendritic spines, displayed distorted spines, giant spines and abnormal spine protrusions were noticed in large number of Purkinje cells in the vermis and the hemispheres.

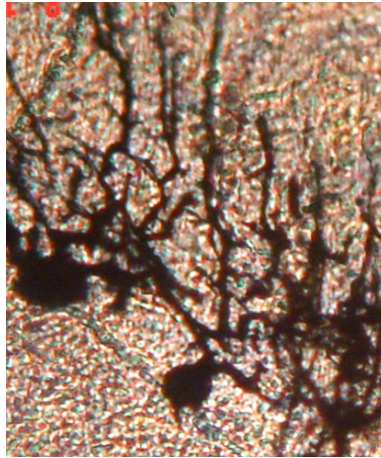


Figure 1: Purkinje cell of the vermis in case of Alzheimer's disease. The tremendous loss of dendritic branches is obvious. Golgi silver impregnation technique (Mag.3,600X).

The decrease in the number of spines was the obvious phenomenon seen all over the cerebellar cortex.

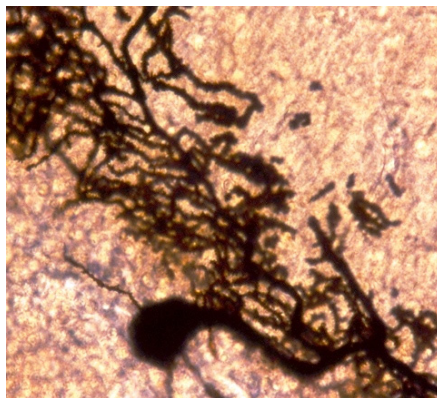


Figure 2: Purkinje cell of the superior surface of the cerebellar hemispheres in case of Alzheimer's disease. The loss of the majority of the apical dendritic branches is obvious. Golgi silver impregnation technique (Mag.3,600X)

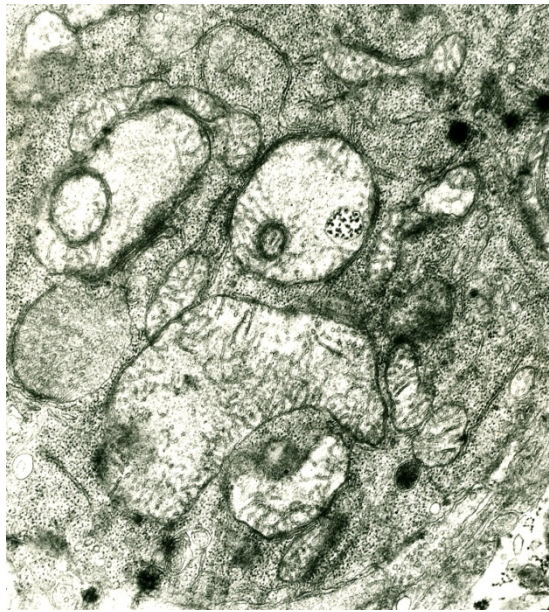


Figure 3: Abnormal mitochondria in the soma of a Purkinje cell of the vermis in case of Alzheimer's disease. The impressive polymorphism of the mitochondria and the abnormal arrangement of the cristae are obvious. (Electron micrograph Mag. 384, 000 X).

Electron Microscopy

Electron microscopy is instrumental for the study of the morphology of the organelles, the synapses, the dendritic spines, the axons, the myelin sheath, the neuron-glia relationships, the pericytes and the blood brain barrier in normal and in pathological conditions of the brain.

Electron microscopy revealed in this study the morphological changes of the mitochondria and the Golgi apparatus as well as the morphological changes of the dendritic branches and the pathology of the dendritic spines.

Mitochondria

The ultrastructural study of the cerebellar cortex, revealed a significant polymorphism of the mitochondria in the soma, the axonal and dendritic profiles of Purkinje cells, the climbing fibers, the mossy fibers and the synapses (Fig. 3). The mitochondria demonstrated a wide variation of size and shape in comparison with those of the normal control brains. Numerous were small, round

or elongated (Fig. 4). A substantial number of mitochondria showed disruption of the cristae, though others included osmiophilic material or showed an unusual polymorphism in the pattern of the cristae, which rarely demonstrated a concentric arrangement or they were arranged according to the long axis of the organelle. In addition pathological changes of mitochondria were noticed in the dendritic spines in the majority of Purkinje cells in the vermis and the cerebellar hemispheres, associated with marked accumulation of multivesicular bodies and decrease of the size of spines.

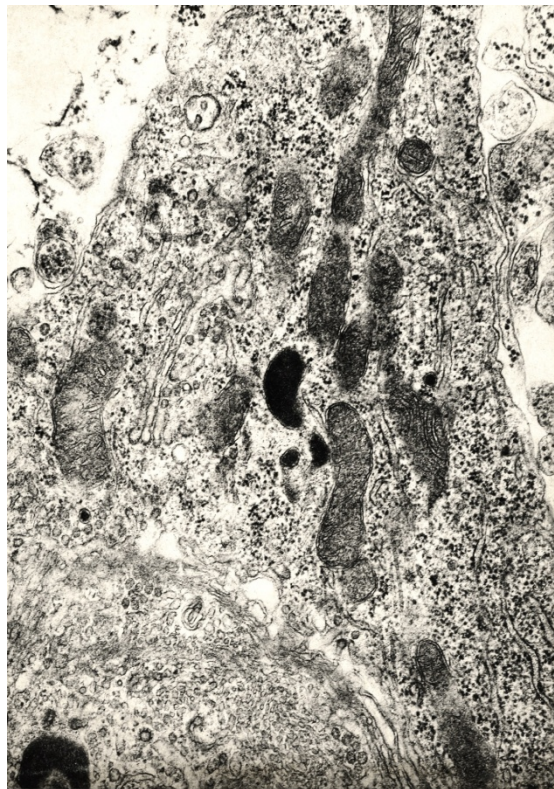


Figure 4: Very long abnormal mitochondria and fragmentation of the cisternae of Golgi apparatus are seen in the primary branch of the apical dendrite of a Purkinje cell of the superior surface of the cerebellar hemispheres in a case of Alzheimer's disease (Electron micrograph Mag. 28, 000 X).

Mitochondria showing morphological changes were also found in the cell body (Fig. 5), the perivascular astrocytes and the astrocytic sheaths around the Purkinje cell dendrites in Alzheimer's brains, whereas in normal controls the mitochondria looked normal.

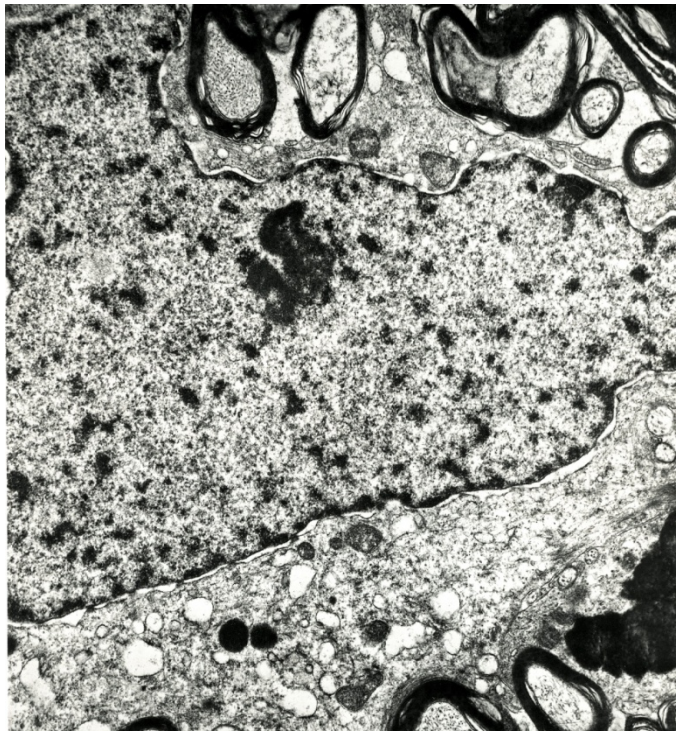


Figure 5: Abnormal mitochondria, dilatation of the smooth endoplasmic reticulum and fragmentation of the cisternae of Golgi apparatus of an astrocyte of the granule cell layer of the vermis of a case of Alzheimer's disease (Electron micrograph. Mag.20, 000X).

In a morphometric estimation the mitochondria in normal controls appeared to have an average diameter of 250 to 650 nm and a mean axial ratio of 1.9 ± 0.2 . The round or global mitochondria in normal controls appeared to have a mean mitochondrial radius of 350 nm. In Alzheimer's disease, ellipsoid mitochondria of Purkinje cells appeared to have an average diameter of 250 to 510 nm and a mean axial ratio of 1.7 ± 0.2 . Round mitochondria were characterized by a mean radius of 280 nm.

Golgi Apparatus

The ultrastructural study of the Purkinje cells of the vermis and the cerebellar hemispheres revealed dilatation of cisternae of the smooth endoplasmic reticulum and marked fragmentation of the cisternae of the Golgi complex in a large number of Purkinje cells (Fig. 6). The same phenomenon was also seen in granule cells in the vermis as well as in the cerebellar hemispheres.

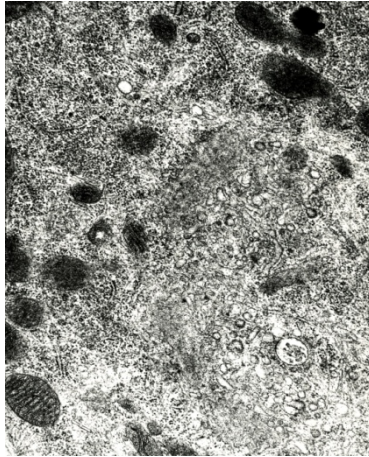


Figure 6: Fragmentation of Golgi apparatus is observed in the soma of Purkinje cell of the inferior surface of the cerebellar hemispheres in case of Alzheimer's disease. Mitochondrial alterations are also seen (Electron micrograph Mag.68, 000X).

The stacks of the cisternae of the Golgi complex were shorter in correlation with normal controls. The number of vesicles associated with the Golgi complex, were found to decrease in most of the Purkinje and granule cells. Many endocytotic polymorphic vesicles, were also observed in the perikaryon of Purkinje cells, granule cells, stellate cells (Fig. 7), astrocytes, as well as the endothelial cells of the capillaries of the cerebellar cortex.

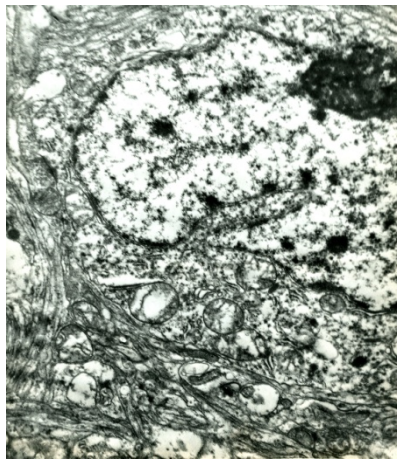


Figure 7: Fragmentation of Golgi apparatus is observed in the soma of a stellate cell of the molecular layer of the inferior surface of the cerebellar hemispheres in case of Alzheimer's disease. Mitochondrial alterations are also observed. The stellate cell is surrounded by large number of parallel fibers (Electron micrograph Mag.25, 000X).

Dendritic Spines

The dendritic spines of the Purkinje cells were also reduced in number. Numerous smooth surfaces of Purkinje cell tertiary dendritic profiles were seen in the molecular layer of the cerebellar cortex. Rarely, unattached Purkinje cell dendritic spines were observed in the molecular layer. The unattached spines were embedded in the soma of astrocytes and had undergone postsynaptic differentiation of the plasma membrane.

Many Purkinje cell spines included sizable multivesicular bodies, abnormal mitochondria dense bodies and abnormal spine apparatus. Giant elongated spines were frequently seen in a substantial number of Purkinje cells in the vermis and the hemispheres (Fig. 8).

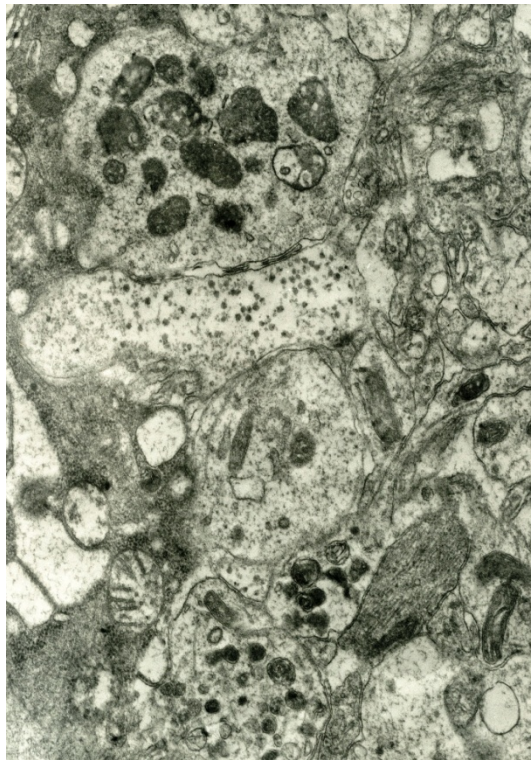


Figure 8: Dendritic profiles of a Purkinje cell, dendritic spines, pre synaptic terminals of parallel and climbing fibers are seen in the molecular layer of the cortex of the inferior surface of the left hemisphere in case of Alzheimer's disease. Abnormal mitochondria, dense bodies, dilated cisternae of the smooth endoplasmic reticulum and unattached spines are seen (Electron micrograph Mag.128, 000X).

In many presynaptic terminals of parallel and mossy fibres in the cerebellum in AD, electron microscopy revealed a marked polymorphism of the few synaptic vesicles (Fig. 9).

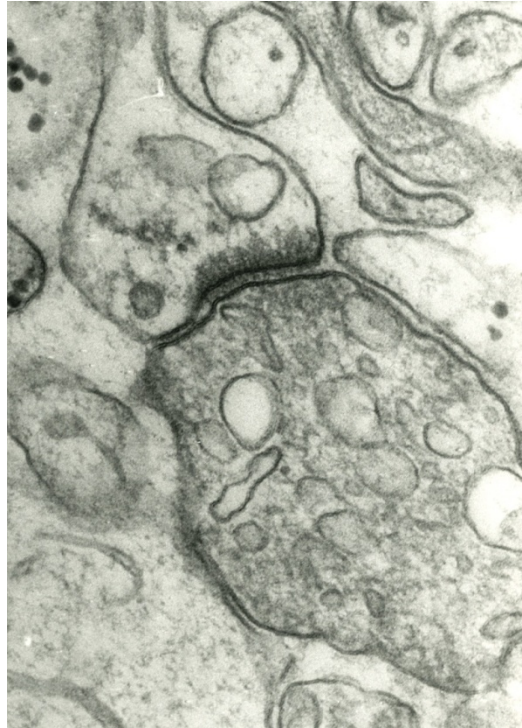


Figure 9: Synapsis between parallel fiber and Purkinje cell dendritic spine is observed in the molecular layer of the nodule of the vermis in case of Alzheimer's disease. The postsynaptic component (the dendritic spine) shows a marked density of the postsynaptic membrane and a small abnormal mitochondrion. The presynaptic component, a parallel fiber's terminal, demonstrates large number of dilated cisternae and polymorphic vesicles (Electron micrograph Mag.680,000X).

Astrocytic Proliferation

Astrocytic proliferation was obvious in the ganglionic layer of the cerebellar cortex (Fig. 10). Thick astrocytic sheaths surrounding the main dendritic branches of the Purkinje cells were extended to the molecular layer, between the parallel fibers and the terminal Purkinje cell dendritic branches. Perivascular astrocytic processes were observed including numerous sizeable pinocytotic vesicles. Endocytotic vesicles, which demonstrated a marked polymorphism were also noticed in the perikaryon of the endothelial cells.

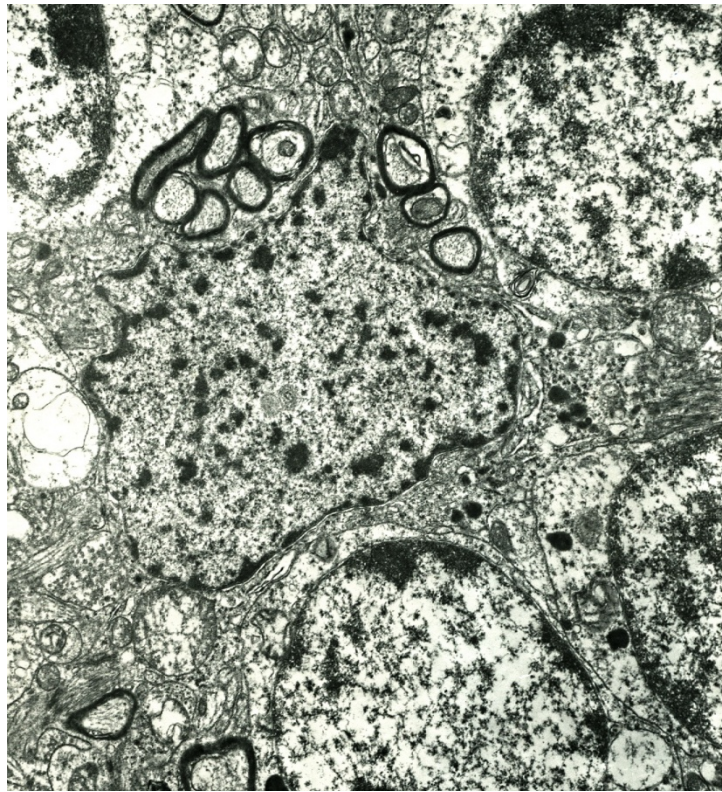


Figure 10: Astrocyte in the granule cell layer, among granule cells in the left flocculus of the cerebellum in case of Alzheimer's disease. Abnormal mitochondria, endocytotic vesicles, dilatation of the smooth endoplasmic reticulum and fragmentation of the cisternae of Golgi apparatus are observed (Electron micrograph. Mag.20, 000X).

DISCUSSION

Mitochondria are the non-nuclear constituents of the cell having their own DNA (mtDNA) and the property of synthesizing RNA and proteins. They are essential to energy equilibrium, since they provide most of the energy by oxidative phosphorylation of glucose, and by involving in other metabolic pathways. The shape and the size of the mitochondria are highly variable [45]. Their morphology is sometimes controlled by the cytoskeleton, the neurofilaments and the microtubules [46]. Normally, approximately one-third of the mitochondria are in motion along with microtubules and actin filaments [47], transported to regions where energy requirement is particularly high. The number of the mitochondria is adjusted, according to the requirement of energy by the cell.

Mitochondria and mtDNA [48] are very sensitive to oxidative damage and inversely mitochondrial alterations may induce or increase the existing oxidative stress, suggesting that there is an intimate and early association between oxidative stress and mitochondrial abnormalities. The combined effect of high calcium ions with oxidative stress may also damage mitochondrial function [49] and has been implicated as a cause for apoptosis in many systems [50, 51].

Some observations [52,53] indicated that increased oxidative damage, decrease in energy production and change of cytochrome c oxidase (CytOX) activity are among the primary events in Alzheimer's disease, emphasizing that the dysfunction of the mitochondria and the oxidation of ion channels [54] may play roles in the pathogenesis of most of the devastating diseases of the brain [18,19,55,56]. Reduced cytochrome oxidase activity has also been reported in platelets [57] as well as in post mortem brain tissue, obtained from patients of Alzheimer's disease [58].

It is important to underline that mitochondrial cytochrome c oxidase may be inhibited by a dimeric conformer of A β 42, a mechanism which is copper dependent [59,60,61]. Oxidative stress, is reasonably associated with amyloid β (A β) accumulation in the neocortex, [62, 63], a fact which plays a crucial role in the pathogenesis of Alzheimer's disease, inducing alterations to the cytoplasm of sensitive cells [64] by increasing mitochondrial reactive oxygen species (ROS) production [65,66]. This would cause further mitochondrial dysfunction [67], since the lack of histones in mitochondrial DNA makes them very sensitive to oxidative stress[68,69].

Mitochondrial changes are also clearly associated with the over expression of the amyloid precursor protein (APP) [70], amyloid- β or expression of the APP751 form in cultured cells [71]. It is well documented that generation of amyloid- β may occur in the cisternae of the endoplasmic reticulum (ER), in the Golgi complex, the lysosomes as well as on the cell surface [72, 73], missing word here author assistance required been accumulated in the endosomes, the lysosomes, the multivesicular bodies [74] and the mitochondria [75]. In Alzheimer's disease intraneuronal amyloid precursor protein and amyloid- β are mostly localized to mitochondria [75]. Mitochondrial uptake of amyloid- β is mediated by the

translocase, which is found on the outer mitochondrial membrane (TOM) [76]. The binding site for amyloid beta is identified in the matrix of the mitochondria, as alcohol dehydrogenase (ABAD), which participates in the metabolism of aldehydes and its deficiency may be involved in the generation of oxidative radicals and in mitochondrial toxicity [77]. Amyloid- β may also induce mitochondrial dysfunctions by interaction with cyclophilin D, which is a subunit of the mitochondrial permeability transition pore [78]. A β PP cleaved by mitochondrial γ -secretase [79] is usually found in a transmembrane-arrested orientation in the mitochondria, in contact with the mitochondrial translocation complexes [80]. Accumulation of transmembrane-arrested A β PP may impede protein translocation and seriously disrupt mitochondrial function. In addition, alterations in the lipid composition of cellular membranes may influence proteolytic processing of A β PP and increase the release of Alzheimer's amyloid beta-peptide from membranes [81].

In addition, mitochondrial interactions and interconnections with neurofilaments and microtubules have been described at the level of electron microscopy as well as in fluorescence microscopy, dynamic light scattering, atomic force microscopy and sedimentation assays [82] which clarify that mitochondria may play a role in plotting the spectrum of morphological alterations in Alzheimer's disease [19,83,84].

The defective mitochondria in Alzheimer's neurons may not supply adequate levels of Adenosine Triphosphate (ATP), which is very important at the synaptic level for normal neural communication. The low levels of cellular ATP at nerve terminals may lead to the loss of synapses and synaptic function [83,85], causing cognitive decline ultimately.

Morphometric studies of the mitochondria in non-nerve cells in Alzheimer's disease revealed a significant reduction in mitochondria density in endothelial cells [86] as well as in fibroblasts and other cells obtained from patients with AD [87]. Mitochondria from fibroblasts grown in tissue culture from skin samples taken during autopsy of patients of Alzheimer's disease, took significantly less calcium than did fibroblast mitochondria from age matched normal controls, suggesting that Alzheimer's fibroblast mitochondria have impaired calcium

transport processes and showed increased sensitivity to oxygenic free radicals [88].

It is important to mention that the mitochondrial genome plays a role in risk for Alzheimer's disease [89,90] and maternal family history is associated with Alzheimer's disease biomarkers [91]. Many proteins are also important in mitochondrial morphological integrity and in binding to the cytoskeletal components [92]. Porin is a protein in the outer-membrane of the mitochondria that forms voltage dependent anionic channels between the mitochondrial inter membrane space and the cytosol. Porin may play crucial role in binding to cytoskeleton [93], because porin rich domains mostly contain binding sites for MAP2. In addition, recent evidence suggest that amyloid β increases the contact points between endoplasmic reticulum and mitochondria, a phenomenon that occurs in cellular stress [94], which usually increases ER-mitochondrial coupling[95].

In all of the cases it was noticed that morphological alterations of mitochondria are frequently associated with fragmentation of Golgi apparatus [31] in the Purkinje cells, granule, stellate cells and the climbing and mossy fibres of the cerebellar cortex [96].

Morphological alterations of the Golgi apparatus in neurons have been described in early cases of Alzheimer's disease, mostly in the hippocampus, the temporal isocortex as well as parietal and frontal cortex. The major processing activity of the Golgi apparatus is glycosylation of proteins, which is based on a large number of sequential steps, each requiring its own enzymes [97]. In addition, substantial functions of the Golgi apparatus receive molecules from the endoplasmic reticulum, process the molecules within their structure, and control sort and traffick proteins to their final destination, some of them are destined for secretion, and others for the extracellular matrix [98,99,100]. In a parallel way, proteins and lipids, which might serve as signaling components, are also associated with the regulation of Golgi organization and function [101].

Protein sorting and trafficking is a continuous process in neurons in health and disease. Any abnormal modification or deviation of protein trafficking may play

an important role in inducing a stream of phenomena which may lead to serious disorders [102,103]. A β PP during its trafficking to the cell surface and in the endocytic pathway, generates amyloid- β through sequential cleavages by β -secretase, [104] and γ -secretase, an intramembranous protease containing presenilin and three other membrane proteins, nicastrin, Aph-1 and Pen-2 [105]. The fragmentation of the Golgi complex, which occurs even in the early stages of Alzheimer's disease, pleads in favor of the hypothesis that impairment of trafficking in Golgi cisternae and the endosomes may be a decisive factor in amyloidogenesis. Moreover, the trans-Golgi network (TGN) is the principal sorting system of the secretory pathway and the main site of intersection with the endolysosomal system [106]. Alterations of the endosomal retrograde sorting pathway may promote the production of A β . The alterations of Golgi apparatus may also play substantial role in protein glycosylation, since the trans-Golgi network is associated with the catalysis of soluble glycoproteins [107].

In the last twenty years, fragmentation of Golgi apparatus was described in neurodegenerative disorders, such as in sporadic and in familial amyotrophic lateral sclerosis (ALS) [108, 109, 110], in human olivary hyperplasia [111]. In the amyotrophic lateral sclerosis, Golgi apparatus examined through immunohistochemical methods with an antibody against MG-160 protein demonstrated a substantial decrease in the number of its elements and fragmentation of its cisternae in the anterior horn cells, as well as in Betz cells of the motor cortex [112,113]. In transgenic mice, expressing a mutant human SOD1 (G93A), and fragmentation of the Golgi complex were also observed which were associated with vacuolization of mitochondria and of the smooth endoplasmic reticulum [114].

The fragmentation of Golgi complex and the decrease of the vesicles in cis- and trans-Golgi network in Purkinje cells of the cerebellum in early cases of Alzheimer's disease both coincide with the synaptic loss and the shortage of the dendritic branches and the pathological alterations of the spines. [96,115].

Understanding the role of mitochondrial factor in the etiopathogenetic cascade of Alzheimer's disease [116,117,118] may introduce new strategies inducing protection to mitochondria [119, 120], which might be beneficial in the treatment of early cases of Alzheimer's disease.

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Declared none

CONFLICT OF INTEREST

The author confirms that this chapter content has no conflict of interest.

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