

Alzheimer's Disease & Treatment

Chapter 3

Mitochondria in Alzheimer's disease: An Electron Microscopy Study

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Abstract

Alzheimer's disease is a progressive neurodegenerative disorder leading gradually in profound dementia, with all the tragic consequence on the everyday life and the social behavior of the patients. The pathogenetic background of the disease is quite enigmatic, implicating several innate mechanisms and additional exterior factors formatting a chain of pathogenetic processes, resulting in synaptic loss, selective neuronal loss and serious decline of the mental faculties eventually. Among the pathogenetic factors the oxidative stress and the mitochondrial dysfunction may play a substantial role in the initial stage of the disease. Mitochondrial alterations may be induced by amyloid toxicity, which by the oxidative stress initiates a chain of pathological procedures resulting in profound dementia. In early cases of Alzheimer's disease we attempted to describe in electron microscopy the mitochondrial alterations in the perikaryon of neurons and astrocytes as well as in the dendritic profiles, the dendritic spines and the presynaptic terminals. The most frequent morphological alterations of the mitochondria consisted of disruption of the cristae and the accumulation of osmiophilic material seen mostly in the synaptic components and the dendritic profiles even in areas where minimal Alzheimer's pathology was observed, such as in the cerebellum and the hypothalamus. A substantial number of neurons demonstrated, in addition to alterations of the mitochondria an obvious fragmentation of the cisternae of Golgi apparatus. Since mitochondrial pathology plays an important role in the pathogenetic mechanism of Alzheimer's disease, we would feel that any therapeutic strategy aiming at protecting the mitochondrial integrity would be beneficial in the treatment of early cases of Alzheimer's disease.

Keywords: Alzheimer's disease; Mitochondria; Golgi apparatus; Astrocytes; Synapses; Electron microscopy.

Abbreviations: AD: Alzheimer's disease; APP: Amyloid Precursor Protein; CytOX: cytochrome C oxidase; mtDNA: Mitochondrial DNA

1. Introduction

Alzheimer's disease (AD) is a multidimensional heterogeneous neurodegenerative condition of the presenility and senility, resulting in a profound irreversible dementia, which predicts one of most tragic epilogues of the life of the patients. The number of suffering people from Alzheimer's disease increases from decade to decade [1], creating a serious global socioeconomic and ethical problem [2,3]. Although the clinical phenomena don't coincide exclusively with the pathological findings [4], the accumulation of A β -depositions in the form of dendritic plaques and the tau pathology, appearing as neurofibrillary tangles characterize the neuropathological pattern of the disease in light microscopy [5,6,7].

Among the pathogenetic mechanisms the oxidative stress [8] associated with mitochondrial changes [9] seems to play a key role in shaping the wide spectrum of the functional and morphological alterations [10], which progressively occur in the brain even from the preclinical stage of the disease to the final one ceaselessly [11,12].

The clinical manifestation of Alzheimer's disease may start as a mild cognitive impairment [13] progressing to inability to encode new memories, to serious impairment of learning, to dramatic loss of professional capacities and verbal fluency, to an over simplification and regression of the social behavior and emotional interactions, resulting in the isolation of the patients in the framework of a tragic functional incapacity, in a state of profound dementia, concluding in a vegetative state at the end of life [14,15].

The pathogenetic mechanisms of the sporadic cases of Alzheimer's disease are obscure and enigmatic [16]. Thus, it is reasonable that a wide range of hypotheses has been proposed aiming at deciphering the etiology of the irreversible cognitive decline in AD [17]. Some of the hypotheses have attracted the attention and the respect of the neuroscientists for many years, enforcing attempts for a lasting, ongoing research, in the hope of a final verification of their value.

Among the many hypothesis the amyloid hypothesis [18,19], the synaptic dysfunction [20, 21, 22], the translational and post translational neurodegeneration [23], the tau pathology [23,24], the neuroinflammation [25], the cholinergic deficiency [26], the oxidative stress [27], the vascular alteration [28, 29, 30], the glucose hypometabolism [31], the autoimmune reaction [32,33], the endocrine dysfunction [34,35] and the alteration of the cell organelles, such as mitochondria [36, 37], endoplasmic reticulum [38], Golgi complex [39], microtubules [40], and synaptic vesicles [41], are still subjects of serious discussions and further investigation.

However, the etiopathology of the sporadic cases of Alzheimer's disease remains still undetermined in spite of the continuous extensive ongoing research in the fields of genetics [42], molecular biology [43,44], electron microscopy [45,46], neuropathology [47], neurochemistry [48], neuroimmunology [49], pathophysiology [50] and neuroimaging [51]. In an attempt to detect the initial alterations in early cases of Alzheimer's disease, we focused our study in the organelles of the neurons and the neuronal processes in those areas of the brain, which demonstrate, as a rule, the minimal accumulation of A β -depositions and neurofibrillary tangles (NFT), which are considered for years as the pathognomonic hallmarks of Alzheimer's pathology.

2. Material and Methods

2.1. Material

We have proceeded to electron microscopy study on specimens derived from 25 brains of patients who suffered from Alzheimer's disease at the early stages, obtained at autopsy 2 to 7 hours after death at a room temperature of 4o C. The range age of the patients at death was 55-80 years. The patients fulfilled, on repeated clinical examinations and laboratory investigations, all the neuropsychological, psychiatric and neurological criteria of AD, which was diagnosed one to three years prior to the end of their life.

The cognition of the patients was estimated and evaluated by a battery of neuropsychological examinations [52], including mini mental state examination (MMSE) [53, 54], dementia rating scale (DRS) [55,56], ADAS-COX test [57,58] and the brief memory executive test (BMET) [59].

All the patients were fluent in their native language and have had completed 18 years of continual education. In addition to detailed physical examination, the patients underwent an EEG examination and a carotid examination by duplex Doppler. Neuroimaging was performed included computerized tomography (CT), magnetic resonance imaging (MRI) of the brain and a single-photon emission computed tomography (SPECT). All the results of clinical and laboratory investigations were evocative for Alzheimer's disease

The patients passed away due to heart arrest.

Brains derived from apparently healthy individuals of the same age range with the AD patients, were used as normal controls.

2.2. Methods

2.2.1. Electron microscopy

Multiple samples of a small size (2×2×2 mm) were excised from many areas of the

brain including the hippocampus, the prefrontal area of the cortex, the superior parietal lobe, the occipital pole and the visual cortex, the Heschl's gyrus of the temporal neocortex, the hypothalamus, the mammillary bodies and the medial geniculate bodies. Samples were also taken from the cerebellum, including all the lobules of the vermis and the cerebellar hemispheres bilaterally.

All the samples were immersed directly in Sotelo's fixing solution [60], composed of 1% paraformaldehyde, 2.5% glutaraldehyde in a cacodylate buffer 0.1M, adjusted at pH 7.35. Then they were immersed for post-fixation in 1% osmium tetroxide for 30 min. at room temperature and dehydrated in graded alcohol solutions and in propylene oxide twice.

After dehydration, the specimens were embedded in araldite mixture and cut in ultrathin sections by a Reichert ultratome. All the ultrathin sections were contrasted on grids with uranyl acetate and lead citrate, and studied in a Zeiss electron microscope of the type 9aS.

The electron microscopy study was particularly focused on the morphology of the organelles of neurons and astrocytes. The mitochondria were extensively studied and in detail described either in the perikaryon of neurons or in the dendritic profiles, the axons and the synaptic components. In addition, the Golgi complex, the endoplasmic reticulum and the morphology of synapses were also extensively studied.

A morphometric estimation of mitochondria and Golgi complex was accomplished on micrographs of a standard magnification of 56,000 X. The analysis of each macrograph was performed with J image analyzer. The surface area of mitochondria, the volume as well as the circularity ratio (CR) were calculated on a total of 8,000 mitochondria.

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

3. Results

The mitochondria in Alzheimer's brain were characterized by an impressive polymorphism, consisted of wide variation of size and shape, as it was observed in the large majority of neurons at any studied area of the brain and the cerebellum. The cristae were fragmented or disarranged, particularly in mitochondria located in the dendritic branches, the spines and the presynaptic terminals (**Figure 1**). Marked morphological alterations of mitochondria were observed also in areas with minimal Alzheimer's pathology, namely in the cerebellar cortex (**Figure 2**) and the hypothalamus.

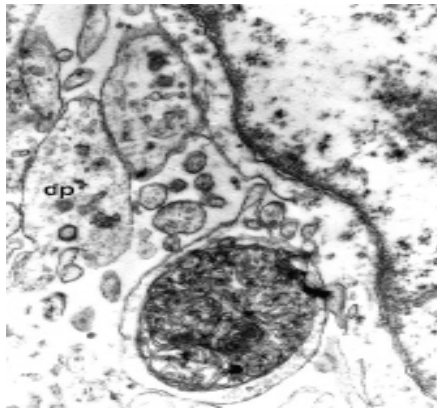


Figure 1: Fragmentation of mitochondrial cristae and complete disarrangement of mitochondrial structure in a dendritic profile (dp) in the molecular layer of the cerebellum in a case of AD. Electron micrograph Mag. 248,000×.

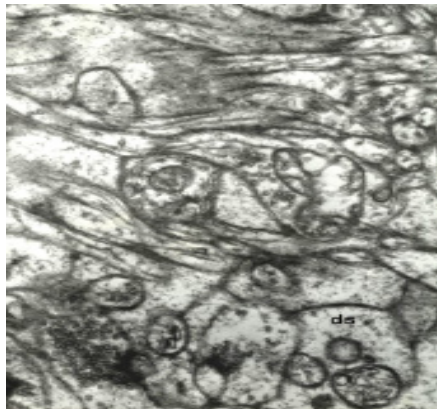


Figure 2: Small round mitochondria in dendritic spines (ds) in the molecular layer of the cerebellum in a case of AD. The disruption of the mitochondrial cristae is obvious. Electron micrograph Mag. 248,000×.

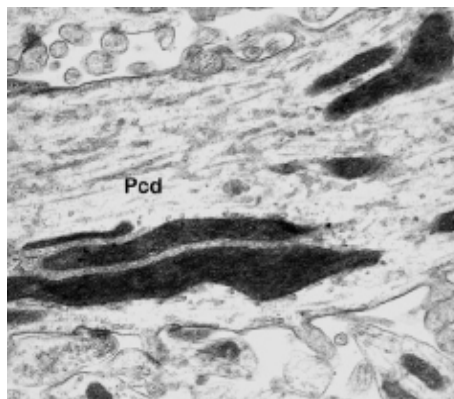


Figure 3: Very elongated mitochondria in Purkinje cell dendritic branch (Pcd) in the molecular layer of the cerebellum in a case of AD. Electron micrograph Mag. 248,000×.

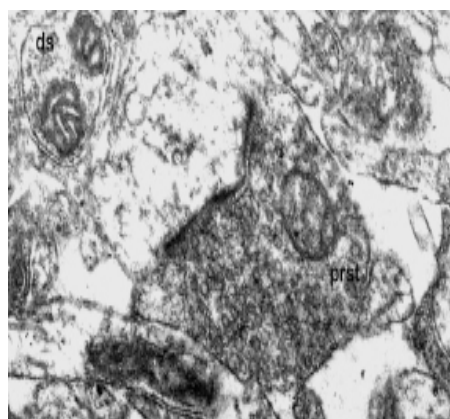


Figure 4: Small round mitochondria in dendritic spines (ds) and presynaptic terminal (prst) in the suprachiasmatic nucleus of the hypothalamus in a case of AD. Electron micrograph Mag. 248,000×.

Very large mitochondria were observed in the soma and the dendritic profiles of Purkinje cells of the cerebellar cortex in the vermis and the hemispheres (**Figure 3**). Disruption of mitochondrial cristae was also observed in a substantial number of pre- and postsynaptic terminal in hypothalamic nuclei (**Figure 4**). Morphological alterations were also seen in granule cells, in climbing and mossy fibers of the cerebellum, in the visual and acoustic cortex, in neurons of the prefrontal area of the cerebral cortex, in medial geniculate bodies, in the mammillary bodies and in the majority of hippocampal neurons. The neurons of the visual cortex contained small round mitochondria in the perikaryon, the dendritic branches and spines showing a substantial disarrangement of cristae. Marked morphological alteration of mitochondria was also observed in astrocytes of the cerebral cortex and the subcortical white matter.

In morphometric estimation the mitochondria, in normal control aged brains 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.

The morphometric estimation of the mitochondria in the soma, the dendrites and the dendritic spines of a considerable number of neurons of the suprachiasmatic nucleus in AD brains revealed that they have an average diameter of 440 ± 250 nm and a mean axial ratio of 1.7 ± 0.2 .

Fragmentation of the cisternae of Golgi apparatus was noticed in the perikaryon of a substantial number of neurons in the cerebral and the cerebellar cortex. The dendritic spines of the majority of neurons in the cerebral and the cerebellar cortex were dramatically reduced in number and size. Most of the presynaptic terminals included small round and dense mitochondria and showed dramatic decrease of the number of synaptic vesicles (**Figure 5**).

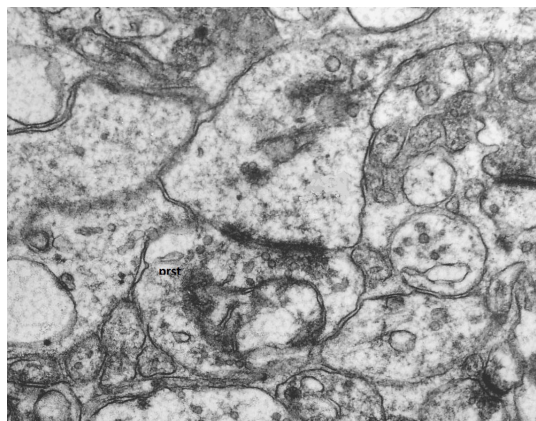


Figure 5: Presynaptic terminal (prst) including deformed mitochondrion with disruption of the cristae in the molecular layer of the vermis of the cerebellum in a case of AD. The presynaptic terminal is characterized by impressive poverty of synaptic vesicles. Electron micrograph Mag. 248,000 \times .

4. Discussion

Mitochondria are very dynamic organelles playing an essential role in energy supply and viability of the cells been involved in many metabolic pathways [61]. Mitochondrial dysfunction, which is normally associated with ageing, may be also a crucial factor in neurodegenerative disorders including Alzheimer's disease, given that mitochondria have a marked sensitivity to cellular stress [62,63].

Mitochondria hypothesis in the pathogenesis of Alzheimer's disease [64] is mainly based on the important role that mitochondrial dysfunction may play in the early stages of Alzheimer's disease by inducing energy deficiency and oxidative stress, which would dramatically increase β -amyloid ($A\beta$) neurotoxicity [65]. In addition, the combined effect of high calcium ions with oxidative stress may induce further impairment of their mitochondrial function, leading to release of cytochrome C and triggering the initiation of the intrinsic pathway for apoptosis [66,67].

It is important that decrease in energy production, altered cytochrome C oxidase (CytOX) activity and calcium homeostasis and oxidative stress are among the earliest detectable phenomena in AD, associated with mitochondrial dysfunction, which have a serious effect on synaptogenesis and neuronal plasticity [68,69,70].

Mitochondria and mtDNA are very sensitive to oxidative damage, due to lack of histones in mitochondrial DNA [123,124] and inversely mitochondrial alterations may induce or enhance the existing oxidative stress, a fact advocating in favor of an intimate early association between oxidative stress and mitochondrial abnormalities [71,72].

Oxidative stress may also enhance the production and the aggregation of $A\beta$ peptide, which contribute extensively in the pathogenetic mechanism of AD, increasing also mitochondrial vulnerability [73,74]. The overproduction of $A\beta$ peptide in AD induces fission and fragmentation of mitochondria, a fact that further increases oxidative stress and causes a considerable decline of energy production, which is associated with the increased expression of Dynamin-related protein 1 (Drp1) [75].

The $A\beta$ peptide enhances the activity of Drp1 protein in neurons, which subsequently induces morphological alteration of the mitochondria and increases the mitochondrial dysfunction in AD. In a parallel way, oxidative stress [76] may induce additional morphological alterations of mitochondria, resulting in deficiency of mitochondrial electron transport proteins, with considerable consequences upon the energy supply of nerve cells, a fact which has been extensively described in Alzheimer's disease and other degenerative conditions of the brain [77, 78, 79, 80].

In Alzheimer's disease intraneuronal amyloid precursor protein and amyloid- β peptide are mostly located in mitochondria [81], where amyloid- β peptide may induce mitochondrial dysfunctions by interaction with cyclophilin D, which is a subunit of the mitochondrial permeability transition pore [82].

Amyloid- β peptide may also interact with A β binding alcohol dehydrogenase (ABAD) on the mitochondrial membranes and induce further mitochondrial dysfunction [83]. Moreover, alterations in the lipid composition of cellular membranes may influence proteolytic processing of APP and increase the release of Alzheimer's amyloid beta-peptide from membranes [84].

Mitochondrial alterations are also closely related with over expression of the amyloid precursor protein (APP) and the accumulation of amyloid- β peptide [85, 86]. The A β peptides are generated either extracellularly or within the cisternae of the endoplasmic reticulum (ER) and the mitochondria [86]. APP is folded and modified in the ER and transported through the Golgi complex to the plasma membrane. Transmembrane arrest of APP causes considerable impairment of mitochondrial function in neurons [85, 87]. Over more, A β peptide inhibits protein influx in the mitochondria, resulting in mutation of mitochondrial DNA (mtDNA), aggravating therefore the mitochondrial dysfunction and their consequent disintegration eventually [88]. Experimental studies, on the other hand, revealed that the soluble form of A β peptide causes a reduced mitochondrial membrane potential (MMP) and energy production [89].

From the morphological point of view, mitochondria in neurons have normally the majority of cristae composed of both tubular and lamellar segments [90]. It is also well known that shape and the size of the mitochondria are highly variable [91], since they undergo continual fission and fusion, which are necessary for cell survival and harmonious adaptation to changing conditions [92], been related, at the same time, with the processes of biogenesis [93] and the mitophagy [94].

In addition mitochondrial morphology is sometimes controlled by the cytoskeleton, namely the neurofilaments and the microtubules [95]. The alteration of the shape of the mitochondria, as a rule, occurs during their course through the axons, the dendrites, and in synaptic terminals, via anterograde transport [96].

Many proteins are also important for the mitochondrial morphological integrity and for binding them to the cytoskeletal components [97]. 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 [98]. Porin, which is also involved in the movement of adenine nucleotides across the outer mitochondrial membrane [99] may play crucial role in binding to cytoskeleton [100,101], since mitochondrial porin can translocate across both endoplasmic reticulum and mitochondrial membranes [102] and porin rich domains mostly

contain binding sites for MAP2 [103] . In addition, recent evidence suggest that amyloid β peptide increases the contact points between endoplasmic reticulum and mitochondria, a phenomenon that occurs in cellular stress, which usually increases ER–mitochondrial coupling [104].

Normally, one third of the mitochondria are in motion along with microtubules and actin filaments [105, 106], being in continuous transportation to regions where energy requirement is particularly high and urgent. The number of the mitochondria is adjusted, according to the requirement of energy by the neuron. It is expected reasonably that the dysfunctional mitochondria may undergo mitophagy [107], a fact which is associated with neurodegeneration [108] and many devastating conditions of the brain.

It is worth to emphasize that morphological alterations of the mitochondria in AD are also observed in areas of the brain with minimal Alzheimer's pathology, such as in the cerebellum, the hypothalamus, the mammillary bodies [109] in neurons lacking neurofibrillary tangles [110], suggesting that mitochondrial pathology is independent of the accumulation of neurofibrillary tangles and dendritic plaques and might be among the earliest phenomena of Alzheimer's neuropathological alterations.

The most dramatic morphological alterations of the mitochondria are seen in dendritic profiles and the synaptic terminals. The defective mitochondria in AD neurons may not supply adequate levels of Adenosine Triphosphate (ATP), which is very important factor at the level of the synapses for the normal neural communication. The low levels of cellular ATP at nerve terminals may lead to loss of synapses and considerable decline of synaptic function, causing serious cognitive impairment.

Morphometric studies of the mitochondria in non-nerve cells in AD revealed a significant reduction in mitochondrial density in endothelial cells [111] as well as in fibroblasts and other cells obtained from patients with AD [112].

Mitochondria from fibroblasts of skin samples taken during autopsy from men who suffered from AD, grown in tissue culture, revealed significantly less calcium uptake than did mitochondria of fibroblasts from age matched normal controls, suggesting that Alzheimer's fibroblast mitochondria have impaired calcium transport processes and showed increased sensitivity to oxygenic free radicals [113].

Mitochondrial alterations in AD are also observed in astrocytes in the cortex and the subcortical white matter. Astrocytes participate actively in the degradation of neuronal mitochondria via the process of trans-cellular mitophagy [114,115], which occurs following internalization of axonal mitochondria by astrocytic processes, which normally contain very small mitochondria [116, 117]. The mitochondrial alterations of the astrocytes in early case

of Alzheimer's disease enhance the noxious role of the A β peptide on the function and the integrity of the astrocytes [118], with serious implications on neuroprotection [119], due to increased excitotoxicity, which would be a reasonable consequence of the disruption of glutamate/GABA-glutamine cycle [120].

The fact that maternal influence seems to be a risk factor for Alzheimer's disease morbidity, according to epidemiologic studies [121,122] and to combined neuropsychological and neuroimaging investigations [123] plead in favor of the substantial role that mitochondria may also play in the pathogenetic cascade of Alzheimer's disease.

In all of the studied cases it was noticed that the morphological alterations of mitochondria in neurons and astrocytes are frequently associated with the fragmentation of Golgi apparatus and the decrease of the vesicles in cis- and trans-Golgi network [124, 125,126]. It was also observed that the morphological alterations of the mitochondria and the fragmentation of Golgi complex coincide with the dendritic and synaptic pathology in early cases of Alzheimer's disease [127,128,129].

Understanding the important role that mitochondrial pathology plays in the etiopathogenetic background of Alzheimer's disease, inducing particularly the degeneration of dendritic branches and spines new therapeutic strategies aiming at protecting the mitochondria and preventing oxidative stress, calcium imbalance and eventual apoptosis might be beneficial in the treatment of early cases of AD.

5. References

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