Dendritic pathology in Alzheimer's disease

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ABSTRACT

Dendritic pathology and decrease of dendritic spine density are prominent phenomena in early cases of Alzheimer's disease, which correlate significantly with the progressive decline of the mental faculties. In previous studies we have described the pathological alterations of the dendrites and the dendritic spines in the prefrontal area of the cortex and the cerebellum. In this study we attempted to describe the morphological alterations of the dendrites and the dendritic spines, quantifying them in the acoustic and the visual cortices of eleven cases of Alzheimer's disease, applying Golgi staining and electron microscopy. In addition, describing also the ultrastructural changes of the mitochondria in the dendrites and the dendritic spines we noticed that mitochondrial pathology correlates substantially with the dystrophic dendrites, the loss of dendritic branches and the pathological alteration of the dendritic spines. We would hypothesize that mitochondrial alterations may play a very important role in dendritic degeneration and the loss of dendritic spines and we should have thought that therapeutic strategies protecting the mitochondria may be beneficial in Alzheimer's disease.

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1. Introduction

Alzheimer's disease is the most common age dependent progressive dementia, involving a number of cellular and biochemical mechanisms resulting in synaptic alterations [12], neurofibrillary degeneration, extracellular deposits of \( \alpha \) \( \beta \) peptide and selective neuronal loss.

From the neuropathological point of view neurofibrillary tangles (NFT) and extracellular amyloid accumulations, defined as neuritic plaques (NP) are the main hallmarks of the disease [3,4].

Synaptic alterations, which are prominent even in the early stages of Alzheimer's disease, may play a very important role in the gradual decline of the higher mental faculties, since they correlate better with cognitive impairment than tau pathology, neuronal loss and neuritic plaques [5].

In two independent studies it has been estimated that the correlation between synaptic density and cognitive status is approximately 0.7 [5,6]. Synaptic alterations are associated with dendritic pathology, which has been noticed long ago, since dystrophic dendrites have been described as a frequent finding in Alzheimer's disease already in the late sixties [4].

The cause of Alzheimer's disease remains enigmatic, in spite of the continuously ongoing research on the field. There is, however, a substantial body of increasing evidence, which pleads in favor of the possible implication of mitochondrial dysfunction in the pathogenesis of late-onset neurodegenerative disorders, including Alzheimer's disease [8–11].

Mitochondria are vital organelles, for the controlling of cell's homeostasis and viability, by virtue of providing most of the energy for the cellular processes. It is important that mitochondria play a critical role in maintaining cellular calcium homeostasis and cellular signaling cascades for both apoptotic and necrotic cell death pathways [12–14].

The proper intracellular distribution of mitochondria is adapted to cellular physiology. High concentration of mitochondria occurs, therefore, in subcellular regions with high metabolic requirements, such as in the vicinity of active growth cones of developing neurons [15]. Normally, the number of mitochondria in dendrites correlates with synapse development and may play an important role in the morphogenesis of the spines and the synaptic plasticity [16].

In the present study, we attempted to proceed in morphological and morphometric estimation of dendritic pathology in Alzheimer's disease, correlating also the morphological alterations of the dendritic spines with the mitochondrial alterations.

2. Materials and methods

Brain tissue was obtained at autopsy from eleven cases, five men and six women, aged 55–84 years, who fulfilled the clinical, neuropsychological and laboratory diagnostic criteria of Alzheimer's disease. All the patients have had a definite history of dementia, which lasted three to seven years. The mean education of the patients was 15.5 years and all of them have spoken the native language fluently.

Screening procedures included medical history, medical examination, cardiological investigation, clinical neurological assessment, psychiatric and neuropsychological examinations. All the patients underwent EEG, carotid duplex Doppler, CT scanning and magnetic resonance imaging.

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resonance imaging (MRI) of the brain, and single photon emission
computed tomography (SPECT). The mental status of the patients was
assessed by the Mini-Mental State Examination (MMSE) and demen-
tia rating scale (DRS). The neurological and neuropsychological
estimations as well as the laboratory investigation of the patients
were evocative of Alzheimer's disease.

After the death of the patients, the autopsy was performed within
3 h. The brains were excised at a temperature of 4 °C.

Ten additional brains macroscopically intact, derived from cogni-
tive normal individuals, who died accidentally, were used as normal
controls. The brains of the patients who suffered from Alzheimer's
disease were matched with control brains age by age.

Multiple samples from the visual cortex (Brodman 17 area, V1
cortex) and the acoustic cortex (Heschl gyri, Brodman 22 area) were
excised bilaterally and immersed immediately in Sotelo's fixing
solution, consisted of 1% paraformaldehyde, 2.5% glutaraldehyde in
cacodylate buffer 0.1 M, adjusted at pH 7.35, where remained for 3 h at
4 °C.

Then the specimens were postfixed by immersion in 1% osmium
tetroxide for 30 min at room temperature and dehydrated in graded
alcohol solutions and propylene oxide. The specimens were
embedded in araldite mixture.

Thin sections at silver interference colour, were performed in a
Reichert ultratome, electron contrasted with uranyl acetate and lead
citrate and grids were studied in a Zeiss 9aS electron microscope.

We studied the morphology of the mitochondria in the dendritic
profiles and the dendritic spines in the majority of neurons of the
acoustic and the visual cortex. Micrographs of section planes at a low
magnification of 5000× were used for identification of the neurons
and their dendrites and additional micrographs of a standard
magnification of 56,000× were used for the morphometric estimation
of the mitochondria, the dendritic profiles and the dendritic spines.

Circularity ratio (CR) was introduced to represent the shape of the
mitochondria. The CR was calculated through the following formula:

\[
CR = \frac{4 \pi A}{L} \quad \text{(A = area in nm, L = perimeter in nm)}
\]

Statistical analysis was based on the Student's t test on the basis of
600 measured mitochondria from twenty specimens (ten from
Alzheimer's brains and ten from normal controls). Observations of
\( p < 0.05 \) were considered significant.

The remaining parts of the acoustic (Hessl gyri, Brodmann 22 area)
and the visual (Brodman 17 area, V1 cortex) cortices were processed
for staining with haematoxylin and eosin, Bodian's staining [17] and
Gallyas staining [18,19] as well as for silver impregnation techniques,
according to rapid Golgi staining and Golgi-Nissl staining [20]. Thus,
they were cut in coronal sections, after a month's fixaction in formalin
and immersed in potassium dichromate (7 g potassium dichromate in
300 ml water) for ten days. Then the specimens were immersed in 1%silver nitrate for ten days, according to the rapid silver impregnation
technique introduced by Golgi [21,22].

Following a rapid dehydration in graded alcohol solutions, the
specimens were embedded in paraffin and cut, some of them at
100 µm and some at 10 µm, alternatively in order to have on one hand
a complete three dimensional visualization of the neurons in the thick
sections and on the other hand a fine visualization of the dendritic
spines in the thin sections of the 10 µm. We applied the Golgi-Nissl
method (post-staining with methylene blue) [20] on the sections of
10 µm in order to obtain the visualization of the total neuronal
population of each section. The thick sections of the 100 µm were
mostly used in order to obtain a three dimensional visualization of the
neurons, which have been impregnated by silver nitrate.

The sections were mounted in permount, between two cover slips
and studied in a Zeiss Axiolab Photomicroscope. The pictures of the
slices were transferred from the microscope to a computer system,
through a Sony video camera and were presented at the screen.

The neuronal dendrites were quantified by counting the number of
branches at each order starting from the apical dendrite and
continuing with the basal dendrites, according to Sholl analysis [23].

In each one of the brains the density of the dendritic spines was
measured in the apical as well as in the basal dendrites of hundred
neurons in the visual and the acoustic cortex, following a three
dimensional reconstruction of each one of the quantified neurons.
Namely, we measured the number of the spines per 100 µm of
dendrite in the total dendritic surface. The data of the quantifications
were compared with the relevant data of the normal controls.

The mean dendritic spine density was estimated by counting the
number of the dendritic spines on 100 µm of dendritic branches on 10
randomly distributed secondary dendritic branches of the apical and
the basal dendrites. Thus, the total number of spines per dendritic
length per neuron was estimated, as indicative of the dendritic spine
density.

3. Results

3.1. Light microscope; Golgi impregnation technique

The neuropathological study of the brains of patients who suffered
from Alzheimer's disease, verified the clinical diagnosis, since in
addition to neuronal loss, Bodian [17,19] and Gallyas methods [18,19]
visualized neuritic plaques and neurofibrillary tangles, which are the
morphological hallmarks of Alzheimer's pathology.

In Golgi and Golgi-Nissl silver impregnation techniques it was
revealed that the majority of the neurons in the visual and the acoustic
cortices demonstrated a marked abbreviation of the dendritic field
in comparison with normal controls. Most of the neurons have lost the
tertiary dendritic branches and they retained the primary apical
dendrite and some of the secondary dendritic branches (Fig. 1). Large
ten number of neurons in both of the cortical areas demonstrated
dystrophic dendrites.

However, the most impressive finding was the tremendous
decrease of the number of the dendritic spines as well as the loss of
the distal spines (Fig. 2). Large number of the secondary dendritic
branches was conspicuously denuded of dendritic spines. The
branches of the apical part of the dendritic tree as well as the basal
arborizations appeared to be equally affected by the spine depletion.

In addition, the morphometric alterations of the spines in
Alzheimer's disease brains were particularly obvious. The dendritic
arborization in the acoustic and the visual cortices in Alzheimer's 178

![Fig. 1. Neurons from the fifth layer of the visual cortex in a case of Alzheimer's disease. A tremendous decrease of the number of the dendritic branches is noticed. The majority of the tertiary dendritic branches has been degenerated. Rapid Golgi staining (magnification 1000×).](image-url)
brains showed a 45% reduction of the over all covered area by the dendritic fields in relation to normal controls (Fig. 3).

Concerning the dendritic spines a 52% reduction was estimated in the acoustic and visual cortices of Alzheimer’s disease brains as compared to normal controls (Fig. 4).

3.2. Electron microscope

The ultrastructural study of the visual and the acoustic cortices revealed an impressive polymorphism of the mitochondria in the soma of the nerve cells as well as in the axonal and dendritic profiles, in the majority of neurons. A substantial number of mitochondria showed disruption of the cristae and some of them demonstrated small round accumulations of osmiophilic material. The mitochondria demonstrated a wide variation of size and shape in comparison with those of the normal control brains. Some of the dendritic profiles included mitochondria, which showed an impressive polymorphism in the arrangement of the cristae.

Morphological alterations of the mitochondria were seen in the soma of the astrocytes, the perivascular astrocytic processes and the astrocytic sheaths in Alzheimer’s brains in the acoustic and visual cortices, in contrast to normal controls, where the mitochondria were unremarkable.

From the morphometric point of view the ellipsoid mitochondria in normal controls appear to have an average diameter of 650±250 nm and a mean axial ratio of 1.9±0.2. The round or global mitochondria in normal controls appeared having a mean mitochondrial radius of 350 nm. In Alzheimer’s disease brains, the ellipsoid mitochondria of the neurons appear to have an average diameter of 510±250 nm and a mean axial ratio of 1.7±0.2. The round mitochondria have a mean radius of 280 nm.

The dendritic spines in the visual and acoustic cortices in Alzheimer’s disease rarely contain mitochondria; however some of the giant spines included very elongated abnormal mitochondria, dense bodies, and multivesicular bodies, atrophied spinal apparatus and small cisternae of smooth endoplasmic reticulum.

Large number of neurons in the acoustic and visual cortices in Alzheimer’s brains demonstrated dendritic varicosities and distorted spine shapes. The majority of the varicosities contained abnormal polymorphous elongated mitochondria. In a substantial number of neurons in the fifth layer of the acoustic and visual cortices some axonless or unattached spines were seen protruded from the secondary dendritic branches of the apical dendrite. The spinal density, which was estimated also at the ultrastructural level, was dramatically reduced as compared with normal controls (Fig. 5).

4. Discussion

Golgi silver impregnation technique is one of the most efficient methods for the visualization of the dendritic arborization of neurons, in order to analyze dendritic parameters, allowing a three dimensional imaging of the entire dendritic field of the impregnated neurons [18,20]. Using Golgi technique, in spite of the many technical delicate aspects, concerning the number of visualized neurons, we could...
On the basis of the hypothesis that mitochondrial pathology may be associated with the dendritic alterations and the substantial decrease of spine density in Alzheimer’s disease, therapeutic strategies inducing protection to mitochondria [40] might be introduced in the treatment of early cases of Alzheimer’s disease.

References


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