DENDRITIC AND SPINAL PATHOLOGY OF THE PURKINJE CELLS FROM THE HUMAN CEREBELLAR VERMIS IN ALZHEIMER’S DISEASE

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SUMMARY

Background: Alzheimer’s disease constitutes one of the main causes of dementia. It is clinically characterized by memory impairment, deterioration of intellectual faculties and loss of professional skills. Furthermore changes in equilibrium and limb coordination are clinically demonstrable in persons with Alzheimer’s disease. In the present study we tried to figure out possible changes of the Purkinje cells in Alzheimer’s disease brains.

Subjects and methods: We studied the Purkinje cells from the vermis of the cerebellum in 5 Alzheimer’s disease brains Golgi technique.

Results: In the Purkinje cells from the inferior surface of the cerebellar hemispheres severe dendritic and spinal pathology consisting of loss of distal dendritic segments and alterations of dendritic spine morphology can be noticed in Alzheimer’s disease brains.

Conclusions: The morphological and morphometric estimation of the dendrites and the dendritic spines of the Purkinje cells from the inferior surface of the cerebellar hemispheres in Alzheimer’s disease brains revealed substantial alterations of the dendritic arborization and marked loss of the dendritic spines, which may be related to cognitive impairment and motor deficits in Alzheimer’s disease.

Key words: Alzheimer’s disease – cerebellum - Purkinje cells - Golgi method

INTRODUCTION

Alzheimer’s disease constitutes one of the main causes of dementia in senium and presenium. It is clinically characterized by memory impairment, deterioration of intellectual faculties and loss of professional skills (Baloyannis 1993, Perry & Hodges 2000).

AD brains are characterized by significant atrophy, being most obvious in the temporal and parietal lobes (Baloyannis 1993). Light microscopy reveals deposition of senile plaques and neurofibrillary degeneration initially in the entorhinal cortex, the hippocampus, and in the acoustic and visual cortex, in the frontal lobe and the cerebellum in the advanced stages (Baloyannis 1993, Wegiel et al. 1999, Larner 1997, Fukutani et al. 1996).

As we have shown in a previous study of ours the cerebellar cortex is characterized by a unique pattern of Alzheimer-type pathology, while there are only diffuse neuritic plaques and no neurofibrillary changes (Mavroudis et al. 2010). Furthermore, a loss of Purkinje cells and synaptic alterations in the mossy fibers, granule cell dendrites, parallel fibers and Purkinje cell dendrites with substantial loss of dendritic spines, and considerable decrease in number of granule and Golgi cells in the granule cell layer have been reported (Perry & Hodges 2000, Wegiel et al. 1999, Larner 1997, Fukutani et al. 1996).

The current study was carried out to investigate the alterations of the dendritic arborization and the dendritic spines of the Purkinje cells from the inferior surface of the cerebellar hemispheres in AD.

SUBJECTS AND METHODS

The present study is based on the morphological analysis of the dendritic arborizations of the Purkinje cells from the inferior surface of the cerebellar cortex from 5 patients suffered from Alzheimer’s disease and from 5 healthy individuals who died accidentally and were used as normal controls.
All the brains have been offered by the Laboratory of Forensic medicine and Toxicology of the Aristotle University of Thessaloniki.

All the patients have a clinical diagnosis of Alzheimer's disease by a neurologist and Mini-Mental State Examination scores below 12.

Gross examination of the brains was performed by a neuropathologist who was blinded to the participant's cognitive scores and medical history. Tissue blocks from multiple neocortical regions, hippocampus, entorhinal cortex, amygdala, visual cortex, auditory cortex and cerebellum were embedded in paraffin. To assess Braak stage, sections at the range of 10µm were stained with the Gallyas method for neurofibrillary pathology while for staining of neuritic plaques the Bielchowsky method was used. Neuritic plaques scores were determined by semiquantitative estimation of neocortical plaque density using the CERAD criteria. All the Alzheimer's disease brains were on Braak stages V/VI, fulfilled the Khachaturian criteria for definite Alzheimer's disease brains were on Braak stages V/VI, fulfilled the Khachaturian criteria for definite Alzheimer's disease and have indicative CERAD scores for Alzheimer's disease (Table 1).

### Table 1. Demographic features and Braak & Braak staging of the brains used in the present study

<table>
<thead>
<tr>
<th>Brain</th>
<th>Age</th>
<th>Gender</th>
<th>Braak &amp; Braak stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD1</td>
<td>67</td>
<td>Male</td>
<td>V/VI</td>
</tr>
<tr>
<td>AD2</td>
<td>65</td>
<td>Female</td>
<td>V/VI</td>
</tr>
<tr>
<td>AD3</td>
<td>72</td>
<td>Female</td>
<td>V/VI</td>
</tr>
<tr>
<td>AD4</td>
<td>69</td>
<td>Male</td>
<td>V/VI</td>
</tr>
<tr>
<td>AD5</td>
<td>76</td>
<td>Male</td>
<td>V/VI</td>
</tr>
<tr>
<td>NC1</td>
<td>86</td>
<td>Male</td>
<td>I/II</td>
</tr>
<tr>
<td>NC2</td>
<td>72</td>
<td>Female</td>
<td>0</td>
</tr>
<tr>
<td>NC3</td>
<td>75</td>
<td>Female</td>
<td>0</td>
</tr>
<tr>
<td>NC4</td>
<td>80</td>
<td>Female</td>
<td>0</td>
</tr>
<tr>
<td>NC5</td>
<td>69</td>
<td>Male</td>
<td>0</td>
</tr>
</tbody>
</table>

Only one of the brains used as normal controls was on Braak stage I/II, the rest having no NFTs and SPs at all (Table 1).

Histological criteria for the diagnosis of AD were those outlined by the National Institutes of health/ American Association of Retired Persons (NIH/AARP) Research Workshop on the Diagnosis of Alzheimer's Disease (Khachaturian 1985). All cases fulfilled the histological criteria for AD.

The brains after the excision from the skull were immediately immersed in a formaldehyde 10% fixing solution where they remained for at least 25 days. Then we excised small parts from the cerebellar cortex at the level of the vermis, which have been used for Golgi method. The specimens were immersed in a dilution of potassium dichromate (7 g of potassium dichromate and 1 ml of formaldehyde 37% in 300 mL of tap water) at a temperature of 18°C. They remained in that solution for 1 week and then they were immersed in aqueous solution of 1% silver nitrate where they remained for 1 more week at a temperature of 15°C in photoprotected environment. Afterwards the specimens were embedded in low-melting paraffin, cut with a slicing microtome at thick sections at the range of 120µm, covered with entellan and studied with a Carl Zeiss Axioskop plus light microscope.

The morphological criteria that were used for the selection of neurons, were integrity and dark homogeneous impregnation throughout the extent of the dendrites, cell bodies located in the middle part of the section thickness to minimize the number of branch segments cut off the plane of the section, and relative isolation from other impregnated cells, blood vessels, and silver deposits (artifacts) placed nearby (Jacobs et al. 1997).

For each one of the brains 10 cells were selected. The morphological study and morphometric analysis of the selected cells were carried out with the aid of Image J application on 100 digital images which were taken using an Amscope digital microscope camera.

Furthermore the selected cells were analyzed according to Sholl’s method of concentric circles (Sholl 1953); 15 concentric circles were drawn at 15µm intervals centered on the cell bodies, and dendritic intersections with each circle were counted. This procedure provided a measure of dendritic density as a function of distance from the cell body. Furthermore the distance of peak of the development of the dendritic arborization (critical value) and the dendrite maximum which is the maximum of intersections in the peak of the development of the dendritic tree were calculated (Sholl 1953).

Spine counts were carried out on the dendrites of the Purkinje neurons at standard magnifications of X1000. Visible spines were counted on 10 segments (10 mm in length) of the dendritic field. The first 5 segments were located on tertiary dendrites, and the second group of segments was located on quaternary dendritic branches. Dendritic spines were classified according to their morphology in long neck ones and short stubby spines. The densities of dendritic spines, as well as their size were estimated manually with the help of Image J software.

The thickness of the molecular layer of the cerebellar cortex, as well as the diameter of the cell soma, and the number of Purkinje cells has been measured in Nissl-stained specimens with the help of Image J application (Figure 1). Adjacent sections were cut in a microtome at the range of 8µm and used for Holzer method, in order to figure out if there is increased gliosis in AD brains.

### Statistical Analysis

A Student’s t-test was used to determine whether significant differences existed across the independent parameters from Purkinje cells from normal controls and AD brains (significance was taken as P<0.05). Furthermore, a Pearson correlation test was carried out in order to figure out if there is any correlation between Purkinje cell number and Purkinje cell dendritic complexity variables.
Purkinje cells from AD brains showed a noteworthy restriction of the dendritic arborization (Figures 2A, 2B) due to loss of terminal dendritic branches, while the number of primary and secondary dendrites per neurons did not exhibit significant difference between the two groups of the study. Sholl’s analysis demonstrated marked decrease of the number of intersections in the distal circles (Figure 3). The peak of the development of the dendritic arborization occurred at 85µm from cell soma for AD brains and at 130µm for the normal controls. Dendrite maximum was 21 and 56 respectively.

The area that is covered by the dendritic field for each of the Purkinje cells was considerably decreased in AD brains as indicated in Figure 4.

The total number of dendritic spines per Purkinje cell of AD brains was by 50% decreased, compared to normal controls (Figure 5, Figure 6). The majority of the remaining spines on AD brains were of the short-stubby type, while the Purkinje cells of normal controls bear spines of the long-neck type and only a few from the short-stubby type.

Although there was loss of Purkinje cells in AD brains, it was without statistical significancene. On account of the loss of Purkinje cells in AD brains, empty baskets are usually seen in Golgi method.

Pearson correlation test did not exhibit any correlation between Purkinje cell numbers and Purkinje cell dendritic complexity and spine variables.
Figure 3. Sholl’s analysis of the dendritic trees from AD brains (white line) and Normal controls (black line) (p<0.01, error bars indicate standard deviation)

Figure 4. Dendritic field area covered by the arborization of the Purkinje cells from AD brains and Normal controls (p<0.01, error bars indicate standard deviation)

Figure 5. Spinal density (number of spines per 10µm) from the Purkinje cells from AD brains and Normal controls (p<0.01, error bars indicate standard deviation)

Figure 6. (A, B) Higher magnification from a part of the dendritic tree of a Purkinje cell from an AD brain. Marks the loss of tertiary and quaternary branches and the substantial loss of dendritic spines. (C, D) Higher magnification from parts of the dendritic tree of a Purkinje cells from a normal control brain (Golgi method, Magnification 1000X)
DISCUSSION

Cerebellum was thought to be spared by Alzheimer’s disease, however recent studies showed a number of pathological changes, including loss of distal dendritic segments, decrease of the total number of dendritic spines, ubiquitin-immunoreactive dystrophic neurites and microglial proliferation of the Purkinje cells (Larner 1997, Mavroudis et al. 2010, Baloyannis et al. 2000, Dickson et al. 1990, Yamamoto & Hirano 1985, Fukutani et al. 1996). The present study confirms the involvement of the Purkinje cells in Alzheimer’s disease neuropathology.

Distal dendritic segments and dendritic spines were prominently affected in Purkinje cells from Alzheimer’s disease brains. These structures are the most plastic components of the dendritic arborisation (Michmizos et al. 2011); therefore these changes might be related to an impairment of mechanisms underlying neuroplasticity.

Soluble Ab peptide and Ab oligomers unsettle neuroplasticity imbalance, resulting in an impairment of synaptic stabilization (Cullen et al. 1997, Wang et al. 2002) and loss of dendritic spines and synapses in Alzheimer’s disease brains. Hyperphosphorylation of tau protein may also cause deleterious effects of neuroplasticity and may underlie its role in the aetiology of Alzheimer’s disease (Maccioni et al. 1995, Mandelkow et al. 1995). Nevertheless the molecular layer of the cerebellar cortex is characterized by a unique pattern of pathology in Alzheimer’s disease, which includes deposition of diffuse plaques and the absence of typical senile plaques, and only minor neurofibrillary changes (Wegiel et al. 1999, Mavroudis et al. 2010). Consequently there might be more mechanisms, such as oxidative damage, vascular pathology, blood brain barrier abnormalities that participate in the neuronal degeneration in AD (Baloyannis et al. 2000, Mavroudis et al. 2010).

Besides substantial spinal loss, the Purkinje cells from Alzheimer’s disease brains bear short-stubby dendritic spines in 90%; in contrast to normal controls the Purkinje cells of which possess mainly the long-neck type of spines. Spinal shape is modulated by a number of proteins via regulation of the actin cytoskeleton (Jaworski et al. 2009).

It is important to emphasize that the cerebellar cortex is affected in Alzheimer’s disease, despite the fact that neurofibrillary degeneration and typical senile plaques deposition are not prominent. Cerebellar cortex offers a valuable background for the further study of Alzheimer’s disease pathogenesis is and future treatment strategies.

The loss of distal dendrites and dendritic spines seen in thick sections of Golgi stained material, leads to a substantial decrease of the synaptic area and synaptic contacts of the Purkinje cells and this could contribute to the cognitive decline of AD. Furthermore the inferior surface of the cerebellar vermis is related to muscle tone of the antigavity muscles of lower body, which are affected in Alzheimer’s disease (Franssen et al. 1999). Therefore, changes in equilibrium and limb coordination that are clinically demonstrable in persons with Alzheimer’s disease might be correlated to the present findings.

CONCLUSION

The morphological and morphometric estimation of the dendrites and the dendritic spines of the Purkinje cells from the inferior surface of the cerebellar hemispheres in Alzheimer’s disease brains revealed substantial alterations of the dendritic arborization and marked loss of the dendritic spines, which may be related to cognitive impairment, equilibrium and limb coordination deficits in Alzheimer’s disease.

Acknowledgements: None.

Conflict of interest: None to declare.

References


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