Research Journal of Medical Sciences 1 (2): 55-61, 2007

Ultrastructural Features of Beta-Amyloid Plaques in Aged Transgenic Tg 2576 Mouse Brain with Alzheimer Plaque Pathology

[1]A.O. Ihunwo, [2]H.J. Luth, [1] R. Schliebs and [3]F.I.B. Kayanja
[1]Department of Neurochemistry,
[2]Department of Neuroanatomy, Faculty of Medicine, Paul Flechsig Institute for Brain Research,
University of Leipzig, Leipzig, Germany
[3]Department of Anatomy, Histology and Embryology, Faculty of Medicine,
Mbarara University of Science and Technology, Mbarara, Uganda

Abstract: Ultrastructural characteristics for βeta-Amyloid (Aβ) plaque and neuronal morphology were obtained from 24 month old (aged) transgenic Tg 2576 mice brains with Alzheimer plaque pathology using immunocytochemical and electron microscopic techniques. Immunoperoxidase staining showed extensive presence of Aβ plaques in the cortex, hippocampus and subcortical areas. There was a homogenous distribution of plaques in the cerebral cortex from the frontal to occipital pole. The entorhinal cortex showed some remarkable presence of plaques. The Aβ plaque morphology was a typical star-shaped appearance of diffuse amyloid plaque with abundant or numerous bundles of extracellular, non-branching and wavy amyloid fibrils radiating from the central dense core. The amyloid fibrils became less closely oriented at the periphery of the plaque. An amyloid interface was also observed in addition to degeneration neurons and dystrophic neurites. The neurites presented as discontinuous surface membranes of cells in the neighbourhood of neuritic plaques, with or without an amyloid core. Neuronal morphology in the cortex and hippocampus were altered in the vicinity of plaques with degenerating neurons showing more of apoptotic signs such as chromatin condensations, cell shrinkage and hyperchromatic nucleus. These data are comparable with some of the reported features in patients with Alzheimer's Disease (AD) and so the transgenic Tg 2576 mice do represent a reliable animal model for investigating AD related plaque pathology.

Key words: Beta-amyloid plaques, amyloid facing pole, dystrophic neurites, Tg 2576 mice

INTRODUCTION

Alzheimer's Disease (AD) is the most common neurodegenerative disorder in senile dementia and the presence of neuritic plaques and neurofibrillary tangles in various regions of the cortex, hippocampus and amygdala of the brain represents the diagnostic hallmark of AD (Khachaturian, 1985). It affects up to 20 million people worldwide and this is estimated to double by 2025 (Prince, 1997). The accumulation of βeta-Amyloid peptides (Aβ) in the brain triggers the pathological events leading to plaque formation in Alzheimer's Disease (AD) development (Hardy, 1997). Transgenic mouse lines expressing human βeta-Amyloid Precursor Protein (APP) with the 'Swedish' mutation (βAPP695swe, K670N/M671 L) designated Tg 2576, has been established (Hsiao *et al*., 1996).

Evidence is accumulating that the Tg 2576 mouse is a useful animal model for some pathological features of AD (Terai *et al*., 2001). The cerebral cortex is highly vulnerable to the lesions of Alzheimer's disease but the susceptibility varies from region to region as not all cortical areas are equally damaged (Akiyama *et al*., 2001). The Tg 2576 mice demonstrated an age-related accumulation of both senile and diffuse βeta-Amyloid plaques in the neocortex and hippocampus as revealed by thioflavine-S staining and immunolabelling for β- Amyloid (Apelt and Schliebs, 2001).

McGeer *et al*. (1992) reported an electron microscopic study of the beta-amyloid precursor protein sequence in normal and AD brain tissue in humans. Immunoelectron microscopic analysis of the Athena/Exempler mouse PDAPP transgenic model has also been reported by Masliah *et al*. (1996, 2001). An attempt to illuminate the precise nature of the pathology present and clarify the course of events leading to Aβ deposition in the doubly transgenic mouse (APPsw/PS1) model using light microscopy, electron microscopy and immunoelectron

Corresponding Author: Amadi O Ihunwo, School of Anatomical Sciences, Faculty of Health Sciences,
University of the Witwatersrand, 7 York Road, Parktown, Johannesburg, South Africa

microscopy immunocytochemistry has been reported by Kurt *et al*. (2001). Reports of investigation into the ultrastructural features of different transgenic animal models with AD are now beginning to emerge. We had previously reported degeneration of β-amyloid-associated cholinergic structures in transgenic APP (sw) mice (Luth *et al*., 2003). The aim of this study was to obtain additional electron microscopic information of β-Amyloid (Aβ) plaque from an aged transgenic Tg 2576 mouse brain with Alzheimer plaque pathology.

MATERIALS AND METHODS

Experimental animal-transgenic Tg 2576 mouse: The transgenic mouse used in this study were 24 month old and contained the human Amyloid Precursor Protein (APP) 695 with the double mutation Lys670_Asn and Met671_Leu (K670N, M671L), which was found in a large Swedish family with early onset of Alzheimer's disease as developed by Hsiao *et al*. (1995, 1996). The transgene is expressed in C57B6/SJL F1 mice (kindly provided by Dr. Karen Hsiao of University of Minnesota), backcrossed to C57B6 breeders. The mice used belonged to the second (N2) generation. The transgenicity was determined in two months old animals in tail biopsy material by Polymerase Chain Reaction (PCR) as described by Hsiao *et al*. (1995). Animal treatment was according to the procedure approved by the University of Leipzig, Germany.

Tissue preparation: Under deep anaesthesia transcardial perfusion was carried out with saline, followed by 50 mL of fixative (4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4) using a peristaltic pump. Brains were carefully removed from the skull and post-fixed in the same fixative overnight at 4°C. Cryoprotection was carried out by putting the brain tissue in a 30% sucrose solution before sectioning using a freezing microtome (Leica SM2000R model, Germany). The brain was frozen at -40°C and cut in the coronal plane (rostral-caudal direction) at 30 μm thickness for light microscopy and 60 μm for electron microscopy. Serial sections were collected in vials containing 0.1M phosphate buffer.

Immunocytochemistry

Single labelling for β-Amyloid plaque (Aβ): Immunocytochemical staining of βeta-Amyloid plaques were performed using a polyclonal rabbit AS720 (Schering AG, Berlin 1:1000) in blocking solution at 4°C overnight. Tissues were rinsed 3 times for 5 min in TBS, pH 7.4 and incubated with secondary antibody in 2% BSA in TBS using biotinylated-goat-anti-rabbit-AB (1:500) for 1 h at room temperature. The sections were rinsed 3 times for

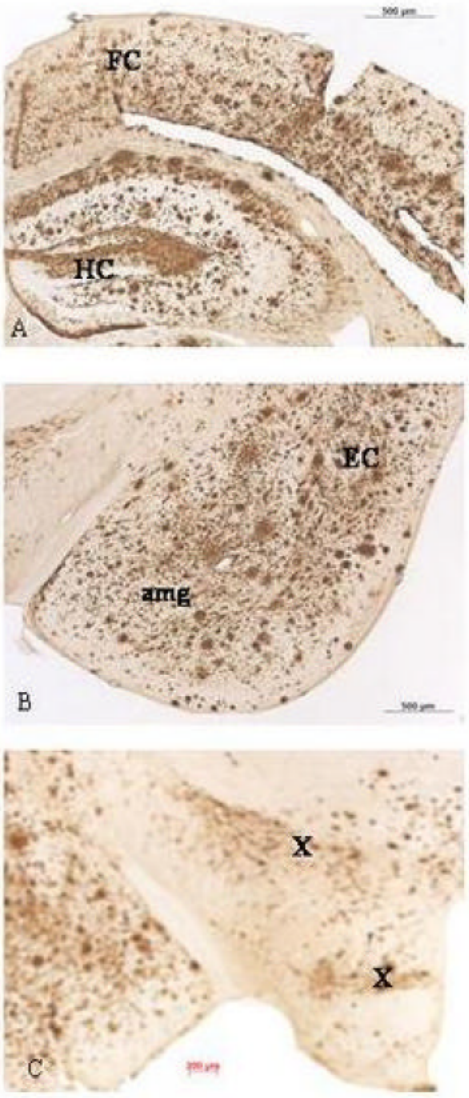

Fig. 1: Photomicrograph of antiserum AS720 immunoreactivity detecting amyloid plaque in 24-month-old transgenic Tg2576 mouse brain. A shows the area of the Frontal Cortex (FC) and Hippocampus (HC). B shows the Entorhinal Cortex (EC) and the amygdale (amg) regions and C, the subcortical regions of the thalamus. Note the extensive distribution of plaque. DAB Staining. Bar scale A and B 500 μm, C: 200 μm.

5 min in TBS, pH 7.4 before the Avidin-Biotin-Complex (ABC) method by incubating in 12.5 μL streptavidin+5 μL biotinylated-Horesradish Peroxidase (bio-HRP) in 2% BSA in TBS, pH 7.4 for 1 h. 3,3'-Diaminobenzidine tetrahydrochloride (DAB) staining was performed using

4 mg DAB in 5mL 0.5M TB pH 7.6 + 4Kl H2O2. The sections were air-dried overnight, passed through toluene for a minute, mounted on albumin-coated slide and cover-slipped using Entellan® (Merck, Germany).

Electron microscope immunocytochemistry: The method was as reported previously (Luth *et al*., 2003). In summary, small piece of the DAB stained entorhinal region of the cerebral cortex and hippocampus were excised and postfixed in 1% osmium tetroxide (OsO4 stock Fluka, Schweiz, Germany) for 60 min in darkness an\t room temperature, dehydrated up to 50% alcohol, counterstained with 1% uranyl acetate, dehydrated in 70, 80 and 100% alcohol and embedded flat in durcupan (Fluka, Buchs, Switzerland). After polymerisation of the resin, the sections were attached to the resin block. Ultra thin sections were cut with an ultra-microtome (Reichert Ultracut, Austria).

Evaluation: Light microscopy analysis of stained brain sections was carried out using a Zeiss Axioplan 2 light microscope (Oberkochen, Germany) fitted with a Sony DXC-930P colour video camera system. Ultrastructural examination and electron micrographs were taken with a 912 Omega transmission electron microscope (Zeiss, Germany).

RESULTS

Immunocytochemistry: DAB staining of 30μm sections showed extensive presence of Aβ plaques establishing Alzheimer plaque pathology in the cortex and hippocampus (Fig. 1). There was a homogenous distribution of plaques from the frontal to occipital pole. The Entorhinal Cortical region (EC) showed some predominance (Fig. 1b). In addition, extensive distribution was also observed in some other sub-cortical regions (Fig. 1c).

Semi-thin sections: Plaques were visible with normal (n) and degenerating (arrows) neurons in the entorhinal cortex (Fig. 2a). Plaque size was between 55-95μm in length and 60-90μm in width in the entorhinal cortex at X400 magnification. Glia cells were also observed. These different cells were usually within the vicinity of plaques. The degenerating neurons (arrows) showed more of an apoptotic than a necrotic process.

In the hippocampus (Fig. 2b and c), a localised glial reaction, consisting of dark atrophic neurons interspersed between histologically normal neurons was observed in the granular layer of the dentate gyrus. No plaque was visible in its vicinity and the surrounding neuropil.

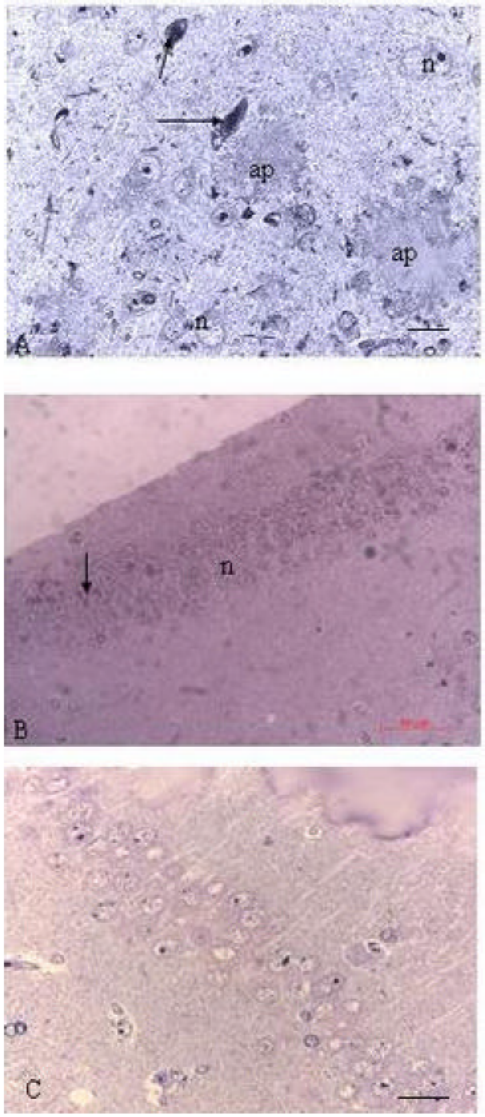

Fig. 2: Photomicrograph of semithin sections of the entorhinal cortex of 24-month-old transgenic Tg 2576 mouse brain showing amyloid plaque (ap), normal (n) and dystrophic (arrows) neurons (Fig. A). Figure B shows a section of the hippocampus showing a normal region (n) and a localised glial reaction with dystrophic neurons (arrow). Note the normal appearance of the hippocampus in Fig. C. Toluidine blue staining. Bar scale 500 μm

Ultrastructural features

Plaque morphology: The section from the entorhinal cortex showed a typical star-shape appearance of diffuse

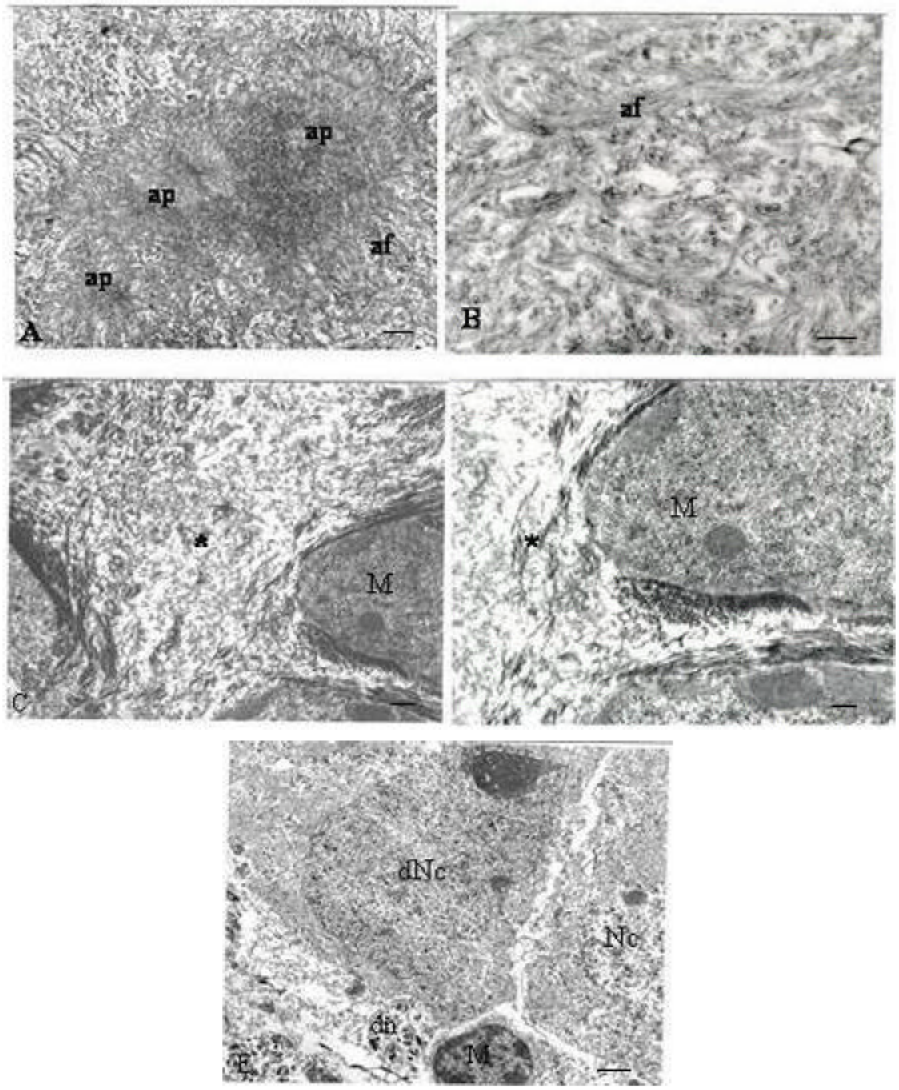

Fig. 3: Electron micrographs of diffuse βeta-Amyloid plaque (ap) in the entorhinal cortex of a 24-month-old transgenic Tg 2576 mouse brain (A). Note the typical star-shaped appearance with amyloid fibrils (af) radiating away from a central dense core of the plaque. The wavy appearance of the amyloid fibrils was distinct at a higher magnification (B). Figures C and D show an amyloid facing pole or interface (asterisk). Note the Microglia (M) in the vicinity and surrounding amyloid fibrils. The microglia is either depositing or attracting and phagocytosing the amyloid fibrils. Neuritic plaque vicinity represented by a neuron with degenerating Nucleus (dNc) besides a normal looking Nucleus (Nc) of an adjacent neuron. Note two dystrophic neurites with discontinous surface membranes (dn) and a microglia (m). Electron microscopy following AS720 - DAB staining. Bar scale A: 0.16 μm; B: 0.05 μm; C: 0.16 μm; D: 0.08 μm and E: 0.20 μm

amyloid plaque (ap) with abundant extracellular, non-branching amyloid fibrils radiating from a dense core (Fig. 3a). The amyloid fibrils (af) became less closely oriented at the periphery of the plaque. Their wavy appearance was established at a higher magnification (Fig. 3b). A Microglia (M) at an amyloid facing pole or interface (asterisk) was observed (Fig. 3c and d). A bundle-like arrangement of amyloid fibrils is visible at the amyloid interface. These fibrils were extracellular in disposition. A discontinuous cell surface membrane was visible at the amyloid facing pole of microglia.

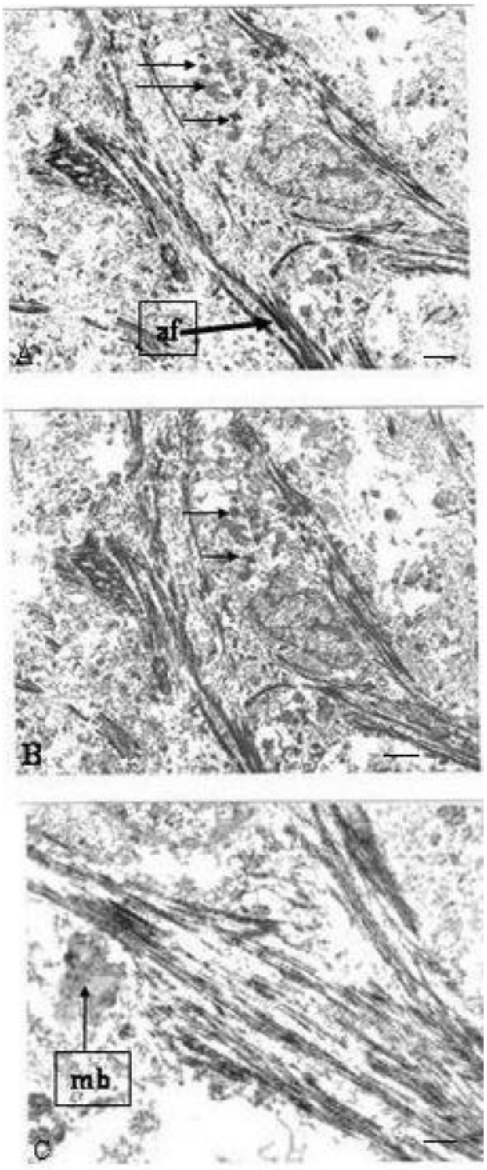

Fig. 4: Electrom micrograph of 24-month-old transgenic Tg2576 mouse brain. Figure A and B, show distinct heterogenous membrane bound bodies, the lysosomes (arrows) indicating some degenerating activity in the vicinity of amyloid fibrils (af). Figure C shows multi-laminated bodies (mb), an indication of an excretory process taking place. Electron Microscopy following DAB staining. Bar scale A: 0.22 μm; B: 0.20 μm and C: 0.62 μm.

In another area a large and dense degenerating Neuron (dNC) was observed (Fig. 3e), beside a normal Nucleus (Nc), two dystrophic neurites (dn) and a Microglia (M) in the same vicinity. Dystrophic neurites presented as discontinous surface membranes of cells usually in the neighbourhood of neuritc plaque with or without an amyloid core. Subsequent analysis of the 24-month-old Tg 2576 mouse showed pathological structures in the cortex in addition to the amyloid plaques. The pathological structures included dystrophic neurites containing dark swollen neurons characteristics of degenerating nerve cells with desegregated amyloid fibrils and several lysosomal inclusions (Fig. 4a and b). Other secondary inclusions were heterogenous granular accumulations characterized by electron dense multi-laminated bodies (Fig. 4c).

DISCUSSION

Ultrastructural features of brain of aged Tg 2576 mice In this study, neurons around amyloid plaques were occasionally pyknotic and/or exhibited clumps of condensed chromatin and irregular membrane structure, morphology typical of dying neurons as defined by Majno and Joris (1995), Calhoun *et al.* (1998). The apoptotic morphological features observed include signs of chromatin condensation, cell shrinkage with a hyperchromatin nucleus (Kerr *et al*., 1972).

Amyloid plaque: One of the criteria for a protein or peptide fibre to be classified as an amyloid fibre is that under electron microscopy, its demonstration should be fine non-branching fibres 6-10 nm in diameter Gorman and Chakrabarty (2001). In the electron microscopic study, the amyloid fibrils observed in the aged Tg 2576 mice were within this diameter and non-branching. A difference between amyloid plaques in the transgenic mouse compared with AD brain, is the lack of paired helical filaments in plaque-associated dystrophic neuritis (Sturchler-Pierrat *et al*., 1997). This was quite evident in this study of Tg 2576 mouse model of AD.

The amyloid facing pole or interface with a discontinous cell surface membrane as in AD (Stadler *et al*., 1999) was visible in this study. Such a consolidated microglia-amyloid space may give rise to leakage of cytotoxic and inflammatory microglial proteins into the surrounding neuropil (McGeer *et al*., 1992). It has been suggested that this type of microglia appears to initiate three types of neuropil response namely, degeneration of neurons, protective activation of astrocytes and attraction and activation of microglial cells sustaining plaque growth (Wegiel *et al*., 2001).

In vitro studies have shown that exogenous amyloid fibrils are digested by microglia, collected and stored in phagosomes and remain for up to 20 days, suggesting a very limited effectiveness of microglia in degrading β-amyloid fibrils (Frackowiak *et al*., 1992). From this study, the microglia in the transgenic Tg 2576 mice displayed characteristic polarity with formation of specific interface with extracellular amyloid as reported in AD (Frackowiak *et al*., 1992) although no amyloid fibril was found within a microglial cell. Indeed, electron microscopic studies of AD brain have almost always failed to detect definite intraneuronal βeta-Amyloid fibrils (Selkoe, 2000). The presence of lysosomes in this study correlates with the report that the lysosomal system is impaired in AD. Brain tissue with established pathologic criteria for AD diagnosis usually presents massive accumulations of lysosomes in neurons, along with changes in their composition and hydrolase content (Nixon *et al*., 1992). Secondary phagosome occurrence, as observed in the 24-month Tg 2576 mice, is evidence of strong lysosomal activity (Peters *et al*., 1991).

Electron microscopic studies of AD brains (Terry and Wisniewski, 1970) showed that dystrophic or degenerating neurites is a constant finding in the classical plaques. Most of these neurites have the morphological features of presynaptic terminals although enlarged postsynaptic elements containing pathological materials are also encountered. Findings from this study of 24-month old Tg 2576 mice did show similar neuropatho-logical features of degeneration as reported by Oster-Granite *et al*. (1996). A number of pathological tissue responses such as activated microglial, astrocyte proliferation and lysosomal debris were visible in close proximity of βeta-Amyloid plaques.

The ultrastructural investigations of brain sections from transgenic Tg 2576 mice in this study also confirmed the presence of plaque-associated inflammatory responses as was recently shown by light microscopy (Apelt and Schliebs, 2001). This study has established some reliable ultrastructural features with respect to βeta-Amyloid plaque in aged transgenic Tg 2576 mouse. These data are comparable to some of the reported features in AD and so the Tg 2576 do present a reliable model for investigation of Alzheimer's disease related plaque pathology.

ACKNOWLEDGEMENT

The authors are indebted to Dr. J Apelt for her assistance during tissue preparations. AOI acknowledges the International Society for Neurochemistry (ISN) and Mbarara University for travel support to Leipzig, Germany.

REFERENCES

Akiyama, H., H. Kondo, K. Ikeda, M. Kato and P.L. McGeer, 2001. Immunohistochemical localization of neprilysin in the human cerebral cortex: Inverse association with vulnerability to amyloid beta-protein (Abeta) deposition. Brain Res., 902: 277-81.

Apelt, J. and R. Schliebs, 2001. Beta amyloid-induced glial expression of both pro- and anti-inflammatory cytokines in cerebral cortex of aged transgenic mice with Alzheimer plaque pathology. Brain Res., 894: 21-30.

Calhoun, M.E., K.H. Weiderhold, D. Abramowski, A.L. Phinney and A. Probst *et al*., 1998. Neuron loss in APP transgenic mice. Nature, 395: 755-56.

Frackowiak, J., H.M. Wisniewski, J. Wegiel, G.S. Merz, K. Iqbal and K.C. Wang, 1992. Ultrastructure of the microglia that phagocytose amyloid and the microglia that produce β-amyloid fibrils. Acta Neuropathologica, 84: 225-33.

Gorman, P.N. and A. Chakrabarty, 2001. Alzheimer β-amyloid peptides: Structure of amyloid fibrils and alternate aggregations product. Biopolymers (Peptide Science) 60: 381-394.

Hardy, J., 1997. Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci., 20: 154-159.

Hsiao, K., D.R. Borchelt, K. Olson, R. Johannsdottir, C. Kitt and W. Yunis *et al*., 1995. Age-related CNS disorder and early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor protein. Neuron., 15: 1203-18.

Hsiao, K., P. Chapman, S. Nilsen, C. Eckman, Y. Harigaya and S. Younkin, *et al*., 1996. Correlative memory deficits, Aß elevation and amyloid plaques in transgenic mice. Science, 274: 99-102.

Khachaturian, Z.S., 1985. Diagnosis of Alzheimer's disease. Arch. Neurol., 42: 1097-1105.

Kerr, J., A. Wyllie and A. Currie, 1972. Apoptosis: A basic biological phenomenon with wide ranging implications in tissue kinetics. Br. J. Cancer. 26: 239-257.

Kurt, M.A., D.C. Davies, M. Kidd, K. Duff, S.C. Rolph, K.H. Jennings and D.R. Howlett, 2001. Neurodegenerative changes associated with β-amyloid deposition in the brains of mice carrying mutant amyloid precursor protein and mutant presenilin-1 transgenes. Exp. Neurol., 171: 59-71.

Luth, H.J., J. Apelt, A.O. Ihunwo, T. Arendt and R. Schliebs, 2003. Degeneration of βeta-Amyloid associated cholinergic structures in transgenic APP(sw) mice. Brain Res., 977: 16-22.

Majno, G. And I. Joris, 1995. Apoptosis, oncosis and necrosis: An overview of cell death. Am. J. Pathol., 146: 3-15.

Masliah, E., A. Sisk, M. Mallory, L. Mucke, D. Schenk and D. Games, 1996. Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F β-amyloid precursor protein and Alzheimer's disease. J. Neurosci., 16: 5795-5811.

Masliah, E., A. Sisk, M. Mallory and D. Games, 2001. Neurofibrillary pathology in transgenic mice overexpressing V717F β-amyloid precursor protein. J. Neuropath Exp. Neurol., 60: 357-368.

McGeer, P.L., H. Akiyama, T. Kawamata, T. Yamada, D.G. Walker, T. Ishii, 1992. Immunohistochemical localization of βeta-Amyloid precursor protein sequences in Alzheimer and normal brain tissue by light and electron microscopy. J. Neurosci. Res., 31: 428-442.

Nixon, R.A., A.M. Cataldo, P.A. Paskevich, D.J. Hamilton, T.R. Wheelock and L. Kanaley-Andrews, 1992. The lysosomal system in neurons: Involvement at multiple stages of Alzheimer's disease pathogenesis. Ann. N.Y. Acad. Sci., 674: 65-88.

Oster-Granite, M.L., D.L. McPhie, J. Greenan and R.L. Neve 1996. Age-dependent neuronal and synaptic degeneration in mice transgenic for the C terminus of the amyloid precursor protein. J. Neurosci., 16: 6732-6741.

Peters, A., S.L. Palay and H.D. Webster, 1991. The Fine Structure of the Nervous System: Neurons and Their Supporting Cells. (3rd Edn.), Oxford University Press, New York.

Prince, M., 1997. The need for research on dementia in developing countries. Trop. Med. Int. Health., 2: 993-1000.

Selkoe, D.J., 2000. Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid β-protein. Ann. N.Y. Acad. Sci., 924:17-25.

Stadler, M., A. Phinney, A. Probst, B. Sommer, M. Staufenbiel, M. Jucker, 1999. Association of microglia with amyloid plaques in brains of APP23 transgenic mice. Am. J. Pathol., 154: 1673-84.

Sturchler-Pierrat, C., D. Abramowski, M. Duke, K.H. Wiederhold and C. Mistl *et al*., 1997. Two amyloid precursor protein transgenic mouse model with Alzheimer disease-like pathology. Proc. Nat. Acad. Sci., USA., 94:13287-92.

Terai, K., A. Iwai, S. Kawabata, Y. Tasaki, T. Watanabe and K. Miyata *et al*., 2001. β-Amyloid deposits in transgenic mice expressing human β-Amyloid precursor protein have the same characteristics as those in Alzheimer's disease. Neuroscience, 104: 299-310.

Terry, R.D. and H.M. Wisniewski, 1970. The Ultrastructure of the Neurofibrillary Tangle and the Senile Plaque. In: Alzheimer's disease and related condition (Eds.), Wolstenholm EGW and O'Connor M. Churchill, London, pp: 145-148.

Wegiel, J., K.C. Wang, H. Imaki, R. Rubenstein, A. Wronska, M. Osuchowski *et al*., 2001. The role of microglia cells and astrocytes in fibrillar plaque evolution in transgenic APPsw mice. Neurobiol. Aging., 22: 49-61.