ROLE OF $\beta$-AMYLOID IN THE DIAGNOSIS OF NEURODEGENERATIVE DISEASES: DIFFUSE LEWY BODY DISEASE VERSUS ALZHEIMER’S DISEASE

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INTRODUCTION

Deposition of $\beta$-amyloid senile plaques in the neuropil and the formation of intracellular neurofibrillary tangles are the histopathological hallmarks of both sporadic and familial forms of Alzheimer’s disease (AD). Excess $\beta$-amyloid, formed from abnormal processing of $\beta$-amyloid precursor protein ($\beta$-APP) coded for on chromosome 21, can also be found in aged individuals with Down’s syndrome (Trisomy 21) as well as in the recently described cases of diffuse Lewy body disease (DLBD) (Gibb et al., 1989; Kosaka, 1993; Kuzuhara and Yoshimura, 1993). Patients with the pure form of DLBD are characterized clinically by progressive dementia with fluctuating confusional states and visual hallucinations, followed later by Parkinsonian symptoms (Byrne et al., 1989; Forstl et al., 1993; Kosaka, et al., 1980; and Perry et al., 1993). Parkinsonism rarely is the presenting symptom. Neuropathological findings include Lewy bodies both in the neuromelanin containing cells of the substantia nigra as well as in the neocortex but few, if any, neurofibrillary tangles. The occurrence of DLBD as well as the Lewy body variant of AD (LBVAD) is not as rare as previously thought (Gibb et al., 1989; Forstl et al., 1993). The LBVAD has features of both DLBD and AD. It has been found that, when combined, both types of Lewy body disease are the second leading cause of non-vascular dementia following classical AD.

Lewy bodies are intraneuronal cytoplasmic inclusions. They occur in the substantia nigra compacta as well as locus ceruleus, nucleus basalis of Meynert, and dorsal raphe nucleus in idiopathic Parkinson’s disease. However, in DLBD, they are found preferentially in the temporal lobe, cingulate gyrus, and insular cortex, in addition to areas involved in idiopathic Parkinson’s disease (Kosaka, 1993; Pollanen et al., 1993). The immunocytochemical staining properties of Lewy bodies have been extensively studied, yet the results are equivocal. In general, antibodies to phosphorylated neurofilaments, particularly the high (NF-H) and
medium (NF-M) molecular weight forms, will stain cortical and nigral Lewy bodies both in tissue sections as well as in partially purified tissue extracts (Bancher et al., 1989; Lennox et al., 1989; Pollanen et al., 1992; Schmidt et al., 1991). This is in contrast to neurofibrillary tangles which stain with antibodies which recognize abnormally phosphorylated tau protein (Galloway et al., 1989). To date, the most sensitive method for detecting both cortical and nigral Lewy bodies is with ubiquitin immunohistochemistry (Bancher et al., 1989; Galloway et al., 1988; Lennox et al., 1989). Recently, it has been shown that in vivo administration of β-amyloid is neurotoxic and can induce abnormally phosphorylated cytoskeletal proteins including tau (for review see Mullan and Crawford, 1993). Based on experiments done in cell culture systems, the neurotoxic effects of β-amyloid have been hypothesized to be due to increased intracellular calcium levels (Mattson et al., 1993). Increases in intracellular calcium can activate a number of protein kinases which are involved in cytoskeletal protein phosphorylation (Drewes et al., 1992). Drewes and colleagues (1992) have shown that mitogen activated protein (MAP) kinase abnormally phosphorylates tau into a state that is similar to that found in neurofibrillary tangles. This kinase recognizes a Lys-Ser-Pro sequence. In addition, Lichtenberg-Kraag et al. (1992) have shown that the Sternberger Monoclonal, Inc (SMI) series of antibodies, originally raised against phosphorylated neurofilaments which stain Lewy bodies, also recognize a similar hyperphosphorylated Lys-Ser-Pro epitope on tau.

Since excess β-amyloid deposition is found both in DLBD/LBVAD and AD, we hypothesized that Lewy bodies may share phosphorylated epitopes that are similar to the neuritic components of amyloid plaques as well as neurofibrillary tangles of classical AD. Using a panel of antibodies against a variety of cytoskeletal proteins, we do in fact conclude that similar epitopes may be abnormally phosphorylated on neurofilament and tau proteins found in Lewy bodies and neurofibrillary tangles, respectively. This suggests that DLBD and AD may represent a spectrum of disease with excess β-amyloid deposition as a common feature or etiological agent.

METHODS

Brain sections from the temporal lobe (Brodmann areas 20,21,22) and frontal lobe (Brodmann areas 8,9), as well as the cingulate gyrus (Brodmann area 24) were obtained at autopsy from two LBVAD, one DLBD and two classical AD cases (Loyola University Brain Bank, Maywood, IL and the Massachusetts Alzheimer Disease Resource Center, Boston, MA). Fresh brain sections were fixed in 2% paraformaldehyde-periodate-lysine (PLP) in phosphate buffer (pH 7.4) for 24 hours. The tissue was then transferred to 20% glycerol/2% dimethylsulfoxide (DMSO) and stored at 5°C. Forty micron sections were cut and placed in a cryoprotectant until stained. The immunocytochemical staining was performed by the avidin-biotin peroxidase (Vector) method. Antibodies used in the present study included: MAP-2 (Boehringer-Mannheim, Indianapolis, IN); SMI 31,32,34,35 (Sternberger Monoclonals, Inc., Baltimore, MD); ubiquitin (Dako Corp, Carpinteria, CA); SE2 (human tau 131-192; courtesy of Dr. K. Kosik); Alz 50 (courtesy of Dr. P. Davies); tyrosine-tubulin and tau-2 (Sigma, St. Louis, MO).

RESULTS

As has been previously described, the ubiquitin antibody stains cortical Lewy bodies preferentially and therefore was used as the baseline (100%) from which all other comparisons were made (Bancher et al., 1989; Lennox et al., 1989). The cortical Lewy bodies were found predominantly in the deep cortical layers (lamina V and VI) in small interneurons. The ubiquitin antibody also stained dystrophic neurites, neuritic plaques, and neurofibrillary tangles.