J. Tokyo Med. Coll., 53(5): 676～681, 1995

Demonstration of β amyloid Protein-containing Neurofibrillary Tangles in the Cerebral Cortex of Elderly Patients with Progressive Supranuclear Palsy

Takahiko UMAHARA[1], Shinsuke KATO, Asao HIRANO and Noriyuki SHIBATA

Division of Neuropathology, Department of Pathology, Montefiore Medical Center, Bronx, NY 10467, USA.

Abstract

Progressive supranuclear palsy (PSP) is characterized at the neuropathological level by the presence of variable amounts of neurofibrillary tangles (NFTs) in several subcortical structures, and by the absence of senile plaques (SPs). NFTs and SPs have been reported to be the present in the cerebral cortex of some patients. In this report we document the presence of β amyloid protein-containing NFTs in the hippocampus and temporal lobe of two elderly PSP patients, and relate these findings to the disease process and to aging.

Introduction

Neurofibrillary tangles (NFTs) and senile plaques (SPs) are histopathological changes associated with aging, Alzheimer's disease (AD), and certain other disorders. SPs are characterized by the accumulation of β amyloid protein[1], but some NFTs in the brains of AD patients do react with the antibody to this protein[2～4]. On the other hand, it is generally accepted that only NFTs, but no SPs are found in certain degenerative conditions such as progressive supranuclear palsy (PSP), parkinsonism-dementia complex (PDC) on Guam and dementia pugilistica. However, recent studies have shown that some elderly patients with PDC on Guam[5] or with dementia pugilistica[6] do have SPs as well as β amyloid protein-containing NFTs (β-NFTs). These findings have raised questions pertaining to the pathogenetic significance of the presence of β-NFTs in these diseases[4)5)7)], and to whether their presence indicates that β amyloid protein represents an original and integral NFT component.

Individuals with PSP usually have variable amounts of NFTs in several subcortical structures, but no SPs. However, NFTs have been identified in the cortex[8～16)], brainstem and subcortical nuclei of certain PSP patients, some of whom have SPs in the corex[14)15)]. In contrast to PDC on Guam[5] and dementia pugilistica[6], no date are available regarding the presence of β-NFTs in PSP. In order to obtain information on the occurrence of such NFTs in this disease, we carried out an immunohistochemical study on archival material from five elderly PSP patients using an antibody against β amyloid protein, and for control purposes, a monoclonal antibody to tau protein. Our results demonstrate that the

Received Feb. 14, 1995, Accepted Jun. 2, 1995

Key words : β amyloid protein, Cerebral cortex, Neurofibrillary tangle, Progressive supraneuclar palsy, Tau protein.

([1]**Present address** : Department of Geriatrics, Tokyo Medical College, Japan.)

(**Present address and mailing address** : Dr. T. Umahara, Department of Geriatrics, Tokyo Medical College, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160, Japan. Tel 03-3342-6111, FAX 03-3342-2305)

hippocampus and temporal lobe cortex of two of our patients did have β amyloid protein-containing NFTs.

Materials and methods

This investigation was performed on formalin-fixed, paraffin-embedded material from 5 patients with PSP (table 1). The diagnosis of PSP was confirmed in each case by neuropathologic examinations, in which conventional procedures, including modified Bielschowsky silver impregnation were used. Samples were obtained from temporal lobes and the hippocampus. Six-μm sections were cut and mounted onto poly-L-lysine-coated slides. The sections were deparaffinized, hydrated and immunostained. As primary antibody we used an affinity-purified rabbit antibody raised against a synthetic peptide corresponding to the N-terminal 28 amino acid sequence of the β amyloid protein[1)17)] (diluted 1 : 1000 with phosphate-buffered saline, pH 7.2, containing 3% bovine serum albumin [PBS-BSA]), and a mouse monoclonal antibody to tau protein[18)] (tau-2, kindly provided by Dr. L. I. Binder ; diluted 1 : 5 with PBS-BSA).

Sections to be incubated with anti-β amyloid protein antibody were pretreated for 10 min with 90.8% formic acid. Incubated with primary antibodies was carried out overnight at 4°C. Sections incubated with PBS-BSA served as reaction controls. Antibody binding was visualized with the respective Vectastain ABC kit for rabbit IgG_1 and mouse IgG_1 (Vector laboratories, Burlingame, CA, USA) ; 3, 3-diaminobenzidine tetrahydrochloride was the final chromogen. The immunostained sections were examined, the number of positively immunostained NFTs and SPs recorded, and their frequency expressed per microscopic field. In those instances in which a larger number of fields had to be examined because of low NFT and/or SP frequency, the results were expressed per average of fields analyzed.

Results

All 5 PSP patients had tau-positive NFTs in the hippocampus (Fig. 1). Their frequency varied from one in every other field to up to 10/field (Table 1). Moreover, the distribution of immunostained hippocampal NFTs in a given patient was not uniform as their number varied from field to field, especially in cases 1 and 2, in which the hippocampus had more tau-positive NFTs than the other three. NFTs immunolabeled by the tau-2 monoclonal antibody were seen in the temporal lobe cortex of 4 patients, but except for case 3, the frequency was rather low (Table 1).

By comparison, NFTs immunostained by antibody to β amyloid protein were found in only 2 cases (Fig. 2), and their incidence, both in the hippocampus and the temporal lobe was significantly lower than that of tau-positive NFTs in these brain structures (Table 1). Thus, 1 β-NFT was seen every second field in case 2, and every fourth in case 3. However, almost none of these NFTs was associated with neuronal nuclei, suggesting that they were extracellular NFTs[4)5)].

SPs immunolobeled by the antibody to β amyloid protein were detected in the hippocampus and temporal lobe of three patients, and, as was the case with tau-positive NFTs, their frequency varied not only from one individual to another but also in a given patient from field to field (Table 1). However, it was readily evident that case 2, which had many tau-NFTs in the hippocampus, also had a considerable number of β-SPs, not only at that location, but also in the temporal lobe, where up to 20 β amyloid protein-containing SPs were seen in one microscope field. On the other hand, in cases 1 and 5 in which the hippocampus had a significant number of tau-NFTs, no β amyloid protein-containing-structure were seen. It was of note that case 4, which had a relatively small number of tau-positive NFTs and an occasional β amyloid protein-containing SPs in the hippocampus and temporal lobe, had no β-NFTs.

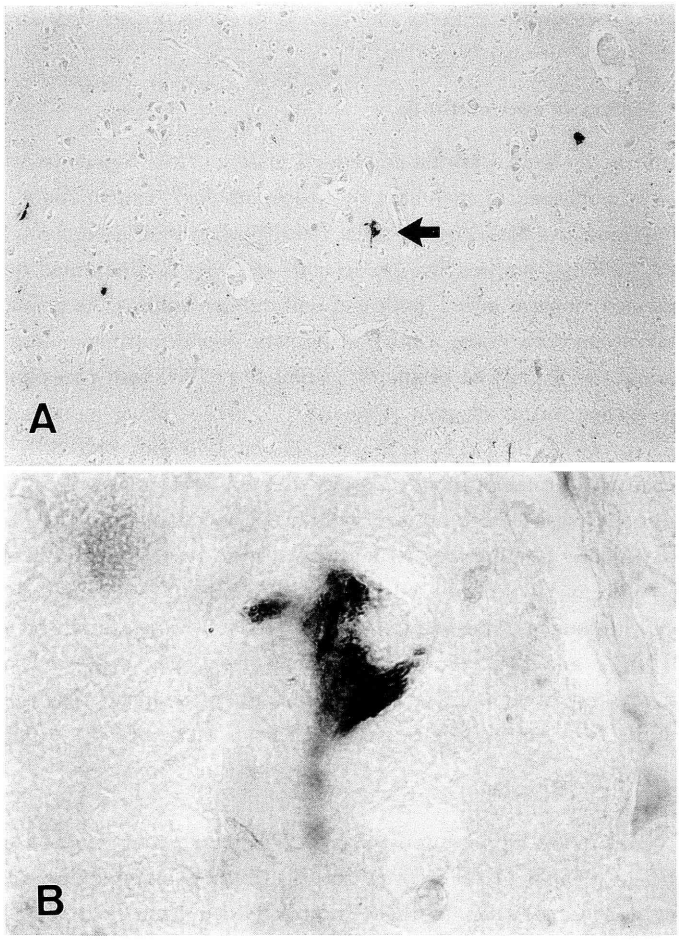

Fig. 1 A Tau-positive neurofibrillary tangles (NFTs) in the hippocampus of case 1 (×65). B Higher magnification of one of the NFTs shown in A (arrow) (×650).

Table 1 Number of cortical neurofibrillary tangles and senile plaques in five patients with autpsy-proven progressive supranuclear palsy

Case	Age/ sex	Clinical diagnosis	Tau-NFTs		β-NFTs		β-SPs	
			Hippo	T-lobe	Hippo	T-lobe	Hippo	T-lobe
1	75 F	AD	3-5/1 f	—	—	—	—	—
2	73 F	parkinsonism	5-10/1 f	1-2/1 f	1/3 f	1/6 f	2-5/1 f	10-20/1 f
3	72 F	PSP	1-2/1 f	1-10/1 f	1/5 f	1/6 f	1/1/1 f	1-4/1 f
4	64 M	parkinsonism	1/1 f	1/20 f	—	—	1/1 f	1/6 f
5	64 F	PSP	1-2/1 f	1/18 f	—	—	—	—

Tau-. β-NFTs : tau-positive, β amyloid protein containing neurofibrillary tangles respectively.
SPs : snile plaqes. Hippo : hippocampus. T-lobe : temporal lobe. AD : Alzheimer's disease.
PSP : progressive supranuclear palsy. f : microscope field.
Each field ×25

Discussion

β amyloid protein is the major component of SPs, which in the aging brain are usually detected earlier than NFTs. β amyloid protein-containing NFTs have been identified in the cerebral cortex of patients

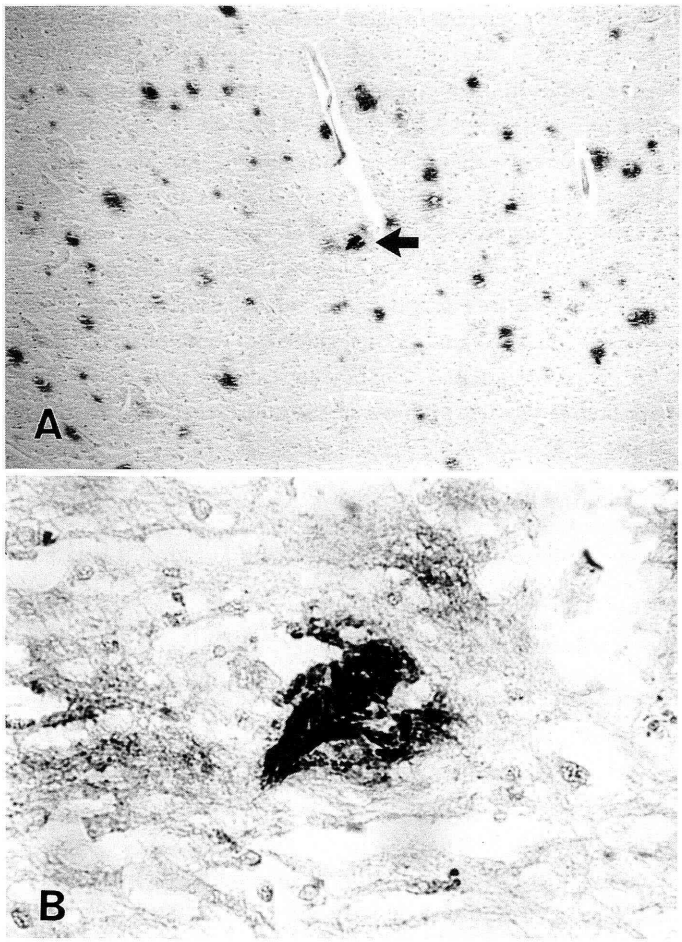

Fig. 2 A β amyloid-containing senile plaques and NFT (arrow) in the hippocampus of case 2 (× 35).
B Higher magnification of the NFT depicted in A (arrow) (× 350).

with AD[2)4)], elderly individuals with PDC on Guam[5)], and patients with dementia puglistica[6)]. These observation led to the notion that β amyloid protein deposits on extracellular NFTs, while recent studies[4)5)7)] have raised the question of whether β amyloid protein is indeed an original and integral NFT component.

Although the presence of a small number of SPs in the cerebral cortex of elderly PSP patients is common, this feature is considered to be a reflection of normal aging[19)]. However, there are some, albeit relatively few patients, with PSP who have numerous NFTs and SPs in the cerebral cortex[12)14)], but these individuals seem to have PSP and AD concurrently. On the other hand, because some NFTs are present not only in the brainstem and subcortical gray matter, but also in the ocrtex, it has been suggested that there is an initial cortical involvement in PSP[8~13)16)].

From the data presented here, it is evident that those PSP patients who had β-NFTs in the hippocampus and temporal lobe, also had β-SPs in these locations. Conversely, individuals in whom no β-SPs were detected, also lacked β-NFTs, even though they had tau-positive NFTs, including one patient (case 1), whose hippocampus had rather significant numbers of the latter. This set of observations suggests the deposition of β amyloid on the NFTs, most likely onto extracellular NFTs, since this was consistently associated with β amyloid deposits in the SPs present in the adjacent cortex.

Several possibilities with respect to each of the patients studied emerge from the results of this investigation. Thus, the neuropathologic findings in case 2, which had numerous tau-NFTs in the hippocampus, and considerable numerous of β-SPs in the hippocampus, and more in the temporal lobe, would suggest that those findings are compatible with pathologic aging and/or AD. On the other hand, the observation concerning case 3 and 4 would indicate that in these patients, PSP was accompanied by normal aging. By contrast, we belived that case 1 and 5, which had cortical NFTs, but no β-NFTs or β-SPs, were less affected by the aging process, and that the presence of cortical NFTs represents a consequence of the PSP disease process. However, it must be emphasized that the number of cortical NFTs in these patients was significantly lower than that found in individuals with AD[3] or PDC on Guam[5]. Nevertheless, as our date demonstrate, β-NFTs and β-SPs, similar to those observed in these two disease, were readily identified in the hippocampus and temporal lobe cortex of two elderly PSP patients. As the ultimate significance of our finding remains to be established, further studies are required to elucidate the precise role of β-NFTs and β-SPs in the PSP disease process.

Acknowledgments

We are grateful to Dr. F. Herz for reviewing the manuscript. We also thank Drs. J. F. Llena and T. Iwamoto for their help.

References

1) Glenner GG, Wong CW. Alzheimer's disease : initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Comm **120** : 885～890, 1984
2) Masters CL, Multhaup G, Simms G, et al. : Neuronal origin of a cerebral amyloid : neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J **4** : 2757～2763, 1985
3) Hyman BT, Van Hoesen GW, Beyreuther K, et al. : A4 amyloid protein immunoreactivity is present in Alzheimer's disease neurofibrillary tangles. Neurosci Lett **101** : 352～355, 1989
4) Yamaguchi H, Nakazato Y, Shoji M, et al. : Secondary deposition of beta amyloid wihthin extracellular neurofibrillary tangles in Alzheimer-type dementia. Am J Pathol **138** : 699～705, 1991
5) Ito H, Hirano A, Yen S-H, et al. : Demonstration of β amyloid protein-containing neurofibrillary tangles in parkinsonism-dementia complex on Guam. Neuropathol Appl Neurobiol **17** : 365～373, 1991
6) Allsop D, Haga S, Bruton C et al. : Neurofibrillary tangles in some cases of dementia pugilistica share antigens with amyloid β-protein of Alzheimer's sidease. Am J Pathol **136** : 255～260, 1990
7) Guiroy DC, Mellini M, Miyazaki M, et al. : Neurofibrillary tangles of Guamanian amyotrophic lateral sclerosis, parkinsonism-dementia and neurologically normal Guamanians contain a 4- to 4. 5-kilodalton protein which is immunoreactive to anti-amyloid β/A4-protein antibodies. Acta Neuropathol **86** : 265～274, 1993
8) Ishino H. : Frequency of Alzheimer's neurofibrillary tangles in the cerebral cortex in progressive supranuclear palsy. (In Japanese) Folia Psychiatr Neurol Jpn **78** : 463～470, 1976
9) Ishino H, Otsuki S. : Frequency of Alzheimer's neurofibrillary tangles in the cerebral cortex in progressive supranuclear palsy (subcortical argyrophilic dystrophy). J Neurol Sci **28** : 309～316, 1976
10) Akashi T, Arima K, Maruyama N, et al. : Severe cerebral atrophy in progressive supranuclear palsy : a case report. Clin Neuropathol **8** : 195～199, 1989

11) Takahashi H, Oyanagi K, Takeda S, et al. : Occurrence of 15-nm-wide straight tubules in neocortical neurons in progressive supranuclear palsy. Acta Neuropathol **79** : 233～239, 1989
12) Amano N, Yagisita S, Yokoi S, et al. : Progressive supra nuclear palsy. On the neurofibrillary tangles observed in Ammon's horn and cerebral cortices-. (In Japanese) Neuropathology **10** : 83～95, 1990
13) Hauw JJ, Verny M, Delaere P, et al. : Constant neurofibrillary changes in the neocortex in progressive supranuclear palsy. Basic differences with Alzheimer's disease and aging. Neurosci Lett **119** : 182～186, 1990
14) Cruz-Sanchez FF, Rossi ML, Cardozo A. : Clinical and pathological study of two patients with progressive supranuclear palsy and Alzheimer's changes. Antigenic determinations that distinguish cortical and subcortical neurofibrillary tangles. Neurosci Lett **136** : 43～46, 1992
15) Gearing M, Olson DA, Watts RA, et al. : Progressive supranuclear palsy : Neuropahologic and clinical heterogeneity. Neurology **44** : 1015～1024, 1994
16) Hof PR, Delacourte A, Bouras C. : Distribution of cortical neuro-fibrillary tangles in progressive supranuclear palsy : a quantitative analysis of six cases. Acta Neuropathol **84** : 45～51, 1992
17) Dickson DW, Crystal HA, Mattiace LA, et al. : Diffuse Lewy body disease : light and electron microscopic immunocytochemistry of senile plaques. Acta Neuropathol **78** : 572～584, 1989
18) Binder LI, Frankfurter A, Rebhun LI. : The distribution of tau in the mammalian central nervous system. J Cell Biol **101** : 1371～1378, 1987
19) Dickson DW, Crystal HA, Mattiace LA, et al. : Identification of normal and pathological aging in prospectively studied nondemented elderly humans. neurobiol Aging **13** : 179～189, 1991

高齢者進行性核上性麻痺例大脳皮質にみられた βタンパク陽性神経原線維変化の検討

馬原孝彦[1] 平野朝雄 加藤信介 柴田亮行

Division of Neuropathology, Department of Pathology, Montefiore Medical Center, Bronx, NY, USA
[1]現 東京医科大学老年病学教室

進行性核上性麻痺は神経病理学的に皮質下諸核および脳幹部における神経原線維変化の出現を特徴とする疾患であるが，皮質病変も報告も散見される．βアミロイド蛋白をその主な構成成分とする老人斑はこの疾患では一般にはみられないが，高齢の場合やアルツハイマー病との合併例では認められる．アルツハイマー病脳や parkinsonisum-dementia complex on Guam や拳闘家脳症では神経原線維変化の一部がβアミロイド蛋白陽性であり，これが二次的変化か固有の構成成分かで議論がなされている．進行性核上性麻痺例におけるβアミロイド蛋白陽性神経原線維変化について検討した報告はなく，今回検討した，対照は高齢進行性核上性麻痺5例で，海馬を含む側頭葉切片を用い，抗tau抗体および抗βアミロイド蛋白抗体を用いた．全例で抗tau抗体陽性の神経原線維変化を認め，3例で老人斑を認めそのうち2例でβアミロイド蛋白陽性神経原線維変化を認めた．このβアミロイド蛋白沈着という所見は加齢による修飾と考えられた．

キーワード：βアミロイド蛋白，大脳皮質，神経原線維変化，進行性核上性麻痺，タウ蛋白．