βPP Participates in PrP-Amyloid Plaques of Gerstmann-Sträussler-Scheinker Disease, Indiana Kindred

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Abstract. Gerstmann-Sträussler-Scheinker disease in the Indiana kindred is pathologically characterized by deposits of PrP-amyloid, neurofibrillary tangles and degenerating neurites. The aim of this study was to investigate seven patients of different ages for βPP and Aβ immunoreactivities associated with PrP-amyloid deposits and degenerating neurites. In one asymptomatic individual with PrP-amyloid deposits, Alz50 and Aβ immunoreactivities were absent. In six symptomatic patients, the degenerating neurites surrounding PrP-amyloid deposits were labeled by Alz50 and by antibodies to synaptophysin, ubiquitin and the N- and C-terminal domains of βPP. In one symptomatic, senile patient, Aβ immunoreactivity was present in the extracellular space, often in association with PrP-amyloid deposits. The analysis of the immunohistochemical findings suggested that in the Indiana kindred the intracerebral accumulation of βPP, synaptophysin and ubiquitinated material most probably revealed a reaction of neurites to PrP-amyloid, whereas the extracellular deposition of Aβ was likely an age-related phenomenon.

Key Words: Aβ; Alzheimer's disease; Amyloid; βPP; Gerstmann-Sträussler-Scheinker disease; PrP-amyloid.

INTRODUCTION

Gerstmann-Sträussler-Scheinker disease (GSS) is a familial amyloidosis with cerebral uni- or multicentric amyloid deposits labeled by antibodies to prion protein 27-30 (PrP) (1-4). A GSS kindred, referred to as GSS, Indiana kindred (GSS-IK) (3-8), has a neuropathologic phenotype characterized by the association of PrP-amyloid deposits, neurofibrillary tangles (NFT) with paired helical filaments (PHF), and degenerating neurites labeled by Alz50 (3, 4, 7). Most PrP-amyloid deposits in the cerebral cortex are surrounded by such degenerating neurites (3-5). This phenomenon results in the formation of PrP-amyloid plaques that are morphologically similar to the senile plaques (SP) of Alzheimer's disease (AD). GSS-IK patients have a mutation at codon 198 of the PRNP gene (9, 10). Furthermore, both PrP and Aβ immunoreactivities have been detected in the brain of a senile patient of the Indiana kindred (11). This observation prompted us to extend the investigation of the PrP-amyloid deposits in patients of different ages, in order to clarify the relationship between PrP-amyloid, Aβ and its precursor βPP, and degenerating neurites.

MATERIALS AND METHODS

The study was carried out on samples of cerebral cortex, thalamus, basal ganglia and cerebellum from two patients of the fifth generation and five patients of the sixth generation of the Indiana kindred. Patient V-WS first developed ataxia and intellectual deterioration and subsequently bradykinesia and diffuse rigidity; this patient died at the age of 73 years, 7 years after the onset of the disease (11). Patients VI-DK, V-LB, VI-JB, VI-RB and VI-LD developed postural abnormalities and intellectual impairment in presenile age and died at ages 63, 59, 55, 51 and 49 years, respectively (3-6). VI-PG was asymptomatic when she died at 44 years of age (12). Tissue samples used for the study were taken at autopsy 3-20 hours after death, fixed in formal (1:10) or Carnoy's fixative and, when possible, periodate-lysine-paraformaldehyde (PLP) (13).

For light microscopy, serial 7 μm thick sections from para- plast-embedded blocks were alternately (i) stained with routine methods (hematoxylin & eosin, cresyl violet, Heidenhain-Woelecke, Congo red and thioflavine S); (ii) impregnated with silver nitrate according to the Gallyas method for NFT, neurites with PHF, and neuritic threads; and (iii) labeled by the following antibodies: (a) anti-PrP (1:200, after 98% formic acid), polyclonal, to PrP purified from scrapie-infected hamster brains; (b) anti-SP28 (1:50, after 98% formic acid), polyclonal, to the 28-residue synthetic peptide homologous to the N-terminal region of Aβ (14); (c) anti-β-amyloid (Dako [Glostrup, Denmark], 1:100, after 98% formic acid), monoclonal, for Aβ; (d) anti-preA4 (Boehringer [Mannheim, Germany], 1:100, monoclonal, for an epitope between residues 60 and 100 of βPP (15, 16); (e) anti-SP20 (1:100), monoclonal, to a 20-residue synthetic peptide homologous to the C-terminal fragment of βPP (17); (f) Alz50 (1:10), monoclonal, to a 68-kDa protein present in the brain of Alzheimer patients, for NFT, neurites with PHF, and neuritic threads (18); (g) anti-ubiquitin (Chemicon [Temecula, CA], 1:200), monoclonal, for ubiquitinated proteins, including those related to NFT (19); (h) anti-synaptophysin (Dako, 1:10), monoclonal, to a vesicle-associated protein localized in presynaptic
terminals (20). Double labeling was carried out using anti-PrP antibody and Alz50 to investigate PrP-amyloid plaques, and using anti-PrP and anti-β-amyloid antibodies to investigate PrP-amyloid plaques for the presence of both PrP and Aβ. Polyclonal antibodies were evidenced by the peroxidase-antiperoxidase (PAP) method with swine anti-rabbit immunoglobulins, PAP complex (Dako) and 3,3',diaminobenzidine as a chromogen. Monoclonal antibodies were revealed by an avidin-biotin system with biotinylated anti-mouse serum raised in sheep, alkaline phosphatase-conjugated streptavidin (Amersham, Amersham, UK) and naphthol-AS-MX-phosphate/fast blue BB as chromogen. Sections of frontal cortex, basal ganglia and cerebellum of patient V-WS, labeled by anti-PrP and anti-β-amyloid antibodies, were subjected to a semiquantitative evaluation using an image analyzer Ibax (Kontron-Zeiss).

For electron microscopy (EM), several PLP-fixed samples of frontal cortex and putamen of patient V-WS and patient VI-DK were embedded in LR white resin (Polyscience [Warrington, PA]), cut with an Ultracut-E (Reichert-Jung) ultramicrotome and stained with uranyl acetate and lead citrate. Immunoelectron microscopy was carried out in serial ultrathin sections mounted on collodion-coated nickel grids and processed for postembedding immunostaining as follows (21). Sections were alternately incubated with normal goat serum and anti-PrP antibody (1:20), and with normal goat serum and anti-SP28 antibody (1:20), reincubated with goat anti-rabbit serum conjugated to 10 nm colloidal gold particles (GARG 10, Biocell [Rancho Dominguez, CA], 1:20), and stained with uranyl acetate and lead citrate. A Zeiss 109 electron microscope was employed for scanning.

For both light and EM immunohistochemistry, the immunoreactions were tested for specificity with normal mouse and rabbit sera as primary antibodies and by peptide (SP28 and SP20) absorption.

RESULTS
PrP-Amyloid Deposits
(Fig. 1A, D, and Fig. 2A)

The neuropathologic features of senile patient V-WS matched closely those of the symptomatic presenile patients V-LB, VI-JB, VI-RB, VI-LD (3–5, 7) and VI-DK. All patients had uni- and multicentric PrP-amyloid deposits in the cerebral and cerebellar cortices, basal ganglia and thalamus. In the cerebral cortex, basal ganglia and thalamus, most PrP-amyloid deposits were surrounded by neurites impregnated with silver and labeled by Alz50 and anti-ubiquitin antibody. Neurofibrillar tangles and neuropil threads unrelated to PrP-amyloid deposits were
Fig. 2. Gerstmann-Sträussler-Scheinker disease in the Indiana kindred. (A) Patient VI-LD aged 49 years, frontal cortex. Double immunostaining with polyclonal anti-PrP antibody (brown reaction product) and monoclonal Alz50 (blue) shows that degenerating
also present, particularly in the inner cortical layers. In
the cerebellar cortex, PrP-amyloid deposits were more
numerous over the molecular than the granular layer and
ranged up to 100 μm in size, whereas Alz50-immuno-
reactive neurites were absent. In the asymptomatic in-
dividual VI-PQ, PrP-amyloid deposits were numerous in
the cerebellar cortex and rare in the cerebral cortex. Fur-
thermore, they were related to ubiquitin-immunoreactive
profiles. Neurofibrillary tangles and Alz50-immunoreac-
tive neurites were absent in this patient.

**βPP-Immunoreactive Neurites**

(Fig. 1B, C, E, F)

Neurites associated to PrP-amyloid deposits were la-
beled not only by Alz50 and anti-ubiquitin, but also by
anti-synaptophysin, anti-SP20 and anti-preA4 antibod-
ies. These antibodies had similar immunostaining pat-
terns, represented by round or rod-shaped profiles up to
15 μm. In the cerebral cortex, basal ganglia and thalamus,
such profiles were mostly localized around PrP-amyloid
cores, whereas in the cerebellar cortex they were smaller
and were associated to both PrP-amyloid cores and the
surrounding tissue.

**Extracellular Aβ Immunoreactivity**

(Fig. 2B-G, Fig. 3)

Senile plaques and preamyloid deposits (22) were ev-
edenced by both anti-SP28 and anti-β-amyloid antibodies
in the cerebral cortex, basal ganglia, and thalamus (an-
terior and external reticular nuclei) of the senile patient.

Double immunostaining carried out with polyclonal
anti-PrP and monoclonal anti-β-amyloid antibodies
showed that PrP and Aβ immunoreactivities often were
associated in the same lesion; in particular, Aβ- immu-
noreactive material was either intermingled with scat-
tered amounts of PrP-amyloid or localized around dense
PrP-amyloid cores. A semiquantitative analysis disclosed
that the percentages of deposits containing both PrP-amy-
lloid and Aβ or only one of them varied in different
regions of the brain; in fact, double immunolabeled de-
posits were more numerous than those labeled by either
anti-β-amyloid or anti-PrP antibodies in frontal cortex
(60, 20 and 20 percent, respectively) and basal ganglia
(100, 0 and 0, respectively), whereas deposits exclusively
labeled by anti-PrP antibody were more numerous in the
molecular layer of the cerebellar cortex (10, 0 and 90,
respectively). The distribution of immunolabeled depos-
ts within the frontal cortex varied according to immu-
noreactivity; most deposits labeled only by anti-β-amy-
loid antibody were localized over the outer layers of the
cortex, whereas double immunolabeled deposits and de-
posits labeled only by anti-PrP antibody were localized
over the inner layers.

By immunoelectron microscopy it was found that anti-
PrP immunogold only decorated straight, 4–8 nm wide
fibrils, whereas anti-SP28 immunogold decorated both
amorphous material and fibrils. Furthermore, immuno-
electron microscopy showed that the Aβ-immunoreactive
rings localized around PrP-amyloid cores were made up
of amorphous material, whereas the cores were fibrillar,
and that fibrils and amorphous material were gently in-
termingled along the border between the core and the
ring.

Immunostaining with anti-SP28 and anti-SP29 antibod-
ies was abolished by absorption of the antisera with the
corresponding peptides. Immunostaining was absent
when normal mouse and rabbit sera were used as primary
antibodies.

Results of the immunohistochemical study are sum-
marized in Table 1.

**DISCUSSION**

The study showed that βPP-immunoreactive material
was associated with PrP-amyloid plaques in the central
nervous system of symptomatic GSS-1k patients. This
material was labeled by antibodies to both N- and C-
terminal domains of βPP, and was related to degenerating
neurites that were also labeled by Alz50, anti-synapto-
physin and anti-ubiquitin antibodies. In the senile pa-
tient, additional amorphous and fibrillary material la-
beled by anti-SP28 and anti-β-amyloid antibodies was
present in the extracellular space. Findings comparable
to ours have been reported concerning two Swedish sib-
lings aged 67 and 78 years (23), who carried a mutation
at codon 217 of the PRNP gene (10). In these patients,
Aβ immunoreactivity was associated to PrP-amyloid de-
posits and NFT were present. However, no anti-βPP an-
tibodies were used for the study of these patients. Fur-
thermore, it has recently been shown that in four GSS
patients aged 60 to 68 years extracellular Aβ and intra-
cellular βPP immunoreactivities were associated to PrP-amyloid deposits. Neurofibrillary tangles were absent in these patients (24, 25).

Degenerating Neurites and Intracellular βPP

Degenerating neurites of PrP-amyloid plaques in GSS-Ik shared antigenic determinants with those of SP in AD. In both diseases, such neurites are labeled not only by Alz50, anti-ubiquitin and anti-synaptophysin antibodies (19, 26, 27), but also by antibodies to epitopes in the N- and C-terminal domains of βPP (28-31). βPP immunoreactivity is not unique to degenerating neurites in AD and GSS-Ik, since it has been found in axonal swellings that develop in the central nervous system following axonal transection, metabolic disorders and aging (32). In AD, βPP accumulation is associated with cytoskeletal abnormalities as revealed by the presence of Alz50 immunoreactivity (30). At variance with AD, the relationship between cytoskeletal abnormalities and βPP accumulation in degenerating neurites is not consistently evident in GSS. In fact, it has been observed that, in patients from GSS families other than the Indiana kindred, degenerating neurites are labeled by anti-βPP antibodies, but not by Alz50 (24). Intracellular βPP, synaptophysin and ubiquitin immunoreactivities might reflect increased protein content in degenerating neurites involved with SP and PrP-amyloid plaques. This speculation is based on the observation that under experimental conditions Aβ stimulates neuritic growth and induces nerve cell degeneration (33, 34). Similarly, PrP-amyloid might stimulate the synthesis of cellular proteins, includ-
TABLE 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>VI-PG</th>
<th>VI-LD</th>
<th>VI-RB</th>
<th>VI-JB</th>
<th>V-LB</th>
<th>V-DK</th>
<th>V-WS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>44</td>
<td>49</td>
<td>51</td>
<td>55</td>
<td>59</td>
<td>63</td>
<td>73</td>
</tr>
<tr>
<td>Neurological signs</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fixative</td>
<td>Formol</td>
<td>Carnoy</td>
<td>Formol</td>
<td>Formol</td>
<td>Carnoy</td>
<td>Carnoy PLP</td>
<td>Carnoy PLP</td>
</tr>
</tbody>
</table>

**Amyloids**
- anti-PrP  
- anti-SP28  
- anti-β-amyloid

**Neurites**
- Aβ50  
- anti-synaptophysin nd  
- anti-ubiquitin +  
- anti-preA4 nd  
- anti-SP20 nd  

+, present; -, absent.

*, absent in cerebellar amyloid deposits.

nd, not determined, fixation procedure inappropriate for anti-synaptophysin, anti-preA4 and anti-SP20 immunostaining.

PLP, periodate-lysine-paraformaldehyde.

ing βPP, that are involved with the growth or degeneration of nerve cell processes.

**Aβ and PrP Deposits**

The senile GSS-Ik patient was peculiar neuropathologically for the presence of extracellular Aβ deposits often colocalized with PrP-amyloid. Why Aβ deposition occurred in this patient cannot definitely be answered by the morphological findings. However, three tentative explanations can be formulated. First, the possibility has to be considered that the senile GSS-Ik patient was affected by AD. This hypothesis was first proposed to explain the association of NFT, Aβ and PrP-amyloid immunoreactivities in the two Swedish patients (23). The association of GSS and AD should be regarded as casual. Accordingly, if AD affects 5 to 7 out of 100 individuals older than 65 years of age (35), the chance for the senile GSS-Ik patient of presenting with both diseases was 5 to 7 percent. Second, extracellular Aβ could have resulted from βPP accumulated in degenerating neurites. Following this view, Aβ deposition in GSS-Ik should be regarded as a late consequence of PrP-amyloid present in the neuropil and acting on neurites. Finally, the deposition of Aβ might have been related to aging (11, 25). This possibility is suggested by the fact that cerebral deposits of Aβ are numerous in aged individuals and increase in number with aging. In the eighth decade of life, the overall prevalence of individuals with such cerebral deposits is 44 percent (36). This figure supports the view that in V-WS, who died at 73 years of age, Aβ deposition was an age-related phenomenon superimposed to PrP-amyloidosis. Accordingly, the association of PrP-amyloid and Aβ deposits could have been fortuitous, although it is likely that PrP-amyloid favored the colocalization of Aβ by modifying the physicochemical properties of the neuropil (24). Double amyloidosis with cerebral Aβ deposition is relatively common in aged patients; so far, it has been found not only in aged GSS patients (24, 25), but also in aged individuals with AA, AL, ATTR or Agel amyloidosis, and in eight 68 to 81 year old patients with Creutzfeldt-Jakob disease (24, 25, 37-40). The last two hypotheses are complementary in the sense that aging might have provided the conditions, including time, necessary for increased intracellular βPP to be processed to extracellular Aβ.

In conclusion, this study showed that in GSS-Ik βPP participates in PrP-amyloid plaques not only as a cytoplasmic component largely represented in degenerating neurites, but also as a source of Aβ that accumulates in the extracellular space. Analysis of neuropathologic findings mostly supports the view that accumulation of βPP in nerve cell processes represents a reaction of neurons to extracellular PrP-amyloid, and that extracellular deposition of Aβ is related to the aging process.

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