Parkinson's Disease and Dementia with Neuronal Inclusions in the Cerebral Cortex: Lewy Bodies or Pick Bodies

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Abstract. Cortical Lewy bodies may be difficult to differentiate from Pick bodies by the usual staining methods. This problem is illustrated in a case of progressive dementia with parkinsonian features. Lewy bodies were found, not only in the pigmented nuclei in the brainstem and in the cerebral cortex, but also in the dentate fascia, a predilection site for Pick bodies. The inclusion bodies were compared with the inclusion bodies in a case of Pick's disease. Intense argyrophilia of the cortical inclusion bodies argued in favor of Pick bodies; antibodies to phosphorylated neurofilaments reacted with the cortical inclusions and with classical Pick bodies, but antibodies to paired helical filaments reacted only with the Pick bodies. The most convincing evidence, that the inclusions were Lewy bodies, was obtained by electron microscopy. The filaments in the inclusions showed fuzzy deposits of electron dense material, characteristic for filaments of Lewy bodies. This contrasted with the smooth filaments of the Pick bodies. It is concluded that cortical inclusions in brains from patients with Lewy bodies in the pigmented nuclei of the brainstem most likely represent Lewy bodies. This can be confirmed by examining the ultrastructure of the inclusions.

Key Words: Argyrophilia; Dementia; Electron microscopy; Immunocytochemistry; Lewy bodies, cortical; Parkinson's disease; Pick bodies.

INTRODUCTION

Certain neurodegenerative disorders, including several with a clinical picture of progressive dementia, are associated with intracytoplasmic inclusions involving cytoskeletal elements. Most of these inclusions are distinctive for the disease they represent and may serve as a diagnostic marker, and also as a clue to the disease process in each disorder. Although it is possible that the same biochemical and molecular abnormality may lead to different forms of structural change within the nerve cell, structural differences are more likely to reflect differences in pathogenesis. Thus, neurofibrillary tangles and senile plaques in the cerebral cortex are required for the diagnosis of Alzheimer's disease (AD), Pick bodies (PB) for diagnosis of classical Pick's disease, and Lewy bodies (LB) in the pigmented nuclei of the brainstem for Parkinson's disease (PD). However, in dealing with cortical LB there is a special difficulty. Because such LB usually have a less pronounced central core and may lack a halo, they may be mistaken for PB. Moreover, cortical LB are especially common in cases of dementia, often in connection with other clinical and pathological features of PD, or of AD (1-4). In the case to be described the identification of the inclusions was particularly difficult, because the cortical intraneuronal inclusion bodies were intensely argyrophilic and because they were present also in the granule cells of the dentate fascia of the hippocampus. This is a predilection site for PB (5, 6) but not for LB.
CASE REPORT

This 73-year-old man had a prostatectomy in 1981 for adenocarcinoma of the prostate. At that time he was mildly demented, depressed and rigid with a shuffling gait. By 1983 his mental status had deteriorated and he was oriented only to person and had marked rigidity. A CT scan of the head was normal. During his last admission in December 1984 there was evidence of further mental deterioration. Treatment with levodopa and bromocriptine resulted in slight improvement, but he developed pneumonia and died in January 1985.

The general autopsy showed bilateral bronchopneumonia, locally recurrent adenocarcinoma of the prostate and a small adenocarcinoma of the left kidney.

The brain weighed 1,400 grams and showed atrophy only of the hippocampal gyri. The ventricles were slightly dilated. The substantia nigra was pale.

On histological examination there was severe nerve cell loss in the substantia nigra with increased extraneuronal melanin, fibrillary gliosis and a few typical Lewy bodies (LB) (Fig. 1a). The paranigral nucleus in the ventral tegmental area suffered the same degree of nerve cell degeneration as the substantia nigra compacta. The locus ceruleus was moderately depleted of nerve cells. A few typical LB were found here and in the dorsal motor nucleus of the vagus. The basal nucleus of Meynert showed a moderately severe nerve cell loss and several LB which were also present in other predilection sites in the brain, but intracytoplasmic inclusions in nerve cells were particularly numerous in the amygdala and the limbic cerebral cortex. The LB were especially prominent in the parahippocampal gyri, in the pyramidal cell layer of the hippocampus (Fig. 1b), and in the granule cells of the dentate fascia. Marked nerve cell loss, rarefaction of tissue and gliosis were present in the pyramidal cell layer of the hippocampus. Inclusion bodies were found in all of the limbic areas, including the cingulate gyrus and the olfactory tubercle. In the neocortex, where they were found mainly in the deep cortex only a few inclusions were found per slide. Senile plaques could be seen in the cerebral cortex of all cortical lobes, and in the amygdala, but neurofibrillary tangles were few and present only in the hippocampus. There was no congophilic angiopathy, and no other lesions were noted.

MATERIALS AND METHODS

Light Microscopy

Eight-micrometer (μm)-thick paraffin sections from the temporal cortex with hippocampus and parahippocampal gyrus were stained with the following methods: Luxol fast blue (LFB) combined with cresyl violet (LFB-CV) or with periodic acid-Schiff reagent (LFB-PAS); Bielschowsky and LFB-Bielschowsky; Congo red; phosphotungstic acid hematoxylin (PTAH); Holzer stains; trichrome stain.

Immunocytochemistry

The immunocytochemical procedures were performed on 8-μm-thick paraffin sections, using the peroxidase–antiperoxidase method (7). The hippocampal–parahippocampal gyrus from a recent case of typical Pick’s disease was immunostained and compared with the similarly stained hippocampal–parahippocampal gyrus from the present case. Usually the immunocytochemical procedures were performed simultaneously on both cases.

The following antibodies were used: Monoclonal antibody directed against the 200 kD phosphorylated neurofilament (07-5 antibody, courtesy of Dr. L. A. Sternberger (8)); and two polyclonal antibodies raised against paired helical filaments, kindly supplied by Dr. Dennis Selkoe. These antibodies were used in dilution 1:500. Immunostaining with antibodies for glial fibrillary acidic protein (GFAP) (courtesy of Dr. L. F. Eng) and preimmune sera were
used for control. Diaminobenzidine was used as chromogen for the reactions for neurofilaments and for GFAP; aminoethylkarbazol (BioGenex Laboratories) was used for the reactions with Dr. Selkoe’s antibodies. The sections were lightly counterstained with hematoxylin.

Electron Microscopy

Small pieces of formalin-fixed tissue from the hippocampus were postfixed in glutaraldehyde and osmium tetroxide and embedded in epon-araldite. Selected blocks were thin-sectioned, stained with uranyl acetate and lead citrate and viewed in a Philips 201 electron microscope. Electron microscopy of formalin-fixed tissue from the hippocampus from a case of familial PD and dementia (9) with cortical Lewy bodies and from three cases of typical Pick’s disease, including the recent case employed for immunocytochemistry, were used for comparison.

RESULTS

The cortical inclusion bodies displayed the usual characteristics of cortical LB (1–4). In contrast to the few typical LB in the brainstem, with their central core and peripheral halo (Fig. 1a), the cortical inclusions were, for the most part, less eosinophilic and less well defined, without a central core (Fig. 1b). The inclusions displaced the nucleus to the periphery of the cell. In Bielschowsky or LFB–Bielschowsky silver impregnation, the majority of the cortical inclusions, including those in the dentate fascia, were heavily impregnated (black) (Fig. 1c), whereas LB in the brainstem were brown or pale. Other staining methods, such as PTAH, PAS, trichrome, and Congo red, did not show any affinity for the inclusions.

Immunocytochemistry

Both inclusions in the present case and PB reacted with the neurofilament antibody, but the reaction was more intense in the Pick’s disease case (Fig. 2a, b, with a
negative control shown in Fig. 2c). Typical brainstem LB do not always react with this particular antibody, but when they do the reaction is usually stronger and in a peripheral band (10). The PB reacted strongly with the two polyclonal antibodies to paired helical filaments (Fig. 3b) (11). This contrasted with a negative or faint reaction in the present case (Fig. 3a).

Electron Microscopy

Low power view of the inclusions demonstrated no clear difference between the inclusion bodies in the present case and the PB or the cortical LB (Fig. 4a–c, shown with a classical brainstem type LB with radiating filaments in Fig. 4d). All of the cortical inclusions contained criss-crossing filaments with variable amounts of granular material and other organelles admixed, but on higher magnification (Fig. 5a–d) important distinguishing characteristics emerged. The filaments in the PB were in all three cases straight filaments without sidearms or fuzzy material attached to them (Fig. 5b). In contrast, the filaments in the present case had varying amounts of fuzzy electron dense deposits (Fig. 5a). The quality of preservation of the tissue was not sufficient to establish the identity of this granular material. Similar fuzzy deposits were seen on the filaments from the cortical LB in the case of familial PD (Fig. 5c, shown with filaments from a LB in the substantia nigra in Fig. 5d). The diameter of the filaments in the three Pick’s disease cases varied from 9 to 20 nm, with an average of 15–18 nm. Because of the ill-defined margins of the filaments in the present case, and in the LB from the cases of PD, measurements were less accurate, but again ranged from 9 to 20 nm. The diameter of filaments therefore did not seem to be an important deciding factor in the differential diagnosis between PB and LB.
Fig. 3. a. The inclusion bodies (arrows) do not react with polyclonal antibodies to paired helical filaments (Selkoe). b. This contrasts with the markedly positive reaction with the PB (arrows) in the case of Pick's disease. Both types of inclusion bodies are shown in the dentate fascia. Hematoxylin counterstain. ×550.

DISCUSSION

Cortical neuronal inclusion bodies in cases with LB in the pigmented nuclei of the brainstem have been the subject of many recent reports (1–4). Because these neuronal inclusions most often are found in patients with dementia, their role as indicators of nerve cell degeneration in the cerebral cortex and their importance for intellectual deterioration have been the subject of much discussion. The inclusion bodies outside of the substantia nigra and locus ceruleus in PD may display considerable deviation from the morphology of typical LB. In the dorsal motor nucleus of the vagus, the hypothalamus, the basal nucleus of Meynert and the sympathetic ganglia, these inclusions may have bizarre shapes and are often found in nerve cell processes. In the cerebral cortex the inclusions are almost always confined to nerve cell perikarya. The type and location of the nerve cell affected by the disease process probably influences the arrangement of the abnormal filaments and other organelles that make up the inclusion body. The presence or absence of neuromelanin may also be an important factor.

Fig. 4. a. Inclusion body in the hippocampus of the present case. Uranyl acetate and lead citrate. ×5,390. b. Pick body in the hippocampus. ×8,500. c. Lewy body in the parahippocampal gyrus from a case of familial PD with dementia (see reference 9). ×7,000. d. Classical Lewy body with radiating filaments in the substantia nigra in a case of PD. ×7,500.
Fig. 5. Filaments (arrowheads) are shown at the same magnification from an inclusion body in the present case (a), from filaments in a PB (b), and from filaments in LB: cortical LB in c, and in the substantia nigra in d; c and d are from the inclusions shown in Figure 4c and d. Only the filaments in the PB are free of deposits of electron dense material or side arms. $\times$ 58,000.
The close relationship of these inclusion bodies to classical, or typical, LB has generally been accepted for two reasons: 1) because Lewy originally found the peculiar, often elongated bodies in extranigral locations (nucleus basalis of Meynert, hypothalamus and the dorsal motor nucleus of the vagus) (12), and 2) because they are found in connection with typical LB in the substantia nigra and locus ceruleus (13).

In the cerebral cortex, where Lewy did not describe inclusions, the situation is particularly complex. Munoz-Garcia and Ludwin (14) have described inclusion bodies in the generalized variant of Pick’s disease, that bear considerable resemblance to cortical Lewy bodies, especially by electron microscopy, but are not associated with parkinsonism or with classical LB in the pigmented nuclei of the brainstem. Other authors have described inclusions in juvenile amyotrophic lateral sclerosis (15), that have some similarities, but also differences (basophilia) compared to the inclusions in the present case.

The examination of the relationship between cortical Lewy-like bodies and other inclusions that involve abnormalities of the cytoskeleton may yield insights into the nerve cell degeneration in these varied and often overlapping disorders.

This case here reported presented an opportunity for a comparison of PB and cortical LB. As the results have shown, not all the evidence pointed in the same direction. Neither PB nor cortical LB present a very striking appearance in hematoxylin and eosin (H&E)-stained sections. The PB may be faintly basophilic, while Lewy bodies are a pale pink color, but both types of inclusions have a fairly homogenous texture. Finding inclusions with suggestion of a central core helps in the identification of inclusions as LB. In the neocortex LB tend to be most numerous in the deeper cortical layers (2, 4), while PB are more common in the superficial cortical layers; since this difference in location is less pronounced in the entorhinal cortex (2), where inclusions were most numerous in the present case, this feature was less helpful. Moreover, Arima (5) in a recent study of three cases found the fifth and sixth cortical layers to be the second most common site for PB. In most doubtful cases silver impregnation methods also settle the question. The PB are intensely argyrophilic and stain black, in contrast to the usually brown or pale LB in Bielschowsky stains.

In the present case we had two reasons for questioning the LB identity of the cortical inclusions: their marked argyrophilia, and the presence of inclusions in the dentate fascia of the hippocampus. Because of these two features we turned to immunocytochemistry and to electron microscopy. The positive staining of both PB and the inclusions in the present case (Fig. 2a, b) with antibody to phosphorylated neurofilaments was no surprise, since both PB and LB were known to react with this particular antibody (10), although typical Lewy bodies may present a different staining pattern and a more intense staining reaction. The neurofilament antibody staining was therefore of no help in the diagnosis. The reaction with the two antibodies to paired helical filaments (donated by Dr. Selkoe) was more helpful, since both antibodies reacted intensely with PB, but hardly at all with the cortical inclusions in the present case (Fig. 3a, b). This was in agreement with findings by Rasool and Selkoe (11), who also found positive staining of PB, but not of LB, using a similar antibody. These results therefore favored an identification of the inclusions in the present case as LB.

The most convincing arguments in favor of calling our inclusions cortical LB came, however, from electron microscopy. As Munoz-Garcia and Ludwin have pointed out (14), the filaments in the PB in the classic variant of Pick’s disease have
a smooth contour without sidearms or fuzzy deposits along their course (Fig. 5b), whereas fuzzy deposits are characteristic for the inclusions in the generalized variant of Pick's disease. Lewy bodies in cortex as well as in the brainstem also have fuzzy deposits (Fig. 5c, d). The filaments in the present case clearly displayed these fuzzy contours (Fig. 5a). The slight differences between LB and PB in the diameter of the filaments are probably of less importance than the character of the filaments, especially since published reports have described widely varying diameters for both types of filaments (14, 16–18).

How does one reconcile these results with the intense argyrophilia of the inclusion bodies in question and their location in the dentate fascia? Weak or absent argyrophilia, with argyrophilia defined as a black impregnation, is probably not a consistent characteristic of LB. Most textbooks and reviews, dealing with inclusion bodies, describe other important staining reactions, but do not mention how LB react with silver stains (19, 20). This contrasts with the emphasis on the argyrophilia of PB. On the other hand, Greenfield and Bosanquet in a table of staining reactions listed Lewy bodies as argyrophilic (with a darker core) (21). They also suggested that the confusion between LB and PB (in the brainstem) "appears to have arisen from the too exclusive use of silver stains" (21, p. 220). In the Palo Alto VA Medical Center neuropathology laboratory, we have also observed black impregnation of LB in Bielschowsky and LFB–Bielschowsky stain in the basal nucleus of Meynert in a few brains from patients with Parkinson's disease, and also occasionally in cortical LB (L. S. Forno, unpublished observation). Kosaka (22) has listed argyrophilia as present in both cortical and brainstem type LB.

The location of the cortical type LB in the dentate fascia is certainly unusual, but LB can be present in small neurons, for example in the amygdala and in the paranglial nucleus. Dickson et al have recently reported the finding of PB-like inclusions in the dentate fascia in cases of AD (23). Although PB and AD neurofibrillary tangles probably are more closely related than PB and LB (10), the finding by Dickson et al (23) provides evidence that the dentate fascia is susceptible to inclusions in conditions other than Pick's disease.

In the present case, our conclusion that we are dealing with cortical Lewy bodies is strengthened by the fact that there was no lobar atrophy, a feature rarely absent in Pick's disease.

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