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Pick-body-like inclusions in corticobasal degeneration differ from Pick bodies in Pick's disease

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Abstract In corticobasal degeneration (CBD), tau-positive cytoplasmic inclusions resembling Pick bodies (PBs) appear in the cerebral cortex. Tau immunoreactivity in PBs is expressed mainly on filamentous elements, whereas that of PB-like inclusions in CBD is expressed on granules, which are densely packed mainly in the periphery of inclusions. PBs are clearly detectable by conventional Bodian silver impregnation but negative for the Gallyas–Braak (G–B) method and for PS262, which recognizes phosphorylation at Ser 262 of the entire tau sequence. Almost all PBs are negative for Ex10, which recognizes 4-repeat tau specifically. In contrast to PBs, PB-like inclusions in CBD could not be detected by the Bodian method, but were positive for the G–B method, PS262 and Ex10. In summary, PBs and PB-like inclusions exhibit distinct differences. These results are useful for the argument of an overlap between PiD and CBD as well as discussion of the phenotypic resemblance of PB-like inclusions bearing types of FTDP-17 to Pick's disease.

Keywords Pick body-like inclusions · Pick bodies · Corticobasal degeneration · Pick's disease · FTDP-17

Introduction

Corticobasal degeneration (CBD) and Pick's disease (PiD) are both tauopathies. Neuropathological differentiation of CBD and PiD is thought to be easy in ordinary cases; however, overlapping of these two diseases is noted based on common features of frequent laterality and/or variation in cerebral lesions [5, 12]. Another common finding is the existence of Pick body-like inclusions in CBD, which resemble Pick bodies (PBs), the hallmark of Pick's disease. PB-like inclusions are also reported in some exonic mutations of familiar frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) and their phenotypic resemblance to PiD is argued based on the existence of PB-like inclusions [2, 8, 9, 11, 14]. We report morphological and biochemical characteristics of PB-like inclusions in CBD to contribute to further discussion.

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Materials and methods

Paraffin-embedded 10-μm sections from multiple brain regions of seven patients with CBD and two patients with PiD were stained with Bodian and Gallyas–Braak (G–B) silver impregnation methods and surveyed for PBs or PB-like inclusions. All cases were diagnosed on a clinical basis and confirmed in every case by routine neuropathological examination. For immunohistochemical study, small brain blocks fixed in 4% paraformaldehyde in 0.1 M phosphate buffer from two patients with CBD and two patients with PiD were used. The blocks were cut on a freezing microtome to 30-μm thickness. For primary anti-tau antibodies, Ex10, which specifically recognizes 4-repeat tau [1], AT8 (phosphorylation dependent, Innogenetics), human-tau pool 2 (phosphorylation independent, rabbit polyclonal) and PS 262, a polyclonal antibody specific to phosphorylated Ser 262 [4], were used. Immunolabeling was detected using the ABC system coupled to a diaminobenzidine (DAB) reaction intensified with nickel ammonium sulfate to yield a purple precipitate. To clarify the type of cells carrying PB-like inclusions, double immunostaining was performed using human-tau pool 2 for the first immunohistochemical cycle and then incubated with antibodies for the second cycle except that nickel ammonium sulfate was omitted from the DAB solution. Antibodies used for the second cycle were: Neu-N (mAb, Chemicon), a neuron specific marker, anti-GFAP (rabbit, IgG, Dakopatts) and CD44 (mAb, IgG, Binding Site) for astrocytes, or anti-C4d (mAb, IgG, Quidel) for oligodendroglia. Brain blocks obtained from two cases each of CBD and PiD were also analyzed by immunoblotting to elucidate which isoforms of tau they were composed of. The details of the results have been described in a previous report [1]. For immunoelectron microscopic examination of PB-like inclusions and PB, small specimens were obtained from each patient with CBD or PiD. The specimens were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, then cut into 30-μm thick sections. The sections were incubated with AT8 antibody for 3 days at 4 °C, visualized by the avidin–biotin method using DAB and embedded in

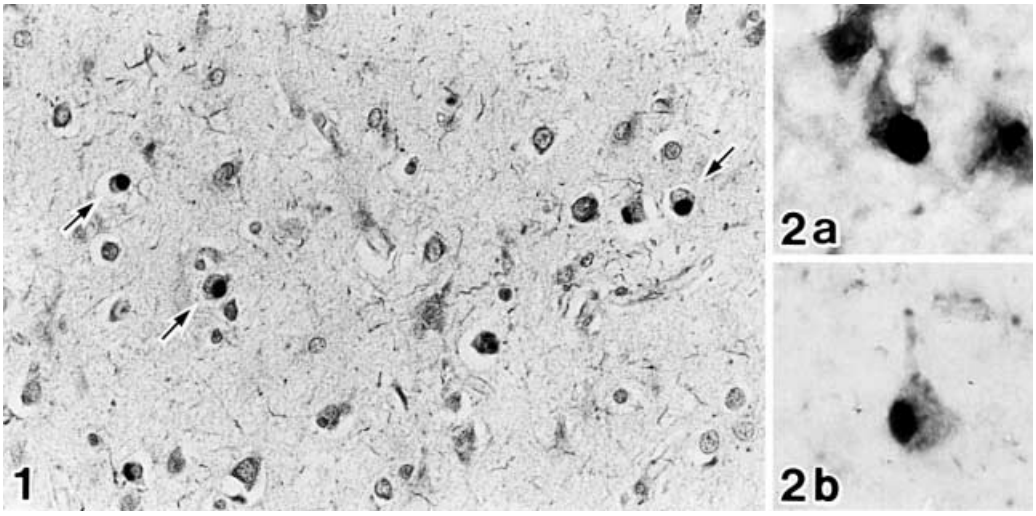

Fig. 1 Tau/G–B-positive PB-like inclusions (*arrows*) were found mainly in the II layer to the upper part of III layer of CBD brain. They were distributed frequently in the frontal convexity where other types of tau/G–B-positive structures are also abundantly deposited. G–B method with Kernechtrot counterstaining. ×420

Fig. 2a, b Double immunostaining using anti-tau and Neu-N antibodies showed the PB-like inclusions in CBD (**a**) as well as PBs in PiD (**b**) to be neuronal. The dark colored tau-positive round-shaped inclusion was originally purple, whereas the light colored Neu-N-positive cytoplasm was originally stained yellow. ×1,050

epoxy resin. The ultra-thin sections were observed under an electron microscope without heavy metal staining.

Results

PB-like inclusions were found in all seven CBD patients by the G–B methods. PB-like inclusions were round or oval and their morphology resembled that of PBs (Fig. 1). These PB-like inclusions could not be detected by the conventional Bodian method. Conversely, PBs in PiD could not be detected by the G–B method, but were demonstrated by the Bodian method (Table 1). On double immunostaining, PB-like inclusions as well as PBs occupied the neuronal cytoplasm, as demonstrated by neuron specific Neu-N antibody (Fig. 2a, b). PB-like inclusions did not coexist with GFAP, CD44 or C4d; however, some of them were found freely in the neuropil in addition to neuronal cytoplasm. The possibility that PB-like inclusions also originate from glia could not be excluded. PB-like inclusions were distributed mainly in the II layer or upper part of the III layer of the cerebral cortex, especially in the frontal convexity, where other type of G–B/tau-positive structures were abundant.

Immunohistochemically, PB-like inclusions in CBD were positive for all examined anti-tau antibodies, AT 8, pool 2, Ex 10 and PS 262 pretreated by formic acid. Meanwhile, AT 8 and pool 2 stained PBs, but Ex 10 recognized only a small number of PBs. PS 262 did not recognize PBs (Table 1). On immunoelectron microscopy, tau-positive eccentric inclusions were found in the cytoplasmic perikarya of both CBD and PiD (Fig. 3a, c). In PB-like inclusions of CBD, the reaction product with AT 8 resided on granules, which incline to lie densely in the periphery of inclusions. The immunoreactivity was poor in the loose central part, where a small number of randomly

Table 1 Differences in the nature of PB-like inclusions in CBD and PBs in Pick's disease (*PB* Pick bodies, *G–B* Gallyas–Braak method, *PS262* anti-tau antibody specific at phosphorylated Ser 262, *Ex10*, anti-tau antibody specific for 4-repeat tau; +, positive; –, negative)

	Bodian	G–B	AT8/pool2	PS262	Ex 10	Immunoblot analysis [1]	Electron microscopy
PBs in PiD	+	–	+	–	–[a]	3-repeat tau ⩾ 4-repeat tau	Accumulation of randomly oriented straight filaments intermingled with paired twisted profile. Granular structures are trapped among the filaments. Tau immunoreactivity resides on these structures
PB-like inclusions in CBD	–	+	+	+	+	4-repeat tau	Densely packed granules in the periphery, while a small number of randomly oriented straight filaments were intermingled with granules in the central part of inclusion. Tau immunoreactivity resides on peripheral granules

[a]A small number of PBs were positive for Ex 10

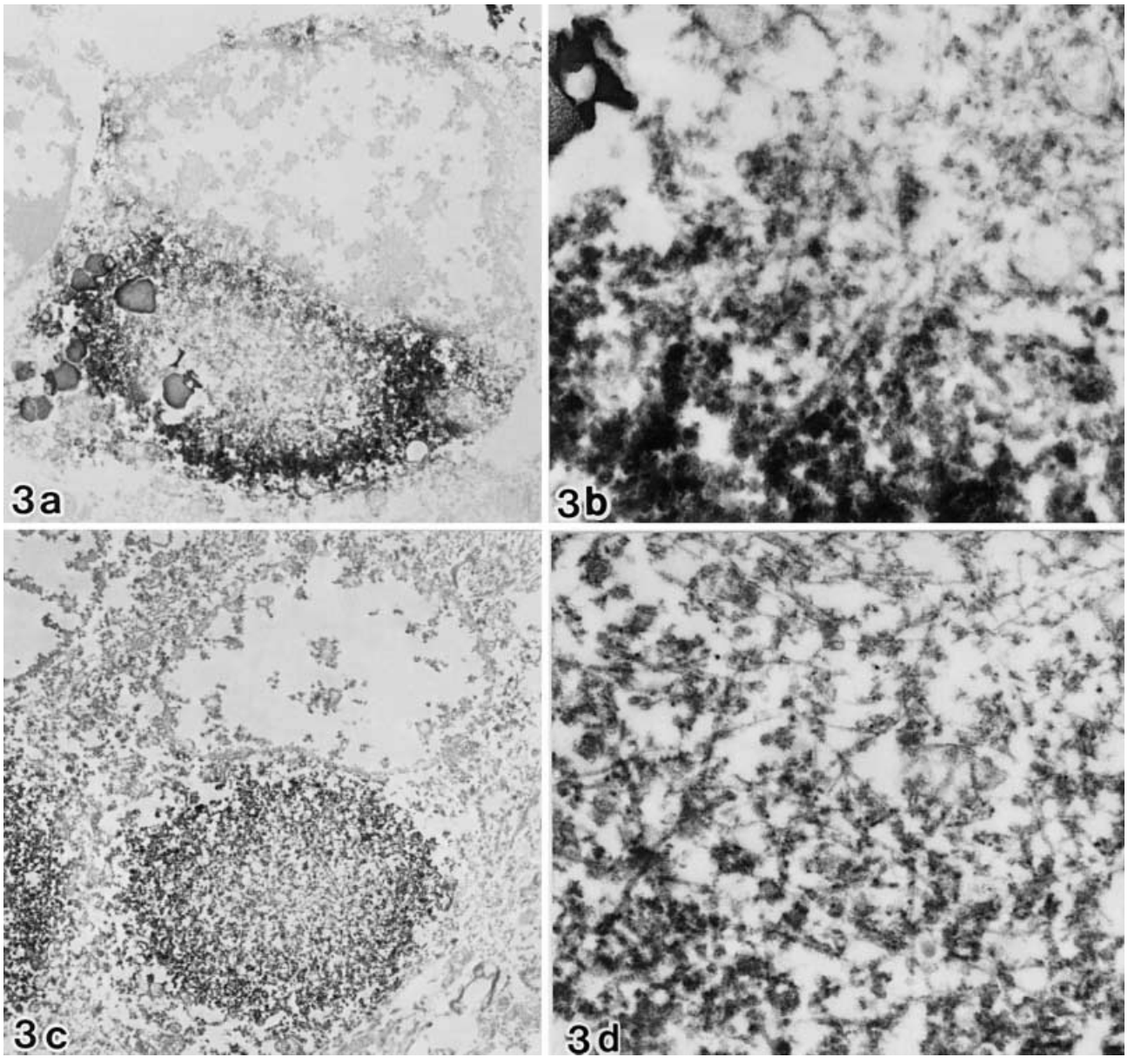

Fig. 3 On immunoelectron microscopy of PB-like inclusions in CBD and PBs in PiD, tau-immunoreactivity was found in the eccentric inclusion, which occupied most of the cytoplasm (**a, c**). At higher magnification, the reaction product with tau on PB-like inclusions in CBD resided on densely packed peripheral granules. Immunoreactivity was faint in the central part of the inclusion, where a small number of randomly oriented straight filaments intermingled with granules (**b**). In contrast with PB-like inclusions, tau-immunoreactivity in PBs in PiD was found on randomly oriented filaments and granules, which were trapped among filaments (**d**). **a, c** ×6,200, **b, d**: ×31,000

oriented straight filaments intermingled with the granules (Fig. 3b). There was no obvious boundary between the central and peripheral part of the inclusions. Immunoreactive granules in PB-like inclusions were similar to AT 8-positive granules in pre-tangles, which were occasionally observed in the same sections. Glial filaments could not be seen. In contrast with PB-like inclusions, the AT 8-immunoreactivity in PBs was found mainly on randomly oriented filaments and granules, which were trapped among filaments (Fig. 3d).

Discussion

PBs in PiD mainly consisted of randomly oriented AT 8-positive straight filaments, which coincided with a previous report [7]. A small number of filaments were scattered in the central part of the PB-like inclusions in CBD; however, tau-immunoreactivity lies not on these filaments but on densely packed peripheral granules, which coincided with the major component of pre-tangles. Differences between PBs and PB-like inclusions were also noted in the results from immunohistochemical and immunoblotting analysis. Delacourt and his colleagues first reported that abnormal tau in PiD consists of 3-repeat tau, while that in

CBD consists of 4-repeat tau [3, 13]. Our follow-up immunoblot study of sarkosyl insoluble fraction of PiD and CBD nearly agreed with their results, although minor bands of 4-repeat tau were also noted in PiD [1]. Immunohistochemical results for Ex10 (4-repeat tau specific antibody) were consistent with our immunoblot data, namely, Ex10 recognized some PBs, thorn-shaped astrocytic inclusions, but did not immunostain the majority of PBs and coiled bodies in PiD. Neuronal and glial tau-positive structures including PB-like inclusions in CBD vigorously labeled by Ex10 and their isoforms were confined to 4-repeat tau on immunoblot analysis [1]. To date, the phosphorylation-dependent antibody at Ser 262 has reportedly not recognized abnormal tau products in PiD [3, 10] and argyrophilic grain disease [15]. Our result was consistent with previous reports. As summarized in Table 1, corticobasal PB-like inclusions and PBs differ in several points. Therefore, the argument regarding the overlap of PiD and CBD based on the presence of PB-like inclusions in CBD should be viewed cautiously.

Recently, PB-like inclusions have been reported in some cases of FTDP-17 [2, 8, 9, 11, 14]. PB-like inclusions in FTDP-17 seem to be divided into two groups, mutations in exon 10 [2, 9] or in other exons of tau [8, 11, 14]. The neuropathology of P301L and P301S mutations in exon 10 is reminiscent of CBD pathology [2, 6, 9]. PB-like inclusions in P301S consist of 4-repeat tau and are not detectable by the Bodian method [9], similar to the PB-like inclusions in sporadic CBD. PB-like inclusions in patients with P301S mutation are positive by the Bodian method [2]; however, in printed photographs the inclusion looks like globosed-type neurofibrillary tangles. We reported a discrepancy between massive tau immunoreactivity and poor argyrophilia by the Bodian method in CBD, which may be based on poor fibril formation of tau aggregates in CBD [16]. Meanwhile, G389R mutation in exon 13 [8] and G272V or K257T mutation in exon 9 [11, 14] exhibit phenotypic resemblance to PiD. Detailed clinicopathology results for G389R as well as K257T mutations are consistent with those for PiD. In particular, the tau isoforms found in the K257T mutation reportedly show a preponderance of 3-repeat tau. PB-like inclusions are basophilic, they are negative for 12E8, and other characteristics also coincide with PBs [11].

Elucidating the phenotypic correspondence between FTDP-17 and sporadic tauopathies, albeit broadly, may provide a clue for further study of sporadic cases. This study documents basic findings of PB-like inclusions, contributing to further discussion.

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