Selective Deposition of 4-Repeat Tau in Cerebral Infarcts

Kazuaki Ichihara, MD, PhD, Toshiki Uchihara, MD, PhD, Ayako Nakamura, Yoshio Suzuki, MD, PhD, and Tomohiko Mizutani, MD, PhD

Abstract

The tau deposits found in neurodegenerative diseases are classified based on their isoforms, that is, 3-repeat (3R) tau and 4-repeat (4R) tau. These isoforms are distinguishable using the antibodies RD3 and RD4, respectively, and Gallyas (Gal) and Campbell-Switzer (CS) silver staining methods, respectively. Tau is also deposited in cerebral infarcts. To characterize the tau profile in these lesions, 21 brains from autopsied patients with cerebral infarcts were analyzed using immunohistochemistry with RD3, RD4, and the anti-paired helical filament antibody AT8 and with Gal and CS staining; all of these techniques identify Alzheimer disease-type neurofibrillary tangles. Fluorescence labeling followed by silver staining in mirror-section pairs was also used to compare the staining patterns. Neurons in and around ischemic foci exhibited the 4R-tau epitope until 34 days postinfarction; argyrophilia with Gal staining persisted longer. The 4R-tau/Gal-positive neurons were negative for 3R-tau and AT8 epitopes and lacked fibrillary structures and argyrophilia by CS staining; they are, therefore, distinct from neurons with neurofibrillary tangles. Positivity for 4R tau/Gal and negativity for 3R tau/CS were also seen in astrocytes and microglia around infarcts. Although this staining profile is characteristic of degenerative processes with 4R-tau deposition, lack of AT8 immunoreactivity and of fibrillary structures in neurons, astrocytes, and microglia indicates that selective 4R-tau deposition represents a stage without tau phosphorylation or fibril formation in cerebral infarcts.

Key Words: 4-Repeat, Campbell-Switzer, Cerebral infarct, Dark neurons, Gallyas staining, Tau.

INTRODUCTION

Tau is a microtubule-associated protein involved in the assembly and stabilization of the microtubule network (1, 2). Tau deposits are one of the major neuropathologic hallmarks of several neurodegenerative disorders including Alzheimer disease (AD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), and Pick disease. Understanding the pathogenesis of these diseases has been greatly enhanced by identification of the biochemical distinction between 3-repeat (3R) and 4-repeat (4R) pathologically phosphorylated tau isoforms and their disease-specific associations (3–5). It is now considered that 4R tau is preferentially deposited in brains with PSP, CBD, and argyrophilic grains, whereas 3R tau is a major component of Pick bodies (6–8). Interestingly, deposits of 4R tau exhibit argyrophilia with Gallyas (Gal) staining and those of 3R tau exhibit argyrophilia with Campbell-Switzer (CS) staining in these conditions. These parallel patterns (i.e. 4R/Gal and 3R/CS) are mutually exclusive except in the case of neurofibrillary tangles (NFTs) of the AD type, which contain both 3R and 4R tau and exhibit argyrophilia with both CS and Gal (9). We have previously reported the presence of Alz-50 (10) and tau2 immunoreactivity (IR) (11) in neurons and microglia/macrophages, respectively, in immunohistochemical studies on human brains with cerebral hemisphere infarcts (CI). Thus, tau deposition in human brains is not a specific phenomenon restricted to neurodegeneration. Some of these ischemic neurons exhibit not only tau IR but also argyrophilia with Gal (10). To elucidate mechanisms of tau deposition, phosphorylation, and fibril formation, and their possible relationship to neurodegeneration, we obtained a more detailed staining profile of these ischemic neurons using antibodies specific to either 4R tau (RD4) or 3R tau (RD3) (12) in combination with Gal and CS.

MATERIALS AND METHODS

We studied the brains of 21 patients with CI autopsied at the Department of Pathology, Asahi General Hospital, Chiba, Japan. The diagnosis, onset, and location of CI in these patients were determined from their clinical histories, neurological manifestations, and computed tomography or magnetic resonance imaging of the brain (Table). Brains were fixed in 10% phosphate-buffered formalin, and sections were embedded in paraffin. Six-micrometer-thick sections from various brain regions were stained with hematoxylin and eosin, Klüver-Barrera, Gal, and CS silver staining methods. Immunohistochemistry was performed on 6-μm-thick sections with RD3 (mouse monoclonal anti-3R-tau antibody, 1:3000; Upstate Biotechnology/Millipore, Billerica, MA) or RD4 (mouse monoclonal anti-4R-tau antibody, 1:1000; Upstate Biotechnology/Millipore) (12) and an anti–paired helical filament tau (PHF-tau, AT8, monoclonal, 1:10,000; Innogenetics, Zwijndrecht, Belgium) (13) antibodies using...
the avidin-biotin peroxidase method (ABC Elite, Vector Laboratories, Burlingame, CA). RD3 antibody was applied after pretreatment of the sections in an autoclave followed by formic acid for 30 minutes. Subsequent treatment with 1% periodic acid was needed for RD4 staining. Relative abundance of the lesions was assessed with an objective of 20× in the most affected foci in each preparation. A semiquantitative scale for RD4-positive neurons was based on their number/microscopic field as follows: −, 0; +, <5; ++, 5 to 10; ++++, >10. For GAL-positive neurons: −, 0; +, <5; ++, 10 to 20; ++++, >20 (see Materials and Methods section).

Comparisons between different stainings were made on serial sections. For identifying the cell types, mirror-section pairs (3 μm thick) were used to compare RD4 IR and argyrophilia with Gal for ischemic neurons (9) and RD4 IR and glial fibrillary acidic protein (GFAP) (rabbit anti-cow GFAP, DAKO, Glostrup, Denmark, 1:1000) IR and anti-CD68 (mouse monoclonal anti-human CD68, 1:1000, DAKO) IR and argyrophilia with Gal for glial cells. For more precise colocalization, mirror-section pairs (3 μm thick), after the pretreatment, were incubated at 4°C for 7 days with the mixture of RD4 (1:300) and anti-GFAP (1:300). The target epitopes were visualized with an anti-mouse IgG conjugated with Alexa 488 (1:200), Molecular Probes, Eugene, OR) and anti-rabbit IgG conjugated with Alexa 633 (1:200, Molecular Probes), respectively. The section pair counterparts were stained with Gal. Other mirror-section pairs after the same pretreatment were incubated at room temperature for 2 days with RD4 (1:300), and the target epitope was visualized with the anti-mouse IgG conjugated with Alexa 488 (1:200). The section pair counterparts, after autoclaving, were incubated at room temperature for 2 days with anti-CD68 (1:300), and the target epitope was visualized with the anti-mouse IgG conjugated with Alexa 488 (1:200) because the CD68 epitope is much attenuated after the pretreatment with formic acid, necessary for RD4 immunohistochemistry. The sections were observed under the confocal microscope, photographed, and then stained with Gal. The microscopic fields, first photographed as fluorescence images for CD68, were matched subsequently in the Gal-stained sections.

RESULTS

Among the 21 patients, RD4 IR neurons were prominent in and around ischemic foci in 12 patients from 2 days until 34 days postinfarction (Fig. 1; Table). Neurons with intense RD4 IR were rare at 2 days (Fig. 1A); RD4 IR became more intense in neuronal cytoplasm at 7 days, and the numbers as well as the staining intensities of the RD4-positive neurons increased in and around ischemic foci (Fig. 1C). The RD4-positive neurons were numerous between 7 and 34 days postinfarction (Fig. 1G), but they were essentially absent after 50 days postinfarction (Fig. 1I). Like RD4 IR, argyrophilia with Gal was faint in ischemic neurons at 2 days (Fig. 1B). There was more intense granular

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**TABLE. Clinical and Histopathologic Data of the Patients With Cerebral Infarction**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, Years</th>
<th>Sex</th>
<th>Days</th>
<th>Location of Infarct</th>
<th>PM Delay, Hours:Minutes</th>
<th>RD4</th>
<th>GAL</th>
<th>AT8</th>
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<td>F</td>
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<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>M</td>
<td>2</td>
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<td>9:19</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>3</td>
<td>68</td>
<td>F</td>
<td>5</td>
<td>Lt frontal lobe</td>
<td>16:55</td>
<td>−</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>87</td>
<td>M</td>
<td>6</td>
<td>Rt motor cortex</td>
<td>1:29</td>
<td>+++</td>
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<td>−</td>
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<tr>
<td>5</td>
<td>96</td>
<td>F</td>
<td>7</td>
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<td>+++</td>
<td>+++</td>
<td>−</td>
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<tr>
<td>6</td>
<td>76</td>
<td>M</td>
<td>9</td>
<td>Lt basal ganglia</td>
<td>9:41</td>
<td>+++</td>
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<td>−</td>
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<tr>
<td>7</td>
<td>93</td>
<td>F</td>
<td>11</td>
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<td>M</td>
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<td>−</td>
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<td>M</td>
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<td>Rt occipital cortex</td>
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<td>5,400</td>
<td>Rt frontal lobe</td>
<td>3:00</td>
<td>−</td>
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Semiquantitative scale for RD4-positive neurons: −, 0; +, <5; ++, 5 to 10; ++++, >10. For GAL-positive neurons: −, 0; +, <5; ++, 10 to 20; ++++, >20 (see Materials and Methods section).

*Days after the ischemic event.

AT8, anti-paired helical filament antibody; F, female; GAL, Gallyas staining; Lt, left; M, male; PM, postmortem; RD4, anti-4R-tau antibody; Rt, right.
cytoplasmic staining and positive neurons were more frequently observed at 7 days and thereafter (Figs. 1D, H, J). In summary, Gal-positive neurons in and around ischemic foci were seen in all 21 cases regardless of the age of infarcts ranging from 2 days to 5,400 days after onset (Table). Gal-positive neurons were more widespread than RD4-positive neurons with respect to extent and chronological extension. Anti-4R-tau antibody IR and argyrophilia with Gal were sometimes scattered diffusely in the cytoplasm.

In contrast to RD4 and Gal staining, neither RD3-positive nor CS-positive neurons were seen in any of the 21 brains examined (data not shown). Although this pattern was evident on separate sections, we examined sections at 7 days (when intense RD4 IR and Gal argyrophilia were frequent) to determine whether the RD4-positive or Gal-positive neurons showed any RD3 IR or argyrophilia with CS. As expected, the RD4-positive neurons lacked RD3 IR (Figs. 1E, 2B), whereas Gal-positive neurons exhibited RD4 IR in the same area on the mirror-section pair (Figs. 2C, D). The presence of NFTs around the ischemic foci in the hippocampus of Patient 1 allowed us to examine how these staining profiles were different between these ischemic neurons and NFTs on the same section. The relation of these argyrophilic structures to AT8 IR was examined on serial fluorescence labeling followed by Gal. The AT8 labeled NFTs but not ischemic neurons (Fig. 2E), whereas both were positive for Gal (Fig. 2F).

This consistent staining profile (positive for RD4 and Gal in parallel but negative for RD3 and CS) of ischemic neurons was shared with nonneuronal cells around ischemic foci (Figs. 2G, H, I, J) identified with RD4 IR in 18 cases or with argyrophilia with Gal in 12 cases out of the 21 cases. Identities of these nonneuronal cells were confirmed by comparing one of the mirror-section pairs stained with either RD4 or Gal and each counterpart stained with glial markers. Some RD4-positive nonneuronal cells (Fig. 3A) around an

**FIGURE 1.** Anti-4R-tau antibody (RD4)- and Gallyas staining (Gal)-positive cells at different time points after cerebral ischemia. (A, B) At 2 days (Patient 2, left frontal lobe), RD4-positive neurons ([A] arrow) are rare, and Gal-positive neurons ([B] arrows, faint staining) are present around ischemic foci. (C-F) At 7 days (Patient 5, left frontal lobe), RD4 immunoreactivity (IR) was more prominent in the neuronal cytoplasm; RD4-positive neurons are more numerous in and around the ischemic focus ([C] arrows). Gallyas-positive granular staining in the neuronal cytoplasm is more prominent, and stained neurons are more numerous ([D] arrows). In an adjacent section, neurons lack Anti-3R-tau antibody (RD3) ([E] hollow arrows) and argyrophilia with CS ([F] hollow arrows); lipofuscin granules are labeled. (G, H) At 28 days (Patient 13, right basal ganglia), RD4-positive neurons ([G] arrows) and Gal-positive neurons ([H] arrows) are seen in and around the ischemic focus. (I, J) At 90 days (Patient 17, left frontal lobe), RD4 IR is absent ([I] hollow arrow) but Gal-positive neurons ([J] arrows) are present in the neighboring section. (A, C, G, I) RD4 antibody immunostaining; (B, D, H, J) Gal; (E) RD3 immunostaining; (F) Campbell-Switzer staining (CS). Scale bar = 50 μm.
ischemic focus were positive for GFAP (Fig. 3B); the RD4-positive GFAP-negative cells might be neurons. Other non-neuronal cells positive for RD4 exhibited CD68 IR (Figs. 3E, F). Similarly, some Gal-positive nonneuronal cells (Fig. 3C arrows, Patient 9) exhibited GFAP IR (Fig. 3D). The Gal-positive astrocytes were located both in the vicinity of blood vessels and in the parenchyma without apparent relation to blood vessels (Figs. 3E–H). Other Gal- or RD4 positive cells had CD68 IR (Figs. 3G, H). To confirm these findings, mirror-section pairs, one stained with RD4 fluorescence and the other with Gal, were combined with multilabeling for glial markers. Gall-positive cells (Fig. 4A) were also positive for RD4 (Fig. 4D); some exhibited GFAP (Figs. 4B, C), indicating that some astrocytes exhibited both RD4 IR and argyrophilia with Gal, and other cells that did not show GFAP IR might be neurons. Some Gal-positive cells around ischemic foci showed RD4 IR (Fig. 4F) and CD68 IR (Fig. 4G).

**DISCUSSION**

We report the selective induction of 4R-tau epitope in ischemic neurons, astrocytes, and microglia in parallel with argyrophilia with Gal staining. Precise comparisons of the same structures by serial labeling and paired mirror sections confirmed that the RD4/Gal-positive cells lacked both RD3 IR and argyrophilia with CS staining. This staining profile was consistent with and is similar to the specific degenerative processes characterized by deposition of 4-repeat tau, that is, PSP, CBD, and argyrophilic grains (14–17). Unlike NFTs, however, the RD4/Gal-positive ischemic neurons did not exhibit AT8-positive fibrillary structures, which are usually positive for Gal, CS, and AT8.

The staining profile was shared with astrocytes and microglia; multilabeling fluorescence studies for precise comparison with the Gal-stained mirror counterpart demonstrated that some of these nonneuronal cells exhibited both RD4 IR and argyrophilia with Gal. In addition to the perivascular localization of Gal-positive astrocytes, as seen in PSP, CBD (18), and normal aging (19), Gal-positive astrocytes associated with ischemia were not restricted to

**FIGURE 2.** Comparison between silver staining and immunohistochemistry. (A, B) Ischemic neurons at 34 days (Patient 14) in and around the ischemic focus are anti-4R-tau antibody (RD4) positive ([A] arrows) but are negative for RD3 ([B] hollow arrows). (C, D) Some Gallyas staining (Gal)-positive neurons ([C] arrows) around an ischemic focus are also positive for RD4 ([D] arrows) on the mirror counterpart in sections from an infarct at 2 days (Patient 1). The same blood vessel is indicated by an asterisk on this mirror-section pair. (E, F) A neurofibrillary tangle (NFT) in the hippocampus around an ischemic focus at 2 days (Patient 1) is positive for anti-paired helical filament antibody (AT8) ([E] asterisk). This section was stained with Gal, and the same NFT ([F] asterisk) is identified. Ischemic neurons are positive only for Gal ([F] arrows), but not for AT8 ([E] hollow arrows). (G–J) Glial cells around an ischemic focus at 14 days (Patient 9) are positive for RD4 ([G] arrows) and Gal ([I] arrows) but are negative for RD3 ([H] hollow arrows) and CS ([J] hollow arrows). Scale bar = 50 μm.
perivascular areas because they were also observed in the parenchyma away from blood vessels. This difference in localization of Gal-positive astrocytes suggests a possible difference in the pathogenetic mechanism (degenerative or vascular) involved in their formation, although the shared staining profile suggests a common pathway.

Previous studies have demonstrated that ischemia induces immunohistochemical emergence of some restricted tau epitopes in different cells in the brain (10, 11, 20, 21). Although biochemical changes of tau induced by cerebral ischemia have not been fully characterized, it has been reported that tau is not hyperphosphorylated after ischemia in human brains (11). In experimental conditions, it is not or is partially phosphorylated (20, 22–25), but its electrophoretic profile is indistinguishable from that of normal tau (11, 25). Previous studies have demonstrated that treatment of cultured neurons with glutamate or colchicine results in dephosphorylation of more heavily phosphorylated tau species (26). When normal human brains are routinely processed, however, they usually do not exhibit tau-like IR, although they contain a significant amount of tau comparable to brains with CI (11, 27, 28). This suggests that some molecular changes of tau itself, other than phosphorylation, are induced by ischemia so that immunohistochemical visualization becomes possible. Because the tau isoform-specific antibodies used in the present study are used to detect tau even in normal brain on immunoblot (29), the emergence of the RD4 IR only after ischemia suggests that 4R tau undergoes some changes for it to be detected by the RD4 antibody on immunohistochemistry. It is notable that the ischemia-induced emergence of the RD4 epitope is tightly correlated with argyrophilia with Gal, as in neurodegenerative processes with 4R-selective tau deposition. Furthermore, the lack of RD3 IR and of argyrophilia with CS is identical to the staining profile characteristic of so-called 4R tauopathies (i.e. PSP, CBD, and argyrophilic grains), with selective involvement of 4R tau by way of phosphorylation (14, 16, 17). The results indicate that similar selective involvement of 4R tau is possible even in non-degenerative conditions such as CI and that this is similarly accompanied by parallel emergence of argyrophilia with Gal but not with CS. Moreover, this staining profile was shared between neurons and glial cells in ischemic brains, suggesting that cellular reactions involving tau after cerebral ischemia is essentially identical, whereas their chronological and spatial extensions were not necessarily identical between neurons and glia.

Although the staining profile (i.e. RD4/Gal-positive RD3/CS-negative) is shared with degenerative processes with 4R-selective tau deposition, none of the ischemic neurons exhibited AT8 IR, which represents phosphorylated PHF tau found in various degenerative lesions with tau deposition (9, 30, 31). Furthermore, none of the ischemic neurons had apparent fibrillary structures similar to NFTs. It has been

**FIGURE 3.** Glial cells positive for either anti–4R-tau antibody (RD4) or Gallyas staining (Gal) in mirror-section pairs. (A, B) Some RD4-positive nonneuronal cells ([A] arrows) around an ischemic focus are positive for glial fibrillary acidic protein (GFAP) ([B] arrows, Patient 9). Anti–4R-tau antibody-positive ([A] arrowheads) and GFAP-negative ([B] hollow arrowheads) cells may be neurons. (C, D) Some Gal-positive nonneuronal cells ([C] arrows, Patient 9) show GFAP immunoreactivity ([D] arrows). These Gal-positive astrocytes were found not only in the vicinity of the blood vessel (arrowheads), but also in the parenchyma without apparent relation to blood vessels (arrows). (E, F) Nonneuronal cells positive for RD4 ([E] arrows, Patient 11) are positive for the microglial marker CD68 ([F] arrows) (Patient 9). (G, H) Other Gal-positive cells ([G] arrows) are CD68 positive ([H] arrows). Panels (A) and (B), (C) and (D), (E) and (F), and (G) and (H) are mirror-section pairs stained for RD4 (A, E), with Gal (C, G), for GFAP (B, D), and CD68 (F, H). Scale bar = 50 μm.
proposed that tau phosphorylation is one of the primary events before tau deposition and subsequent fibril formation in neurodegenerative conditions (27, 32, 33). Indeed, the pretangle neurons in the hippocampal pyramidal layer exhibit granular AT8 IR in the cytoplasm without forming NFTs (32, 34). Similar AT8 IR without forming NFTs is frequently observed in CBD (35, 36); some of the AT8-positive neurons are apparently argyrophilic with Gal even without forming NFTs (30). It remains to be clarified whether these AT8/Gal-positive neurons in CBD evolve into apparent NFTs because NFTs with evident fibrillary structure are exceptional in the cerebral cortex of CBD (36).

The present study indicates that once it is altered by ischemia, nonphosphorylated tau exhibits selective induction of the 4R epitope as well as selective argyrophilia with Gal. This staining profile remained unchanged even in cases with older ischemia. We do not know yet whether this staining profile lacking AT8 IR in ischemic neurons represents the earliest stage of NFT formation, but the present results indicate that deposition of tau takes place in an isoform-dependent manner even without being phosphorylated, and that selective argyrophilia might correspond to this isoform.

Argyrophilic neurons, sometimes referred to as dark neurons, have been described in various pathological conditions other than neurodegeneration including cerebral ischemia (10, 37, 38), brain injury (39-41), and electroshock (42). It has been suggested that dark neurons are formed along a gel-to-gel phase transition (43, 44), glutamate release, and neuronal transmembrane ion fluxes (45). If glutamate toxicity produces a dose-dependent increase in immunocytochemical staining for tau (46, 47), it is possible that dark neurons induced by cerebral ischemia are related to tau deposition mediated by glutamate toxicity (45). It remains to be clarified whether dark neurons, as described in different conditions or with different stains such as toluidine blue (48, 49), cresyl-violet (50, 51), and hematoxylin and eosin (52), are homogeneous with respect to their immunohistochemical and biochemical features. It is likely that they represent a cellular injury or cascade leading to cell death. Interestingly, spinal cord injury by chronic compression induces AT8-positive PHF-like features on electron microscopy in astrocytes (53). Persistent mechanical injury, which is different from instantaneous ischemic insults, might have contributed to the development of PHF in these cases, as seen in neurodegeneration with tau deposition and chronic traumatic encephalopathy (54). It is then possible that the persistence of cellular injury, either mechanical or degenerative, may contribute to the development of aggregated tau deposition to form PHF-like structures that are positive for AT8. In addition, the persistence of cellular injury, which is different from transient injury as seen in CI, may contribute to the development of aggregated 3R-tau and 4R-tau.

FIGURE 4. Multifluorolabeling demonstrates astrocytes and microglia that are positive for both anti-4R-tau antibody (RD4) and Gallyas staining (Gal) in mirror-section pairs. (A-D) Gallyas staining-positive cells (A) around an ischemic focus at 14 days (Patient 9) are positive for RD4 (D) green, arrows). Some of them stain for glial fibrillary acidic protein (GFAP) (red in [C] and yellow in [B] arrows), indicating that some astrocytes exhibit both RD4 immunoreactivity (IR) and argyrophilia with Gal (A-D) arrows). Others not exhibiting GFAP IR (C] red, hollow arrowheads) might be neurons (A, B, D] arrowheads). (E-G) Other Gal-positive cells (E] arrows) around an ischemic focus at 28 days (Patient 13) are positive for RD4 (F] green, arrows) and CD68 (G] red, arrows). The section stained for CD68 (G] was photographed and then was stained with Gal; the same microglia (E] arrows) in the field as in (F] and (G] are identified. (A, E] Gal; (B] merge; [C] red] GFAP immunostaining; (D, F] green] RD4 immunostaining; (E] red] CD68 immunostaining. Scale bar = (A-D) 50 μm; (E-G) 25 μm.
FIGURE 5. Argyrophilic properties and tau immunoreactivity among various neurological disorders. Diseases are classified according to their predominant tau isoforms. Solid lines indicate positive staining. AD, Alzheimer disease; AGD, argyrophilic grains; AT8, anti-paired helical filament antibody; CBD, corticobasal degeneration; CI, cerebral infarction; DNRC, diffuse NFTs with calcification; NFTs, neurofibrillary tangles; PSP, progressive supranuclear palsy; RD3, anti-3R-tau antibody; RD4, anti-4R-tau antibody.

Deposition to form PHF-like structures in neurons that are positive for AT8 (53); nearby astrocytes were selectively positive for 4R tau alone. This contrast will provide essential clues to understand the cascade from tau deposition and phosphorylation to PHF formation. Further dissection of this cascade may clarify what is essential for tau to be phosphorylated to form PHFs from which we may be able to construct a strategy for preventing PHF formation even after the deposition of tau.

As a practical starting point for demonstrating how tau immunostaining and silver staining are interrelated in various pathological conditions, a comparison summary of staining profiles of anti-tau antibodies (RD3, RD4, AT8, Alz 50, tau2, etc) and silver stains (Gal, CS, Bodian and Bielschowsky) is presented in Figure 5. The present study demonstrated the selective appearance of 4R tau in ischemic neurons and its association with argyrophilia with Gal. This parallel positivity was initially recognized after comparing different degenerative conditions (9) and is now extended to CI, further suggesting a molecular link between 4R-tau deposition and argyrophilia with Gal. Future studies to identify underlying molecular events leading to the selective induction of 4R tau and argyrophilia with Gal will clarify the disease-specific cascade shared between neurodegeneration and CI.

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