Dystrophic Neurites in the APP/PS1 Rat Model of Alzheimer’s Disease

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Abstract

Among the three Fluoro-Jade dyes, Fluoro-Jade C (FJC) is the most sensitive fluorescent marker of neuronal degeneration, allowing for the localization not only of degenerating neurites, axons, and axon-terminals. FJC also has a high binding affinity for the core of plaques observed in Alzheimer’s disease (AD), which mainly consists of the amyloid beta (Aβ) peptide. Never characterized before is FJC’s ability to bind to dystrophic neurites (DNs; swollen neural processes). Here, we propose the presence of DNs may correlate with synaptic impairments in AD. Several studies have shown the mechanism of Aβ deposition in AD; however, little attention has been paid to the location of DNs in AD brains. DNs in the AD brain are morphologically recognized by immunohistochemical staining with antibodies specific to amyloid precursor protein (APP) and reticulin fibers. They also contain ubiquitin (UBI), β-site APP cleaving enzyme-1 (BACE1; enriched within a distinct subtype of DNs), endosome/lysosome marker lysosomal associated membrane protein-1 (LAMP-1), and/or CD68. In this study, we localized DNs using FJC in the APP/PS1 rat model of AD. Co-localization immunolabeling on FJC-stained tissues with LAMP-1, CD68, BACE1, RTN3, and UBI were performed. To determine intensity and area of colocalization was highest in ferritin and NU-1, markers of DNs, microglia, and the oligomeric form of total area occupied by the plaque cores, the majority colocalized with Aβ.

Materials and Methods

Animals: Tissue sections (30µm) were obtained from 16-month-old APP/PS1 Swedish double mutation transgenic (APP/PS1 Tg) rats, which are a model for AD (NCTR breeding colony). All animal procedures were approved by the Institutional Animal Care and Use Committee at the National Center for Toxicological Research and were conducted in accordance with the general principles for animal research outlined in the Guide for the Care and Use of Laboratory Animals.

Combined FJC and immunolabeling: Sections were cut on a cryostat, starting at the base of the cortex and removing tissue sections of 10µm in thickness, perpendicularly to the surface of the brain. Tissue sections were washed in 0.1 M PBS (pH 7.4; phosphate buffer) and mounted using a cryo-adhesive solution (mixture of 45% glycerol, 3% ethylene glycol and 52% in 0.1 M PBS with dried water) and subsequently processing. Briefly, floating sections were processed in a scintillation and water (v/v) solution in 5:1 ratio in TBS-RIPA buffer. Sections were rinsed three times with PBST (pH 7.4) and incubated in Triton X-100 for 15 min at room temperature. Following three washes in PBST, sections were incubated with a 1:1 ratio of Triton-X-100 to PBST. A drop of 1:1000 primary antibody was applied onto the sections and incubated for 4 h at room temperature. Following three washes in PBST, sections were incubated with 1:1000 secondary antibody for 3 h at room temperature. After primary and secondary antibody incubation, sections were mounted in gelatin coated glass slides and labeled for FJC with slight modification.

Table 1. Antibody source and dilution factor

Table 2. Numerical characterization of peripheral and core components

Results and Discussion

The plaque peripheries in the cortex displayed: DNs (APP, RTN3) active in multiple pathways (UB1, BACE1, LAMP-1, CD68), axonal degeneration (MBP), the oligomeric form of Aβ (NU-1), as well as microglia (ferritin).

Where plaques with densely FJC-stained cores were observed, DNs, microglia, and oligomeric Aβ were also evident.

In the cortex, 84% or more of stain colocalized with FJC in the plaque periphery. Colocalization with MBP was solely in the periphery.

FJC has a highest affinity for the oligomeric form of Aβ (NU-1) and microglia (light chain ferritin).

The frequency of plaques present varied by brain region (data not shown).

Conclusion

FJC labeled DNs located in the plaque periphery also contain a variety of substances suggesting a myriad of pathways may be involved in the accumulation around plaques and eventual synaptic dysfunction. These processes may be involved in the progression of AD; thus, one of these pathways may serve as a therapeutic target to mitigate disease progression.

Colocalization of multiple markers remains the best identification of DNs as FJC has a higher affinity for other structures and, in this study, the total area occupied by colocalization of FJC with any other marker in the cortex comprised less than 3% of the Aβ0 FVY.

Axonal degeneration (MBP) is unique to the plaque periphery.