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Pharmacologic Inhibition of 5-Lipoxygenase Improves Memory, Rescues Synaptic Dysfunction, and Ameliorates Tau Pathology in a Transgenic Model of Tauopathy

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ABSTRACT

BACKGROUND: 5-Lipoxygenase (5-LO) is a protein widely distributed in the central nervous system where it modulates amyloidosis and memory impairments in transgenic mouse models of Alzheimer's disease. However, no data are available as to whether 5-LO is elevated in human tauopathy or if it directly influences tau pathology in a relevant model of the disease.

METHODS: We assayed 5-LO levels in brain samples from patients with tauopathy and transgenic tau mice, and we evaluated the effect of 5-LO pharmacologic inhibition on the phenotype of these mice.

RESULTS: The 5-LO protein is upregulated in human tauopathy and transgenic tau mice brains. Pharmacologic blockade of 5-LO in tau mice resulted in significant memory improvement, rescue of synaptic integrity and dysfunction, and reduction of tau pathology via a cdk5-dependent mechanism.

CONCLUSIONS: These results establish a key role of 5-LO in the development of the tau pathology phenotype and demonstrate it to be a novel viable therapeutic target for the pharmacologic treatment of human tauopathy.

Keywords: Behavior, 5-Lipoxygenase, Frontotemporal dementia, Synapse, Tau protein, Tauopathy, Transgenic tau mice

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One hallmark feature of Alzheimer's disease is the accumulation of intracellular aggregates of the hyperphosphorylated microtubule-associated protein tau. This type of pathology is also the major signature of another large group of neurodegenerative diseases, collectively referred to as tauopathies, which include, among others, progressive supranuclear palsy (PSP), Pick's disease, and corticobasal degeneration (1,2). Although emerging evidence suggests that alterations in inflammatory processes occur in the brains of humans with tauopathies and mouse models of tauopathies, the involved mechanisms, the source of inflammation, and, most importantly (3–5), whether it is a primary or a secondary event in the disease pathogenesis are unclear.

5-Lipoxygenase (5-LO) is an inflammatory enzyme widely expressed in the central nervous system where it localizes mainly in neuronal cells. Its presence has been shown in cortex and hippocampus, where the levels increase in an age-dependent manner (6). Previously, we showed in transgenic animal models of Alzheimer's disease that this enzymatic pathway plays a functional role in modulating phenotypes (7–11). In these studies, we observed that besides Aβ, the levels of tau phosphorylation were also influenced. For instance, overexpression of 5-LO in vivo increases phosphorylation of specific tau epitopes, and neuronal cells transfected with 5-LO show a significant increase in tau phosphorylation even when their ability of generating Aβ is completely blocked, suggesting that the effect on tau is independent from Aβ (9). Taken together, these data support the novel hypothesis that 5-LO could play an active role in modulating tau metabolic pathways important for the development of tauopathy.

To test this hypothesis, in the present study we used transgenic tau mice (htau) in which the mouse tau gene was replaced by the nonmutated human tau gene (12) and zileuton, a selective and orally available 5-LO inhibitor (13), was administered to the mice. Compared with the htau mice receiving placebo, the mice treated with zileuton manifested a significant improvement in cognition and memory associated with restoration of their hippocampal synaptic function. In addition, pharmacologic inhibition of 5-LO yielded significant decreases in tau phosphorylation, which was mediated by a cyclin-dependent kinase 5 (cdk5) mechanism.

Our findings support a functional and direct role for 5-LO in the development of tau pathologic phenotype in vivo. These findings provide important preclinical evidence that this protein is a viable potential pharmacologic target for the treatment of tauopathies.

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METHODS AND MATERIALS

All animal procedures were approved by the Animal Care and Usage Committee, in accordance with the U.S. National Institutes of Health guidelines. The htau mice express a tau transgene derived from a human Puromycin N-acetyltransferase, H1 haplotype driven by the tau promoter along with a targeted disruption of exon 1 of tau (12). The animals were backcrossed 10 times on the same genetic background of C57BL6/SJL. The wild-type (WT) mice were aged-matched C57BL6/SJL control mice. The animals were kept in a pathogen-free environment on a 12-hour light/dark cycle and fed a normal chow and water ad libitum. Two separate groups of 3-month-old WT and htau mice were randomly assigned to receive zileuton (200 mg/L) or vehicle in drinking water three times per week over 7 months. After the treatment period, at 10 months of age, the mice underwent behavioral tests as described subsequently. The mice were euthanized 1 week later, and the brain was removed, gently rinsed in cold .9% phosphate-buffered saline, and immediately dissected into two halves. One half was immediately stored at −80°C for biochemistry; the other half was fixed in 4% paraformaldehyde in phosphate-buffered saline, pH 7.4, for immunohistochemistry studies.

Behavioral Tests

All the animals were handled for at least 3–4 days before testing. They were tested in the Y-maze and Morris water maze in random order (Supplement 1). The experimenter conducting the tests was unaware of the genotype or treatment.

Biochemistry and Immunohistochemistry Analyses

Extracts from brain homogenates were used for biochemistry analyses, and brain sections were used for immunohistochemistry as previously described (Supplement 1) (9,11,12,14).

Electrophysiology

These studies were performed as previously described (Supplement 1) (11).

Primary Neuron Studies

Cortices from htau mouse pups (P0) were isolated and incubated in .1% papain/Hanks balanced salt solution/.5 mmol/L ethylenediamine tetraacetate without Ca^{++} or Mg^{++} (Fisher Scientific, Waltham, Massachusetts). Cells were plated in Neurobasal-A medium (Gibco Life Technologies, Grand Island, New York) plus 10% fetal bovine serum on poly-D-lysine-coated six-well plates at a density of 106 cells/well and kept at 37°C. At 24 hours after plating, medium was removed and replaced with Dulbecco's Modified Eagle's medium plus B27 supplements and GlutaMAX (Gibco Life Technologies) to promote neuronal survival and inhibit growth of nonneuronal cells. Neurons were used for experimentation 7 days after plating, when at ~70% confluence and treated with 25 μmol/L zileuton for 24 hours. After treatment, supernatants were collected, and cells were harvested in lytic buffer for biochemical analyses.

Data Analysis

One-way analysis of variance, unpaired Student *t* test (two-sided), and Bonferroni multiple comparison tests were performed using Prism 5.0 (GraphPad Software, Inc, La Jolla, California). All data are presented as mean ± SEM. Significance was set at $p < .05$.

RESULTS

Levels of 5-LO Are Elevated in Human and Mouse Tauopathy Brains

To see whether 5-LO is significantly increased in human tauopathy, we measured levels in brain samples from patients with a confirmed diagnosis of PSP ($n = 5$, 70 ± 12 years old) and healthy control subjects ($n = 6$, 82 ± 5 years old). As shown in Figure 1A and B, steady-state levels of 5-LO were significantly higher in the brain frontal cortices of patients with PSP compared with control subjects. Next, we assayed 5-LO levels in brains from htau mice at different ages (2, 6, 10, and 16 months). Compared with WT mice, brain cortices from htau mice showed an age-dependent increase in levels of 5-LO, which became statistically significant by 10 months of age (Figure 1C and D). By contrast, no significant differences were observed between WT and htau mice at any of the same time points when the cerebellum was assayed suggesting a region-specific increase of 5-LO in this mouse model (Figure 1E and F).

Pharmacologic Blockade of 5-LO Ameliorates Cognition

To evaluate the behavioral effect of 5-LO pharmacologic blockade, we administered zileuton, a selective and orally available inhibitor of the enzyme to htau mice starting at 3 months of age for 7 months. At this time point, 10 months of age, mice were initially tested in the Y-maze. No differences were observed between the four groups (WT, WT-zileuton, htau, htau-zileuton) of mice in regard to their general motor activity, which was assessed by the total number of arm entries (Figure 2A). However, when we counted the number of alternations in the same paradigm, we observed that htau mice on placebo had a smaller number of alternations resulting in a significantly lower percentage compared with the WT mice. In contrast, the htau mice receiving zileuton had a higher number of alternations, which were comparable to the WT group, suggesting an improvement of their working memory (Figure 2B). Thereafter, mice were tested for reference spatial memory function by using the Morris water maze. In these studies, we performed visible platform training followed by hidden platform testing with four probe trials per day. All mice in each group were similarly proficient swimmers and able to locate the visible platform (data not shown). However, as previously described (15), htau mice performed significantly worse than WT mice for the first 3 days of training (Figure 2C). Treatment of the htau mice with zileuton eliminated this deficit because this group performed similarly to WT control mice (Figure 2C). In the probe trial, htau mice showed a significant increase in the latency to first entry to the platform (Figure 2D), along with a significant decrease in the number of entries to the target platform (Figure 2E), and spent less time in the target zone (Figure 2F) compared with WT mice. However, the htau mice treated with zileuton were comparable to the WT mice in all the above-mentioned parameters, performing