Molecular Neurobiology (2019) 56:1211–1220
https://doi.org/10.1007/s12035-018-1124-7

Learning Impairments, Memory Deficits, and Neuropathology in Aged Tau Transgenic Mice Are Dependent on Leukotrienes Biosynthesis: Role of the cdk5 Kinase Pathway

Phillip F. Giannopoulos[1] · Jian Chiu[1] · Domenico Praticò[1]

Received: 3 April 2018 / Accepted: 11 May 2018 / Published online: 7 June 2018

Abstract
Previous studies showed that the leukotrienes pathway is increased in human tauopathy and that its manipulation may modulate the onset and development of the pathological phenotype of tau transgenic mice. However, whether interfering with leukotrienes biosynthesis is beneficial after the behavioral deficits and the neuropathology have fully developed in these mice is not known. To test this hypothesis, aged tau transgenic mice were randomized to receive zileuton, a specific leukotriene biosynthesis inhibitor, or vehicle starting at 12 months of age for 16 weeks and then assessed in their functional and pathological phenotype. Compared with baseline, we observed that untreated tau mice had a worsening of their memory and spatial learning. By contrast, tau mice treated with zileuton had a reversal of these deficits and behaved in an undistinguishable manner from wild-type mice. Leukotriene-inhibited tau mice had an amelioration of synaptic integrity, lower levels of neuroinflammation, and a significant reduction in tau phosphorylation and pathology, which was secondary to an involvement of the cdk5 kinase pathway. Taken together, our findings represent the first demonstration that the leukotriene biosynthesis is functionally involved at the later stages of the tau pathological phenotype and represents an ideal target with viable therapeutic potential for treating human tauopathies.

Keywords Tauopathy · cdk5 kinase pathway · Five-lipoxygenase · Leukotrienes · Neuroinflammation · Behavior

Introduction

Neurodegenerative diseases represent a large and heterogeneous group of chronic disorders both sporadic and familial, often characterized by the progressive accumulation of signature protein aggregates, which in most cases provide the basis for their neuropathological classification [1]. To this end, the term "tauopathies" is typically used to define some of these diseases whose main feature is the presence of filamentous accumulations of highly phosphorylated tau protein only in neurons or neurons and glial cells [2, 3]. They comprise several different clinical and pathological entities and have been sub-classified into primary and secondary, depending on whether tau neuropathology is considered the major contributing factor to the pathogenesis or simply associated with it [4].

✉ Domenico Praticò
praticod@temple.edu

[1] Alzheimer's Center at Temple, Lewis Katz School of Medicine, Scott Richards North Star Foundation Chair, Alzheimer's Research, Temple University, 947, Medical Education and Research Building, 3500 North Broad Street, Philadelphia, PA 19140, USA

While pathological post-translational modifications of tau have unequivocally been shown to be able to cause neurodegeneration, the precise molecular and cellular mechanisms whereby this protein is involved in the pathogenesis of these diseases are still poorly understood. Interestingly, besides the accumulation of highly phosphorylated tau, its filaments, and ultimately the neurofibrillar tangles, consistent evidence has demonstrated that both human tauopathies as well as their animal models are also characterized by intense humoral and cellular neuroinflammatory responses [5, 6].

We recently showed that post-mortem brain tissues from subjects with a clinical and pathological diagnosis of progressive supranuclear palsy, one of the most common form of tauopathy, have a significant up-regulation of the 5-lipoxygenase (5LO), an enzyme whose metabolic products, the leukotrienes, are potent pro-inflammatory lipid mediators [7, 8]. Further, in relevant mouse models of tauopathy genetic absence or early pharmacological blockade of 5LO activation resulted in significant improvement of behavioral deficits and delay in the development of tau phosphorylation and pathology [7, 9, 10]. However, all these studies are to be considered as preventative in nature since all of them have used mice at an early stage of their phenotype

before overt pathology has developed. For this reason, whether the pharmacological approach targeting the leukotriene biosynthesis pathway is suitable as an effective therapy for tauopathy remains unknown.

To address this important biological question, we performed a study investigating the effect of chronic administration of zileuton, a specific 5LO inhibitor, to aged tau transgenic mice after their behavior deficits and tau neuropathology were established.

Methods and Materials

Animals

All animal procedures were approved by the Animal Care and Usage Committee and were in accordance with the National Institute of Health guidelines. The htau mice used in this study express a tau transgene derived from a human PAC, H1 haplotype driven by the tau promoter along with a targeted disruption of exon 1 of tau [11]. The animals were backcrossed ten times on the same genetic background of C57BL6/SJL. The wild-type (WT) mice are aged-matched C57BL6/SJL controls. The animals were kept in a pathogen-free environment on a 12-h light/dark cycle and fed a normal chow and water ad libitum. At 12 months of age, htau and WT mice were randomized into two groups: one receiving zileuton (200 mg/l) ($n = 10$, five males and five females per genotype), the other vehicle in their drinking water (n = 10, per genotype, five males and five females) three times per week for 16 weeks. This concentration of the drug was selected based on our previous work in which we showed that it significantly reduced 5LO activation and suppressed leukotriene production [12]. At the end of the treatment period, mice underwent behavioral tests as described below. A week later, they were euthanized, brains removed, and immediately dissected into two halves: one stored at − 80 °C for biochemistry, the other fixed in 4% paraformaldehyde for immunohistochemistry studies.

Behavioral Testing

All the animals were handled for at least 3–4 days prior to testing. They were tested in the Y-maze and Morris water maze in random order, and the experimenter conducting the tests was unaware of the genotype or treatment.

Y-Maze

The Y-maze apparatus consisted of three arms 32 cm (long) 610 cm (wide) with 26-cm walls (San Diego Instruments, San Diego, CA). Testing was always performed in the same room and at the same time to ensure environmental consistency as previously described [13, 14]. Briefly, each mouse was placed in the center of the Y-maze and allowed to explore freely during a 5-min session as a measure of spontaneous alternating behavior. The sequence and total number of arms entered were video-recorded. An entry into an arm was considered valid if all four paws entered the arm. An alternation was defined as three consecutive entries into three different arms (1, 2, 3, or 2, 3, 1, etc.). Percentage of alternation was calculated using the following formula: total alternation number/total number of entries − 2) × 100.

Morris Water Maze

To perform the Morris water maze (MWM), we used a white circular plastic tank (122 cm in diameter, walls 76 cm high), filled with water maintained at 22° ± 2 °C and made opaque by the addition of a nontoxic white paint, as previously described [13, 14]. Mice were trained for four consecutive days to find a Plexiglas platform submerged in water from four different starting points. If they failed to find the platform within 60 s, they were manually guided to the platform and allowed to remain there for 15 s. Mice were trained to reach a training criterion of 20 s (escape latency). Mice were assessed in the probe trial, which consisted of a free swim lasting for 60 s without the platform, 24 h after the last training session. Animals' performances were monitored using Any-Maze™ Video Tracking System (Stoelting Co., Wood Dale, IL) which provided data for the acquisition parameters (latency to find the platform and distance swam) and the probe trial parameters (number of entries in the target platform zone of the platform and time in quadrants).

Biochemical Analysis

Mouse brain homogenates were extracted using radioimmunoprecipitation assay buffer (RIPA) as previously described [13–15]. Briefly, 30 mg of cerebral cortex was sonicated in RIPA buffer added with protease and phosphatase inhibitors cocktail (Santa Cruz Biotechnology) and subsequently ultracentrifuged at 45,000 rpm for 45 min.

RIPA extracts from mouse brain homogenates were assayed for LTB4 levels by using a specific LTB4 ELISA kit (Enzo Life Sciences, Farmingdale, NY), and following the instructions of the manufacturer [12, 14]. Analyses were always performed in triplicate.

Sarkosyl Insolubility Assay

The assay for insoluble tau fraction was performed as previously described [14–16]. Analyses were always performed in triplicate.

Immunoblotting

Western blot was performed as described previously [13–16]. Briefly, samples were separated on SDS page by using a Bis-

Tris gel and then transferred onto nitro-cellulose membranes (Bio-Rad, Richmond, CA). Membranes were incubated with primary antibodies overnight at 4 °C and later with secondary antibody for 1 h at room temperature. Membranes were developed with Odyssey Infrared Imaging System (LI-COR Bioscience). The following primary antibodies were used: anti-5LO (1:200; Santa Cruz Biotech.), HT7 (1:200; Thermo), AT8 (1:100; Thermo), AT180 (1:200; Thermo), AT270 (1:200; Thermo), PHF-1 (1:100; generous gift of Dr. P. Davies), PHF-13 (1:200; Cell Signaling), MC1 (1:100; generous gift of Dr. P. Davies), anti-cdk5 (1:200; Santa Cruz Biotech.), anti-p35/p25 (1:100; Santa Cruz Biotech.), anti-SAP/JNK (1:200; Cell Signaling), anti-pSAP/JNK (1:200; Cell Signaling), anti-PP2A (1:200;Thermo), anti-synaptophysin (1:400; Santa Cruz Biotech.), anti-PSD-95 (1:200; Cell Signaling), anti-MAP2 (1:1000, Millipore), anti-GFAP (1:200; Santa Cruz Biotech.), anti-CD45 (1:100; Thermo), and β-actin (1:1000; Santa Cruz Biotech.). Actin was always used as an internal loading control.

Immunohistochemistry

Brain sections were prepared for immunohistochemistry as previously described [13–16]. Briefly, after antigen retrieval, sections were treated with 3% H_2O_2 in methanol for 30 min to eliminate endogenous peroxidase activity in the tissue, and with blocking solution (5% normal serum in Tris buffer, pH 7.6). Subsequently, sections were incubated overnight at 4 °C with the primary antibodies—HT7, AT8, AT180, PHF-1, PHF-13, SYP, PSD-95, MAP2, GFAP, and CD45—and then incubated with secondary antibody and developed using the avidin-biotin complex method (Vector Laboratories) with 3,3di-aminobenzidine as chromogen.

Statistical Analysis

All data are expressed as mean ± standard deviation. The one-way ANOVA test was used to compare more than two groups. The two-tailed Student's *t* test was used to compare up to two groups. Statistical significance was set at $p < 0.05$.

Results

Zileuton Restores Behavior Impairments in Aged Tau Mice

To investigate whether pharmacological inhibition of 5LO activation modulated cognitive impairments of aged htau mice, we first assessed behavioral response at the beginning

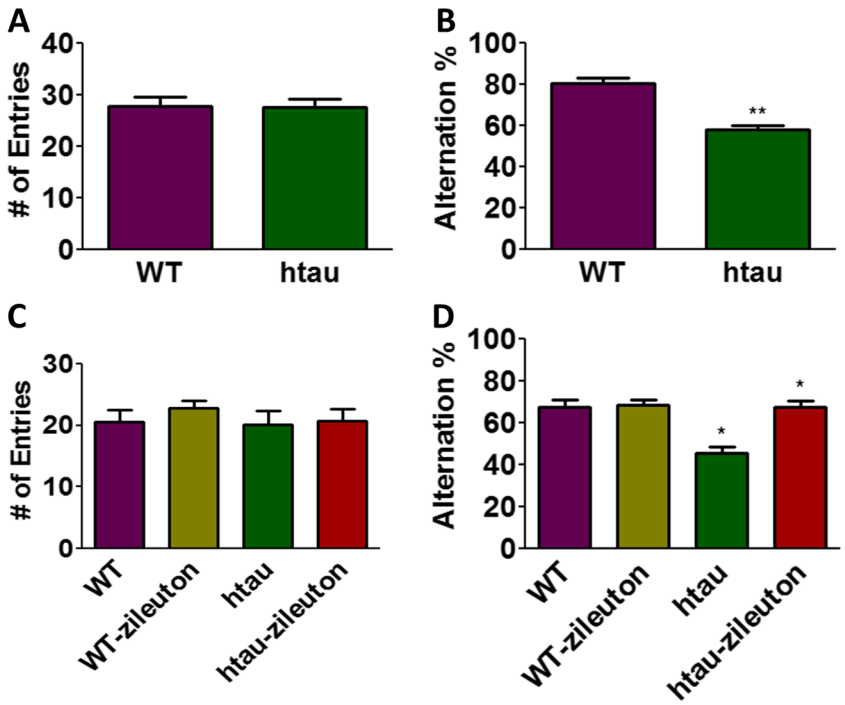

Fig. 1 Chronic administration of zileuton rescues working memory deficits in aged htau mice. Starting at 12 months of age, htau mice and wild-type (WT) mice were first tested in the Y-maze and Morris water maze. Next, they were randomized to receive zileuton or vehicle in their water until they were 16 months old, and then tested in the same two paradigms. **a** Total number of arm entries for WT, and htau mice at 12 months of age. **b** Percentage of alterations for the same two groups of mice (**$p < .001$) ($n = 10$ per group) (*two-tailed Student's t test*). **c** Total number of entries for wild-type (WT; WT-zileuton; htau and htau-zileuton mice at 16 months of age. **d** Percentage of alterations for the same four groups of mice (*$p < .01$) ($n = 10$ per group) (*one-way ANOVA*). Values represent mean ± SEM

of the study (baseline 12 months old), and then after 16 weeks of zileuton treatment (16 months old). As expected, we observed that compared with WT mice, 12-month-old htau mice manifested significant behavioral deficits in both the Y-maze as well as the Morris water maze, which assess working memory and spatial learning and memory, respectively (Figs. 1 and 2). After 16 weeks, while we observed a further reduction in the percentage of alternations for the htau mice control, zileuton-treated mice had a percentage of alternation similar to the WT group (Fig. 1c, d). In the Morris water maze, as expected for their age compared with WT group, the htau mice had a significant increase in the latency to the platform,

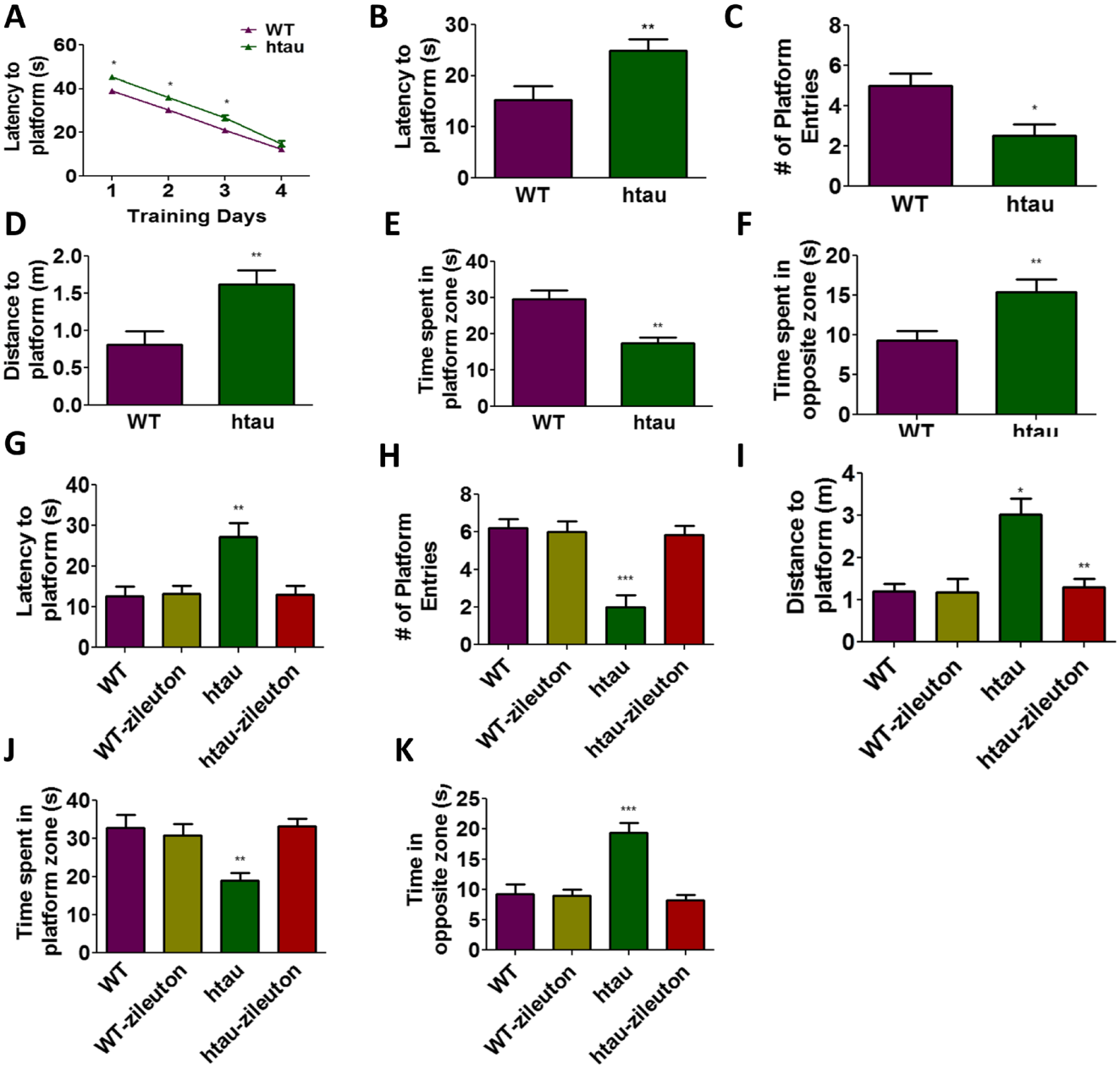

Fig. 2 Chronic administration of zileuton reverses spatial learning and memory impairments in aged htau mice. **a**, **b** Morris water maze training phase: latency to initial platform crossing for wild-type (WT) and htau mice at 12 months of age (*$p < .05$, **$p < .01$). **c** Number of entries to the target platform zone for the same two groups of mice (*$p < .001$). **d** Probe trial: distance traveled to platform zone for the same two groups of mice (**$p < .001$). **e** Time spent in the target platform zone for the two groups of mice (**$p < .001$). **f** Time spent in opposite zone for the two groups of mice (**$p < .001$) ($n = 10$ per group). **g** Training phase: latency to initial platform crossing for WT, WT-zileuton, htau and htau mice receiving zileuton at 16 months of age (**$p < 0.001$). **h** Number of entries to the target platform zone for the same four groups of mice (***$p < .001$). **i** Probe trial: distance traveled to platform zone for the same four groups of mice (*$p < 0.05$, **$p < .0001$). **j** Time spent in the target platform zone for the two groups of mice (**$p < .001$).**k** Time spent in opposite zone for the two groups of mice (***$p < .001$). Values represent mean ± SEM ($n = 10$ per group) (*one-way ANOVA*)

a decrease in the numbers of entries and time spent in this area, and spent more time in the opposite quadrant (Fig. 2a–f). By contrast, all of these impairments were completely reversed in the htau mice treated with zileuton, whose behavior overlapped the WT mice's responses (Fig. 2g–l). No effect *t* of zileuton was observed in the treated WT mice for both behavioral paradigms.

Blockade of Leukotriene Biosynthesis Reduces Tau Phosphorylation in Aged Tau Mice

After completion of the behavioral tests, mice were euthanized and brains harvested for biochemical and immunehistochemical analyses. First, we assessed the pharmacologic inhibitory action in vivo of our drug by measuring brain levels of leukotriene B4 (LTB4), the principal metabolic product of 5LO enzymatic activation. Compared with the untreated group, brains from htau mice receiving zileuton had a significant reduction in the levels of LTB4, but no effect on the steady-state levels of the 5LO protein was observed (Fig. 3a–c).

To test whether the pharmacological treatment influenced tau phosphorylation in aged mice, we measured the levels of total soluble tau, as recognized by the HT7 antibody, and its phosphorylated isoforms at ser202/thr205, thr231/ser235, thr181, ser396/404, and ser396, as recognized by the antibodies AT8, AT180, AT270, PHF-1, and PHF-13, respectively. Compared with controls, no significant differences were observed in the zileuton-treated animals when levels of total soluble tau were assessed (Fig. 3d). By contrast, we observed that leukotriene biosynthesis suppression resulted in a significant reduction in the levels of the phosphorylated isoforms at the epitopes recognized by AT8, AT180, PHF-1, and PHF-13 antibodies (Fig. 3d,e). By contrast, we did not observe any significant change in the immunoreactivity recognized by the antibody AT270 when the zileuton-treated mice were

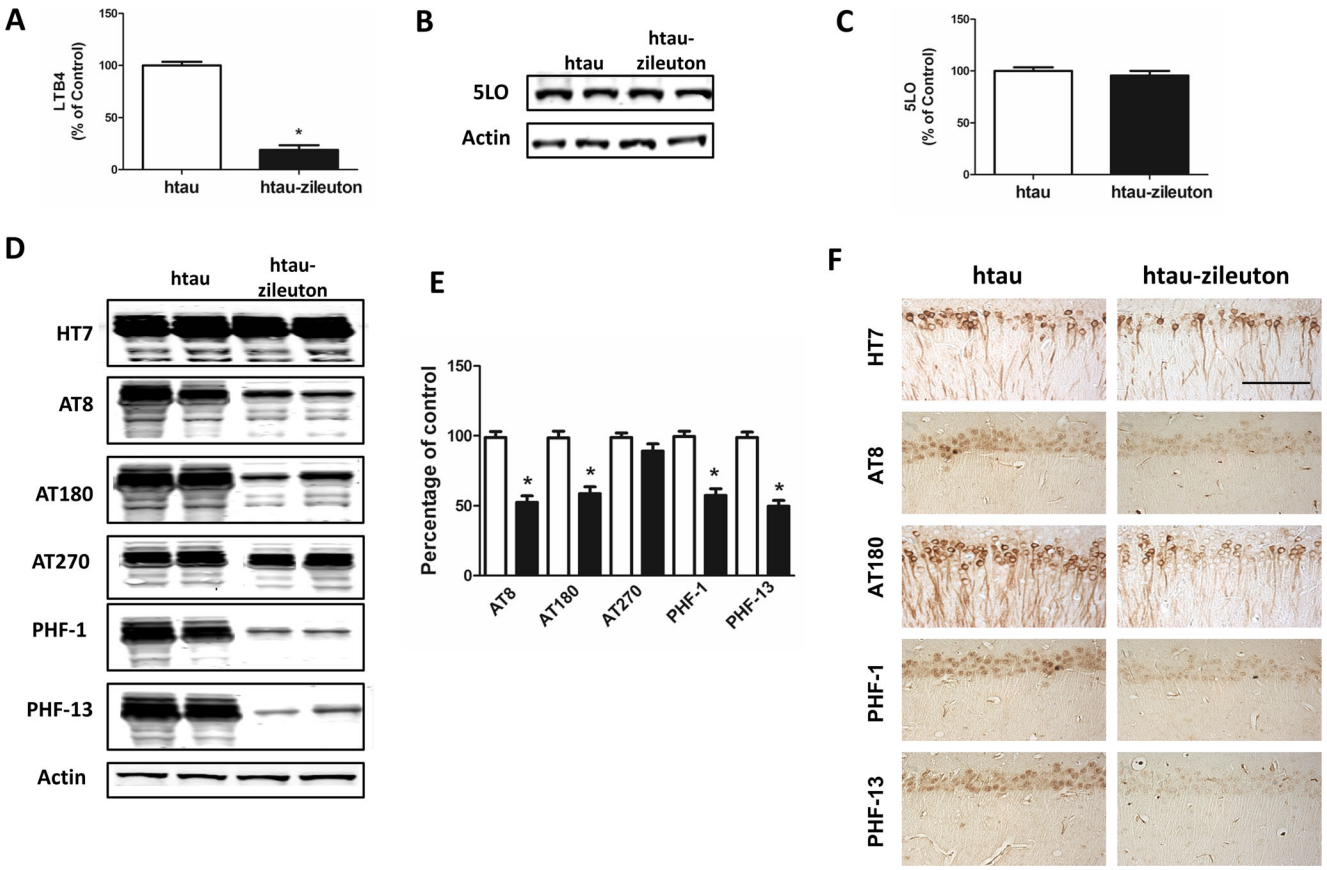

Fig. 3 Leukotrienes biosynthesis suppression reduces tau phosphorylation in aged htau mice. **a** Levels of LTB4 in brain cortex homogenates from htau mice receiving zileuton or vehicle for 16 weeks ($n = 10$ per group) (*$p < .001$). **b** Representative Western blot analysis for 5LO in brain cortex homogenates from the same two groups of htau mice described in the previous panel. **c** Densitometric analysis of the immunoreactivity to the antibody shown in panel **b** ($n = 10$ per group). Values represent mean ± SEM. **d** Representative Western blots of total soluble tau (HT7), phosphorylated tau at residues ser202/thr205 (AT8), thr231/ser235 (AT180), and thr181 (AT270), ser396/ser404 (PHF-1), and ser396 (PHF-13) in brain cortex homogenates from htau mice receiving zileuton or vehicle. **e** Densitometric analyses of the immunoreactivities to the antibodies shown in the previous panel ($n = 6$ per group) (*$p < 0.05$) ($n = 6$ per group). Values represent mean ± SEM (*two-tailed Student's t test*). **f** Representative images of brain sections from htau mice receiving zileuton or vehicle immunostained with HT7, AT8, AT180, PHF-1, and PHF-13 antibodies (*scale bar 100 μm*)

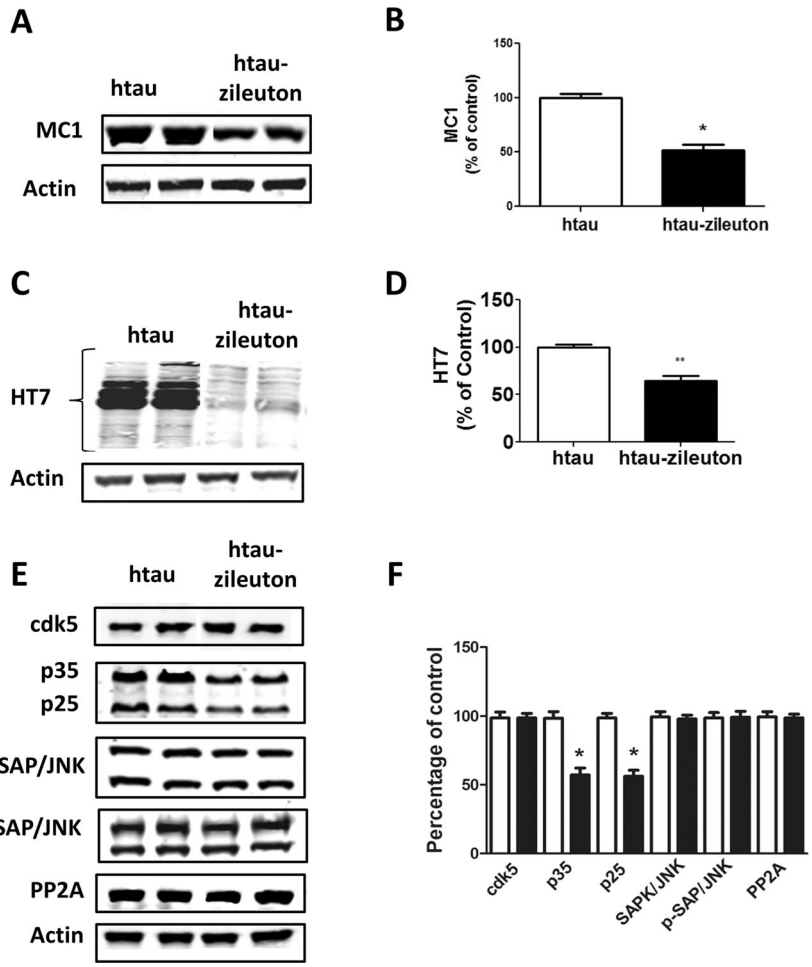

Fig. 4 Leukotriene reduction modulates tau neuropathology via the cdk5 kinase pathway. **a** Representative Western blot analysis for MC1 in brain cortex homogenates of htau mice receiving vehicle or zileuton for 16 weeks. **b** Densitometric analyses of the immunoreactivities to the antibody shown in the previous panel (*$p < 0.05$). **c** Representative Western blot analysis for sarkosyl-soluble tau in brain cortex homogenates from mice receiving vehicle or zileuton. **d** Densitometric analyses of the immunoreactivities to the antibody shown in the previous panel (**$p < 0.05$). **e** Representative Western blot analysis for cdk5, p25, p35, SAP/JNK (JNK1-JNK2/3), p-SAP/JNK (p-JKN1-JKN2/3), and PP2A in brain cortex homogenates from htau mice receiving vehicle or zileuton for 16 weeks. **f** Densitometric analyses of the immunoreactivities to the antibodies shown in the previous panel (*$p < 0.05$). Values represent mean ± SEM ($n = 6$ per group) (*two-tailed Student's t test*)

comparted with the untreated ones (Fig. 3d, e). These data were further confirmed by immunohistochemistry analyses of brain sections from the two groups of mice (Fig. 3f).

Leukotriene Reduction Decreases Tau Pathology in Aged Tau Mice

Next, we assessed immunoreactivity for the antibody MC-1, which identifies aggregation prone conformation of tau protein [17]. As shown in Fig. 4, we found a significant reduction in MC1 levels in zileuton-treated mice compared to controls. Finally, mice receiving the drug had a significant reduction in the levels of total insoluble tau (HT7-sarkosyl soluble) levels when compared with the untreated group (Fig. 4c, d). Next, we investigated whether zileuton had any influence on some of the major kinases that are considered important regulators of tau phosphorylation [18]. While we did not observe any differences between the two groups for the total level of cdk5, zileuton-treated mice had a significant reduction in the level of p25 and p35. By contrast, no significant changes were found for total SAP/JNK, p-SAP/JNK, and protein phosphatase 2A (PP2A) (Fig. 4e, f).

Anti-Leukotrienes Therapy Rescue Synaptic Integrity in Aged Tau Mice

To assess the effect of leukotriene suppression on pre-synaptic and post-synaptic integrity, we measured protein levels of synaptophysin (SYP), post-synaptic density protein-95 (PSD-95), and the microtubule-associated protein 2 (MAP2). At the end of the treatment, compared with the untreated group, mice receiving zileuton had a significant increase in the steady-state levels of all three biochemical markers of synaptic integrity (Fig. 5a, b). These results were further confirmed by immunohistochemistry analyses of brain sections for the two groups of mice (Fig. 5c).

Blockade of Leukotrienes Biosynthesis Reduces Neuroinflammation in Aged Tau Mice

To investigate the effect of pharmacological inhibition of 5LO activity on neuroinflammation, biochemical markers of astrocytes and microglia activation such as glial fibrillary acidic protein (GFAP) and cluster of differentiation 45 (CD45) were assessed. As shown in Fig. 6, while htau mice controls had an elevation for both markers we observed that brain homogenates

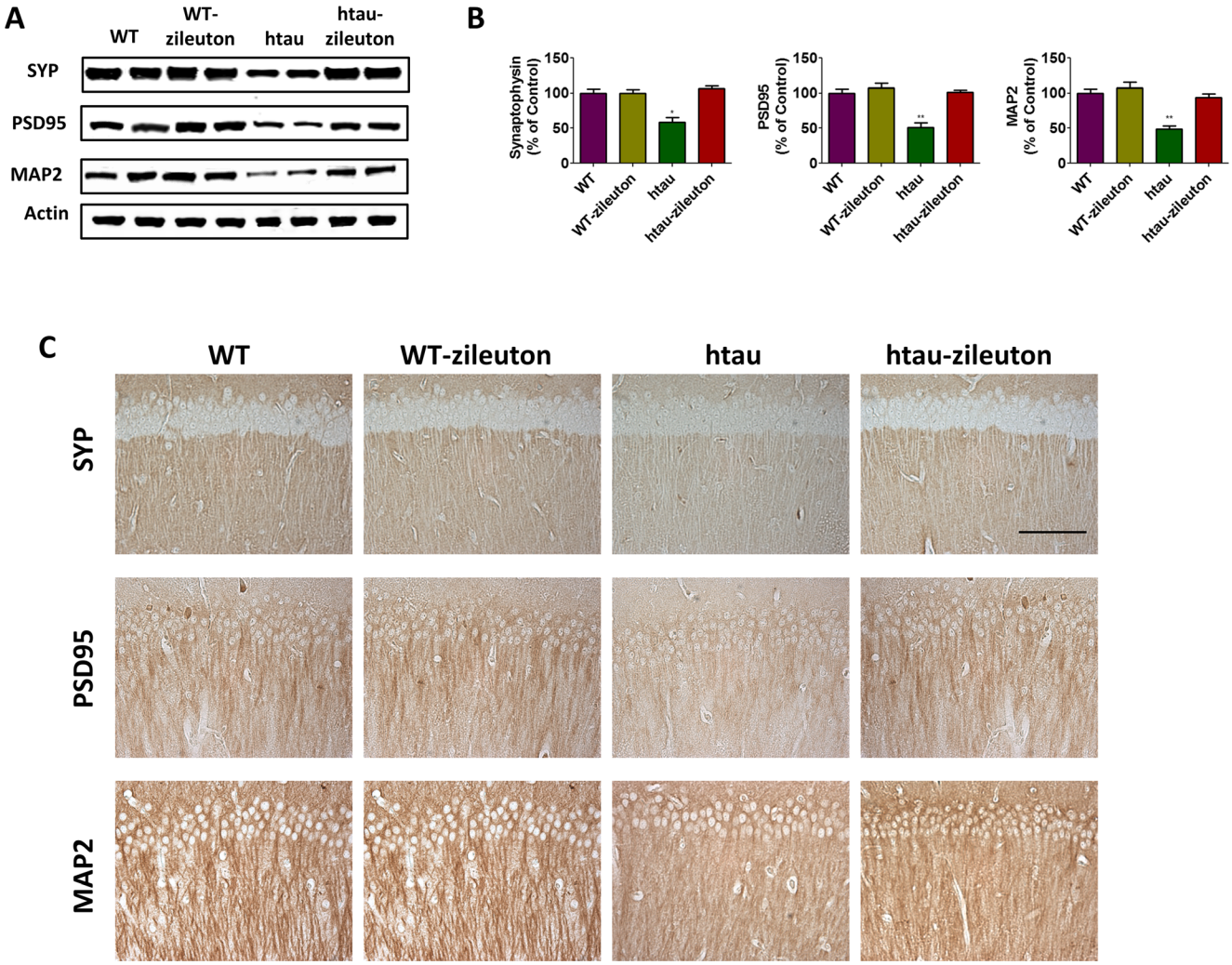

Fig. 5 Anti-leukotriene therapy ameliorates synaptic integrity in aged tau mice. **a** Representative western blot analysis of synaptophysin (SYP), post-synaptic density protein 95 (PSD-95), and microtubule-associated protein 2 (MAP2) in brain cortex homogenates from wild type (WT) and htau mice receiving zileuton or vehicle for 16 weeks. **b** Quantification of the immunoreactivities to the antibodies shown in the previous panel ($n = 6$ per group; $^*p < 0.05$). Values represent mean ± SEM (*one-way ANOVA*). **c** Representative images of brain sections from htau mice receiving zileuton or vehicle immunostained with SYP, PSD-95, and MAP2 antibodies (*scale bar 100 μm*)

for htau mice receiving zileuton had a significant decrease in the steady-state levels of both GFAP and CD45 proteins, which were similar to the levels found in WT mice. Immunohistochemistry analyses of brain sections from the same two groups of mice confirmed these findings (Fig. 6c).

Discussion

The findings presented in this paper demonstrate that chronic administration of a selective and specific inhibitor of 5LO enzyme activation by suppressing the formation of leukotrienes is beneficial in a relevant mouse model of tauopathy even when started after the behavioral deficits and the neuropathology are well established.

Our data provide a strong pre-clinical experimental support to the concept that this therapeutic approach could have a disease-modifying potential for human tauopathies, a large group of neurodegenerative diseases characterized by the progressive accumulation of highly phosphorylated tau protein.

Besides Alzheimer's disease, where tau deposits accumulate together with amyloid-beta peptides, the majority of tauopathies such as progressive supranuclear palsy and corticobasal degeneration manifest only deposits of tau highly phosphorylated as paired helical filaments in the soma and in dendritic processes of neurons and glia cells [19]. Currently, the mechanisms and cellular pathways leading to aberrant tau phosphorylation and tau filaments accumulation are not understood, and no therapy is available to cure or halt the progression of these neuropathological conditions [20].

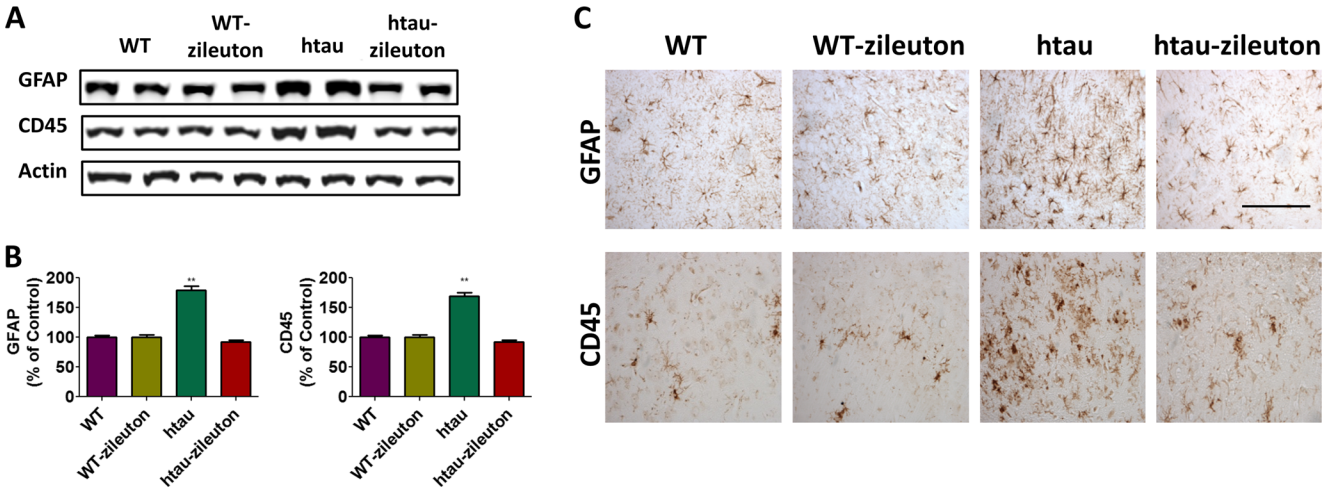

Fig. 6 Anti-leukotrienes therapy reduces neuroinflammation in aged htau mice. **a** Representative western blot analyses of GFAP and cluster of differentiation 45 (CD45) in brain cortex homogenates of wild type (WT) and htau mice treated with zileuton or vehicle. **b** Densitometric analyses of the immunoreactivities to the antibodies shown in the previous panel (*$p < 0.05$) ($n = 6$ per group). Values represent mean ± SEM (*one-way ANOVA*). **c** Representative images of brain sections from htau mice receiving zileuton or vehicle immunostained with antibodies against GFAP and CD45 (*scale bar 100 μm*)

Previously, we have demonstrated that the 5LO protein is significantly upregulated in both human tauopathy and in two transgenic mouse models of the disease. We also showed that its expression levels directly modulate the development of the phenotype in these animal models, and most importantly that early blockade of its activation by suppressing the formation of pro-inflammatory lipid mediators, such a LTB4, delays the onset of memory deficits, tau hype-phosphorylation, and synaptic pathology [7, 9, 10]. Taken together, these results not only clearly supported a preventative action for a therapy aimed at blocking leukotrienes biosynthesis, but also for the first time directly implicated these bioactive lipid mediators in the pathogenesis of neurodegenerative dementing disorders such as Alzheimer's disease and related tauopathy. However, today, we know that in the majority of the clinical cases, the brain pathological changes for these neurodegenerative diseases start a decade or so before any clinical manifestation is evident. This knowledge is at the basis of the common idea that the failure of the various therapeutic approaches attempted so far in these conditions is secondary to the fact that they are initiated too late, and for this reason do not have a real disease-modifying effect. Therefore, an ideal therapeutic drug, even at a pre-clinical level, should demonstrate significant beneficial effects also if it is administered after the establishment of discreet neuropathology [21, 22].

Our study is the first pre-clinical investigation performed in a relevant mouse model of tauopathy that addresses this very important biological question with the intent to fill this gap and provide useful translational information. Thus, the htau mice were treated with the pharmacological probe starting at 12 months of age, a time that is known to coincide with the development of a full tauopathy phenotype, which is characterized by significant behavioral deficits, high tau phosphorylation, and neuropathology. By the end of the treatment, we found that compared with baseline, the untreated htau mice manifested a worsening in both the Y-maze and the Morris water maze, which measure working memory and spatial learning and memory, respectively [23, 24]. By contrast, tau transgenic mice receiving the drug had both behavioral responses similar to the ones observed with the wild-type controls suggesting a rescue of their memory and learning deficits as result of the active treatment. In support of the concept that under our experimental conditions, zileuton had indeed reached its target, we found that the levels of LTB4, the major metabolic products of the 5LO enzymatic activation, were significantly reduced, but as expected, the drug did not influence the steady-state levels of 5LO protein [25].

Having demonstrated that the rescue of the behavioral impairments was associated with a good compliance with the therapeutic regimen, next, we assessed the effect on tau phosphorylation and neuropathology. Compared with untreated mice, we found that zileuton treatment resulted in a significant reduction in the phosphorylation levels at specific epitopes known to be involved in the development of tau fibrils and tangles [26]. However, no significant differences were observed when we assayed the level of total soluble tau in both groups, suggesting that the drug did not have any effect on the transgene. Confirming previous observations, when we looked for some of the major kinases that have been implicated in tau phosphorylation, we observed that only the cdk5 pathway was affected. In particular, while we did not see any variation in the steady-state levels of cdk5 alone, we observed that zileuton-treated mice had significant reduction in its two activators, p25 and p35.

Besides tau phosphorylation, we observed that pharmacological blockade of leukotriene biosynthesis had a beneficial effect also on tau neuropathology, as indicated by the

significant reduction of the total amount of insoluble tau fraction, and the lower levels of MC1, which is known to reflect pathological changes in tau conformation.

The rescue of the behavioral deficits and the significant reduction in the extent of tau neuropathology were also associated with a significant improvement in the synaptic integrity, which is known to represent the biochemical basis for the behavior and at the same time also to be very vulnerable to the development of tau pathology. At the end of the 16 weeks treatment, we observed that compared with controls, tau mice receiving zileuton had a significant elevation in the levels of three distinct synaptic markers, namely synaptophysin, PSD95, and MAP2 [27, 28].

It was no surprise to observe that, in association with the suppression of potent pro-inflammatory lipid mediators such as the LTB4, zileuton-treated mice had a significant reduction in the levels of GFAP, a marker of astrocyte activation, and CD45, a marker of microglia cells activation. This observation is in agreement with previous ones in which the drug we have implemented acts as a potent anti-inflammatory agent both in vitro and in vivo [29, 30].

Taken together, our findings establish a direct and functional role for the leukotrienes in the entire spectrum of the tau neuropathological phenotype. Interestingly, recent epidemiological studies have demonstrated that adult patients with asthma, a chronic respiratory disease characterized by an elevation of leukotriene biosynthesis, are at higher risk to develop dementia and Alzheimer's disease [31, 32]. This fact further supports the concept that this metabolic pathway, which is highly expressed both in the CNS and in the periphery, plays a wider role than previously anticipated.

Finally, our study is not only of translational value, but it is also physiologically relevant since the model we have implemented to test our hypothesis, the htau mice, expresses all six normal human isoforms of tau protein without the confounding presence of human mutant tau variants. Taken together, our findings represent the first pre-clinical evidence that suppression of leukotriene biosynthesis by pharmacologically targeting the 5LO enzymatic activity rescues the entire tau pathological phenotype (memory deficits, tau phosphorylation, synaptic pathology, neuroinflammation) even after its development.

The demonstration that 5LO is functionally involved at the later stages of the tau pathological phenotype further supports the hypothesis that pharmacological inhibitors of this protein enzyme represent a class of drugs with viable therapeutic potential for treating human tauopathies.

Authors' Contributions PFG and DP designed the study; PFG and JC performed the experiments; PFG and DP analyzed the data and drafted the manuscript. All authors have discussed the results and seen the final version of the paper before submission.

Funding Information Domenico Praticò is the Scott Richards North Star Foundation Chair of Alzheimer's research. This study was supported in part by grants from The Wanda Simone Endowment for Neuroscience and the Scott Richards North Star Charitable Foundation.

Compliance with Ethical Standards

Conflict of Interest The authors have no conflicting financial interest to disclose.

References

1. Yerbury JJ, Ooi L, Dillin A, Saunders DN, Hatters DM, Berat PM, Cashman NR, Wilson MR et al (2016) Walking the tightrope: proteostasis and neurodegenerative diseases. J Neurochem 137: 489–505
2. Arendt T, Stieler JT, Holzer M (2016) Tau and tauopathies. Brain Res Bull 126:238–292
3. Kovacs GG (2015) Neuropathology of tauopathies: principles and practice. Neuropathol Appl Neurobiol 41(1):3–23
4. Spillantini MG, Goedert M (2013) Tau pathology and neurodegeneration. Lancet Neurol 12:609–622
5. Ishizawa K, Dickson DW (2001) Microglial activation parallels system degeneration in progressive supranuclear palsy and corticobasal degeneration. J Neuropathol Exp Neurol 60:647–657
6. Laurent C, Dorothee G, Hunot S, Martin E, Monnet Y, Duchamp M, Dong Y, Légeron FP et al (2007) Hippocampal T cell infiltration promotes neuroinflammation and cognitive decline in a mouse model of tauopathy. Brain 140:184–200
7. Giannopoulos PF, Chu J, Sperow N, Li JG, Yu WH, Kirby LG, Abood M, Praticò D (2015) Pharmacologic inhibition of 5-lipoxygenase improves memory, rescues synaptic dysfunction, and ameliorates tau pathology in a transgenic model of tauopathy. Biol Psychiatry 78:693–701
8. Joshi Y, Praticò D (2015) The 5-lipoxygenase pathway: oxidative and inflammatory contributions to the Alzheimer's disease pathogenesis. Front Cell Neurosci 8(436):1–8
9. Vagnozzi AN, Giannopoulos PF, Praticò D (2017) The direct role of 5-lipoxygenase on tau pathology, synaptic integrity and cognition in a mouse model of tauopathy. Transl. Psychiatry 7(12):1288
10. Giannopoulos PF, Praticò D (2017) Overexpression of 5-lipoxygenase worsens the phenotype of a mouse model of tauopathy. Mol Neurobiol. https://doi.org/10.1007/s12035-017-0817-7
11. Andorfer C, Kress Y, Espinoza M, de Silva R, Tucker KL, Barde YA, Duff K, Davies P (2003) Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. J Neurochem 86:582–590
12. Chu J, Li JG, Praticò D (2013) Zileuton improves memory deficits, amyloid and tau pathology in a mouse model of Alzheimer's disease with plaques and tangles. PLoS One 8:e70991
13. Di Meco A, Joshi YB, Praticò D (2014) Sleep deprivation impairs memory, tau metabolism, and synaptic integrity of a mouse model of Alzheimer's disease with plaques and tangles. Neurobiol Aging 35(8):1813–1820
14. Li JG, Barrero C, Merali S, Praticò D (2017) Five lipoxygenase hypomethylation mediates the homocysteine effect on Alzheimer's phenotype. Sci Rep 7:46002. https://doi.org/10.1038/srep46002.
15. Giannopoulos PF, Chu J, Joshi YB, Sperow M, Li JG, Kirby LG, Praticò D (2013) Five-lipoxygenase activating protein reduction ameliorates cognitive deficit, synaptic dysfunction, and neuropathology in a mouse model of Alzheimer's disease. Biol Psychiatry 74:348–356

16. Joshi Y, Chu J, Praticò D (2013) Knockout of 5lipoxygenase prevents dexamethasone-induced tau pathology in the 3xTg mice. Aging Cell 12(4):706–711
17. Weaver CL, Espinoza M, Kress Y, Davies P (2000) Conformational change as one of the earliest alterations of tau in Alzheimer's disease. Neurobiol Aging 21:719–727
18. Kelleher I, Garwood C, Hanger DP, Anderton BH, Noble W (2007) Kinase activities increase during the development of tauopathy in htau mice. J Neurochem 103:2256–22678
19. Wang Y, Mandelkow E (2016) Tau in physiology and pathology. Nat Rev Neurosci 17:5–21
20. Chiti F, Dobson CM (2017) Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. Annu Rev Biochem 86:27–68
21. Li C, Götz J (2017) Tau-based therapies in neurodegeneration: opportunities and challenges. Nat Rev Drug Discov 16(12):863–883
22. Braczynski AK, Schulz JB, Bach JP (2017) Vaccination strategies in tauopathies and synucleinopathies. J Neurochem 143(5):467–488
23. Wahl D, Coogan SC, Solon-Biet SM, de Cabo R, Haran JB, Raubenheimer D, Cogger VC, Mattson MP et al (2017) Cognitive and behavioral evaluation of nutritional interventions in rodent models of brain aging and dementia. Clin Interv Aging 12: 1419–1428
24. Stewart S, Cacucci F, Lever C (2011) Which memory task for my mouse? A systematic review of spatial memory performance in the Tg2576 Alzheimer's mouse model. J Alzheimers Dis 26(1):105–126
25. Steinhilber D, Hofmann B (2014) Recent advances in the search for novel 5-lipoxygenase inhibitors. Basic Clin Pharmacol Toxicol 114(1):70–77
26. Wang JZ, Xia YY, Grundke-Iqbal I, Iqbal K (2013) Abnormal hyperphosphorylation of tau: sites, regulation and molecular mechanism of neurofibrillary degeneration. J Alz Dis 33(suppl.1):S123–S139
27. Alldred MJ, Duff KE, Ginsberg SD (2012) Microarray analysis of CA1 pyramidal neurons in a mouse model of tauopathy reveals progressive synaptic dysfunction. Neurobiol Dis 45:751–762
28. Moreno H, Morfini G, Buitrago L, Ujlaki G, Choi S, Yu E, Moreira JE, Avila J et al (2016) Tau pathology-mediated presynaptic dysfunction. Neuroscience 325:30–38
29. Bennett M, Gilroy DW (2016) Lipid mediators in inflammation. Microbiol Spectr 2016:4(6). https://doi.org/10.1128/microbiolspec.MCHD-0035-2016
30. Giannopoulos PF, Joshi YB, Praticò D (2014) Novel lipid signaling pathways in Alzheimer disease pathogenesis. Biochem Pharmacol 88(4):560–564
31. Chen MH, Li CT, Tsai CF, Lin WC, Chang WH, Chen TJ, Pan TJ, Su TL et al (2014) Risk of dementia among patients with asthma: a nationwide longitudinal study. J Am Med Dir Assoc 15(10):763–767
32. Peng YH, Wu BR, Liao WC, Muo WC, Hsia TC, Kao CH (2015) Adult asthma increases dementia risk: a nationwide cohort study. J Epidemiol Comm Health 69(2):123–128