Original Article

An electrically charged calcium bicarbonate-containing water breaks down and prevents intracellular accumulation of mutant Tau.

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Summary We have previously demonstrated that the electrically-charged calcium bicarbonate-containing water (CAC-717 solution) efficiently inactivated the abnormal isoform of prion protein (PrP^{sc}). PrP^{Sc} is resistant to proteinase-K and constitute β-sheet conformation, a common pathological feature among neurodegenerative diseases including Alzheimer's disease (AD). AD is the most common cause of dementia and is characterized by two pathological features: amyloid plaques (APs) and neurofibrillary tangles (NFTs). AP is an extracellular deposition of β-amyloid protein (Aβ), and NFT is an intraneuronal accumulation of hyperphosphorylated Tau, one of the microtubule-associated proteins. Our previous study showed that CAC-717 succeeded to degrade aggregated Aβ. This finding prompted us to hypothesize that CAC-717 could also break down pathological form of Tau.

Here we demonstrated that CAC-717 effectively broke down pre-aggregated human mutant Tau. Furthermore, we found that CAC-717 treatment led to a significant decrease in both soluble and insoluble human mutant Tau levels even *in vivo*. These findings suggest that CAC-717 could be a novel therapeutic agent capable of breaking down pathological form of Tau.

Key words: Alzheimer's disease, CAC-717, Drosophila melanogaster, Tau

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Received 24 December 2025; Accepted 18 March 2026

Introduction

CAC-717 is a type of calcium bicarbonate particle that is electrically charged and possesses a mesoscopic structure[1]. Previous studies demonstrated that the CAC-717 solution was effective in neutralizing a wide variety of pathogens, including bacteria and both enveloped and non-enveloped viruses[1-5]. Despite having an approximate pH of 12.4, its pH quickly drops to 8.84 when it comes into contact with human skin, and the CAC-717 solution was shown to be innocuous and nonirritating to humans[1]. It is noteworthy that the CAC-717 solution can efficiently inactivate the abnormal isoform of prion protein (PrP^{sc})[6,7]. PrP^{Sc} is resistant to proteinase-K and constitute β-sheet conformation, a common pathological feature among neurodegenerative diseases including AD[8].

Alzheimer's disease (AD) is the most common neurodegenerative disease leading to dementia and is characterized by two pathological features: amyloid plaques (APs) and neurofibrillary tangles (NFTs)[9]. AP is an extracellular deposition of β-amyloid protein (Aβ), which is produced by sequential cleavage of β-amyloid precursor protein[10-12]. Aβ consists of 38-43 peptides, and it is widely accepted that Aβ42 is the most neurotoxic molecule and prone to aggregate[13-16]. NFT is an intraneuronal accumulation of hyperphosphorylated Tau, one of the microtubule-associated proteins[17,18]. Evidence from clinical studies suggests that the progression of Tau pathology is a primary factor in the onset of AD[19]. Recent advances in biomarker study have led to the identification of phosphorylated Tau at the 217 residue, denoted as pT217, as a promising biomarker for AD[20-23]. Plasma levels of pT217 increase throughout the preclinical and early symptomatic phases of AD, and it also highly correlate not only with Aβ but also with Tau accumulation in brain[20-23]. Moreover, autosomal dominant mutations in the *MAPT* gene, which encodes Tau, cause frontotemporal dementias[24-26]. These findings strongly suggest that Tau pathology would have a pivotal role in the pathogenesis of dementia. Evidently, our previous study showed that CAC-717 succeeded to degrade aggregated Aβ[27]. This led us to hypothesize that CAC-717 could also break down pathological form of Tau.

Here we demonstrated that CAC-717 effectively broke down pre-aggregated human mutant Tau. Furthermore, we found that CAC-717 treatment led to a significant decrease in both soluble and insoluble human mutant Tau levels even *in vivo*. These findings suggest that CAC-717 could be a novel therapeutic agent capable of breaking down pathological form of Tau.

Experimental procedures

Antibodies

We used the following antibodies: mouse monoclonal anti-Aβ antibody (82E1; IBL, Gunma, Japan); mouse monoclonal anti-β-actin antibody (Sigma, St Louis, MO, USA); mouse monoclonal anti-Tau antibody (Tau12; Millipore, Temecula, CA, USA); and rabbit polyclonal anti-LC3-II antibody (Cell Signaling Technology, Danvers, MA, USA).

CAC-717 solution

The CAC-717 solution has a pH value of approximately 12.4 and contains 6.9 mM calcium bicarbonate. CAC-717 is produced in accordance with Japan patent No. 5778328, and Food and Drug Administration/USA Regulation No. 880.6890 Class 1 disinfectant. As a negative control, cells were treated with 6.9 mM calcium carbonate solution (pH12.4) at a concentration of 100 μl/ml.

Preparation of aggregated AD proteins

Aβ 1-42 (Aβ42; Peptide Institute, Inc., Osaka, Japan) were dissolved by brief vortexing in 0.02% ammonia solution at a concentration of 250 μM and then diluted with phosphate-buffered saline (PBS) at a concentration of 50 μM. The diluted Aβ42 solutions were pre-incubated at 37℃ for 4 hours to induce aggregation, and then treated with CAC-717 for another 30 min. CAC-717 was added to Aβ42 solution at same, half, or a quarter quantity. Pre-aggregated recombinant human 2N4R Tau (mutated P301S) solution was purchased from

Stress Marq Biosciences, Inc., Victoria, BC, Canada. CAC-717 was added to Tau solution in a same manner as Aβ42.

Plasmids

The cDNAs for 2N4R human Tau mutated P301L (P301L Tau) were subcloned into pCI-neo. Plasmid transfections were performed using PEI-MAX reagent (Polysciences, Warrington, PA, USA), according to the manufacturer's protocol.

Cell cultures and chemical treatments

A neuronal cell line, mouse neuroblastoma Neuro2a cells from ATCC (Manassas, VA), was grown in Dulbecco's Eagle's medium (DMEM) (Sigma) with 5% fetal calf serum. Cells were cultured under humidified air containing 5% CO2 at 37℃. Cells were plated at a density of 2.0×10^4 cells onto 6-well culture plates (Thermo Fisher Scientific, Waltham, MA, USA) and coverslips (Matsunami, Osaka, Japan) coated with 0.1% polyethyleneimine (Wako, Osaka, Japan). To investigate cell toxicity of CAC-717, cells were treated with CAC-717 for 24 h. The final concentration for adding CAC-717 to the culture medium was 25, 50, or 100 μl/ml. As a negative control, cells were incubated with 6.9 mM calcium carbonate solution (pH 12.4) at a concentration of 100 μl/ml. Conditioned media were harvested and examined for LDH assay according to manufactural protocol (Nacalai Tesque, inc., Kyoto, Japan).

Cells were transfected with human Tau-expressing plasmids as mentioned above. 48 h after transfection, cells were treated with CAC-717 for 24 h. As a negative control, cells were treated with 6.9 mM calcium carbonate solution (pH12.4) at a concentration of 100 μl/ml. For chemical treatment studies, cells were incubated with a combination of CAC-717 with 10 μM pepstatin A (Sigma), 10 μM E64d (Cayman Chemical, Ann Arbor, MI, USA), and 10 μM leupeptin (Sigma) for 24 h.

Cells that were plated in 6-well plates were lysed in lysis buffer containing 1% TritonX-100, 150 mM NaCl, 10 mM HEPES (pH 8.0), 2 mM EGTA, and Complete Mini™ proteinase inhibitor cocktail (Roche Molecular Biochemicals). After incubation for 30 min at 4℃ and centrifugation at 20,000 g for 20 min, the supernatants (soluble fractions) were subjected to western blot analyses. The resulting pellets were lysed in a sample buffer solution containing 62.5 mM Tris-HCl (pH 6.8), 2.3% SDS, 0.5% Triton X-100, 2 mM EGTA, 2.5% 2-mercaptoethanol, and then also subjected to western blot analyses as insoluble fractions. We used the following primary antibodies: anti-Tau (1:5,000) and anti-β-actin (1:200,000). We performed three independent experiments (n = 6 for each experimental group), duplicating each experiment. We quantified the optical density of immunoreactive bands obtained from the western blots using Image J[28]. Data are presented as means ± SEM. Statistical significance ($p < 0.05$) was determined using one-way ANOVA, followed by Tukey hsd *post hoc* tests.

For immunocytochemistry, Neuro2a cells that were plated on coverslips were fixed with 4% paraformaldehyde in phosphate buffer, and then permeabilized with 0.1% Triton X-100 in PBS for 10 min. After blocking with 3% bovine serum albumin in PBS and 0.1% Tween20, cells were incubated with primary antibodies overnight at 4℃. After thorough washing, cells were then incubated with AlexaFluor 488-conjugated anti-mouse IgG (Jackson ImmunoResearch, Laboratories, Inc., West Grove, PA, USA) and AlexaFluor 568-conjugated anti-rabbit IgG (Abcam) for 2 h at room temperature, and then counterstained with DAPI. The stained cells were photographed with an LSM700 confocal laser-scanning microscope (ZEISS, Oberkochen, Germany). The quantitative analyses of the Tau-immunoreactive area in a given coverslip area was determined by using Image J (Version 1.54p; https://imagej.net/ij/). The image analysis was performed in six unselected areas, 250 × 250 μm each, from coverslips. Data are shown as means ± SEM. Statistical significance ($p < 0.05$) was determined using one-way ANOVA, followed by Tukey hsd post hoc tests.

For immunocytochemistry, we used the following primary antibodies: anti-Tau (1:1000) and anti-LC3-II (1:1000). We performed three independent

experiments.

Drosophila stocks

Flies were maintained in plastic vials with standard cornmeal-yeast agar medium at 25℃ under a 12-h light/dark cycle. The *nSyb-Gal4*, *pGMR-Gal4*, *tub-gal80*^{ts}, *pUAS-mCD8-GFP* and *P{ninaE.GFP}1* (Express GFP-tagged rhodopsin1 in R1-6 photoreceptor cells under the control of *ninaE* regulatory sequences) were obtained from the Bloomington Drosophila Stock Center (Indiana University). The *UAS-tauR406W* was kindly gifted from Dr. Feany[29].

Induction system (GAL4/UAS/gal-80ts system)

To induce tauR406W in neurons at the adult stage, we have established the following strain: *nSyb-Gal4/Y; UAS-tauR406W/tub-gal80*^{ts}. To induce *tauR406W* in compound eyes at the adult stage, we have established the following strain: *pGMR-Gal4, P{ninaE.GFP}1/Y; UAS-tauR406W/tub-gal80*^{ts}*; +/tub-gal80*^{ts}. These lines were shifted the temperature from 18 to 29℃ before the experiment[30].

As a control, we established the following strains: *nSyb-Gal4/Y; UAS-mCD8-GFP/tub-gal80*^{ts}*; +/+ : pGMR-Gal4, P{ninaE.GFP}1/Y; +/tub-gal80ts; +/tub-gal80*^{ts}.

Method of administering reagents

CAC-717 was diluted with 5% sucrose/H_2O to 5% (v/v). Adult flies were given *ad libitum* access to filter paper soaked with each reagent twice a week, 2-3 days apart, for 15 h each time[31]. Control flies were fed with standard cornmeal-yeast agar medium containing 5% sucrose.

Climbing assay and survival assay

In the climbing assay also referred to as a negative geotaxis assay[32], 80 flies were placed in an empty plastic vial (diameter, 3 cm; height, 20 cm). The vial was tapped gently so that all flies fell into the bottom area, and counting the number of individuals that climbed 5 cm or more within 10 seconds. The test was conducted twice with a 15-second interval between each test, and the average value was used as the measurement result. The test results were statistically analyzed using Welch's t-test in Excel.

For survival assays, flies were maintained at 20 flies/vial on standard medium at 29℃. Flies were transferred to fresh vials every 2-3 days and scored for survival. Each experiment was conducted with at least 20 flies of each genotype. The test results were statistically analyzed using log-rank test in Excel.

Biochemical analysis of fly heads

30 adult fly heads were dissected from flies, and homogenized in the lysis buffer mentioned above. After centrifugation at 20,000 g for 20 min, the supernatants (soluble fractions) were subjected to immunoblot analyses. The resulting pellets were lysed in a sample buffer solution, and then also subjected to western blot analyses as insoluble fractions. We used the following primary antibodies: anti-Tau (1:5,000) and anti-beta-actin (1:200,000).

Degeneration assay of the photoreceptor cells

1% agar medium was melted at 100℃, and anesthetized flies were placed in when the temperature dropped to 65℃. After the agar had completely solidified, water was added so that the entire fly was immersed, and the survival of the photoreceptor cells was observed under fluorescent light using a 60x water depth lens. Fluorescent images were observed and captured with a CCD camera (Olympus, DP-86)[33].

The survival rate of photoreceptor neuron (R cell) was calculated by counting the number of surviving photoreceptor neurons (R-cells) cells (GFP positive cells) relative to the number of ommatidia, and using the following formula: Survival rate of R cells (%) = (number of GFP positive cells / number of ommatidia x 6) x 100

Results

CAC-717 breaks down aggregated Aβ and Tau

In this study, we first analyzed whether CAC-717

could break down aggregated Aβ42 or human mutant Tau *in vitro*. We utilized human mutant Tau as a pathological form of Tau due to its known ability to accelerate fibril formation at a faster rate compared to the wild-type Tau protein[34]. The CAC-717 solution was combined with pre-aggregated solutions of recombinant human Aβ42 or human mutant Tau (P301S) solutions, and subsequently incubated for 30 min. As previously reported, the immunoblot results demonstrated that CAC-717 significantly lowered the levels of high-molecular weight smear bands of Aβ (Fig. 1A)[27]. CAC-717 also clearly decreased the levels of high-molecular weight bands of human mutant Tau (Fig. 1B).

CAC-717 treatment prevents intracellular accumulation of Tau

Since Tau pathology is a characteristic intracellular pathology[17,18], we examined whether CAC-717 could break down pathological form of Tau proteins inside the cell. First, we conducted LDH assay to determine whether CAC-717 could induce cell toxicity in mouse neuroblastoma, Neuro2a cells. Our analyses showed that CAC-717 did not exhibit cell toxicity even at a concentration of 100 μl/ml (Fig. 2A). Neither did we observe any morphological changes in Neuro2a cells with CAC-717 treatment (Fig. 2B). Although CAC-717 has an approximate pH of 12.4, the addition of CAC-717 did not alter the color of culture medium (data not shown). Next, we expressed human mutant Tau with the P301L mutation in Neuro2a cells. In the present study, a mouse monoclonal anti-human Tau antibody, Tau12, was employed since it does not recognize endogenous rodent Tau. Western blot analyses showed that CAC-717 treatment led to a significant decrease in both soluble and insoluble Tau levels in Neuro2a cells at concentrations of 50 and 100 μl/ml (Fig. 2C, D). In contrast, CAC-717 treatment had no impact on the levels of endogenous β-actin (Fig, 2C, D).

CAC-717 decreases intracellular levels of Tau without relying on the lysosomal degradation pathway

It remains unclear how CAC-717 degrades pathological form of proteins such as Prp[sc7,8]. Several studies demonstrated that autophagy plays a role in the breakdown of intracellular pathological form of Tau[35-39]. Therefore, we investigated whether CAC-717 could break down intracellular Tau without relying on autophagy by conducting a study using

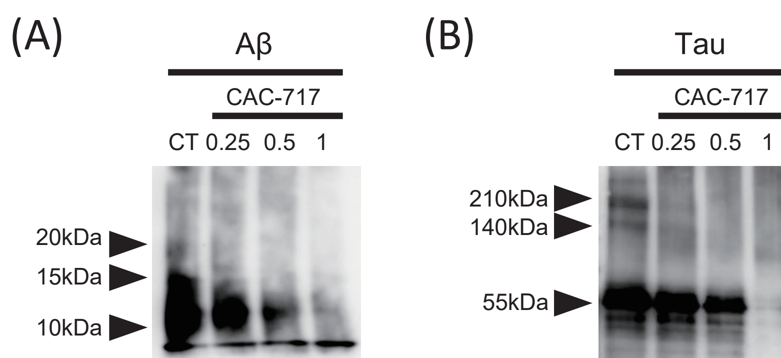

Fig. 1. CAC-717 breaks down aggregated Aβ and Tau.
Immunoblots showing the amounts of pre-aggregated Aβ (A) and Tau (B). In this study, CAC-717 solution was combined with pre-aggregated Aβ or Tau at the ratio of 0.25:1, 0.5:1, and 1:1 respectively. For negative control, pre-aggregated proteins were combined with negative control solution, 6.9 mM calcium carbonate solution (pH12.4), at the ration of 1:1 (control; CT). For Aβ study, human recombinant Aβ1-42 solutions were pre-incubated to induce aggregation, and then treated with CAC-717 for 30 min. For Tau study, pre-aggregated recombinant human P301S mutant Tau solutions were purchased, and then treated with CAC-717 for 30 min. The CAC-717 treatment clearly reduced the levels of aggregated Aβ and Tau in a dose-dependent manner.

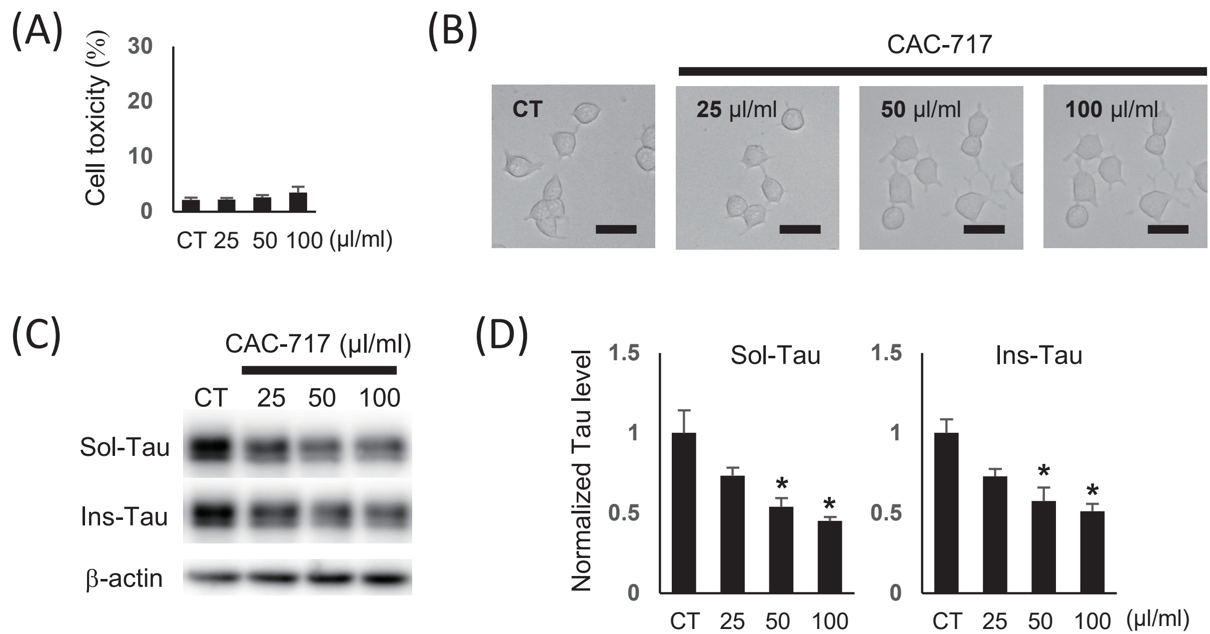

Fig. 2. CAC-717 treatment prevents the accumulation of mutant Tau in Neuro2a cells.
(A) Bar graphs showing the cell toxicity of CAC-717 as determined by LDH assay in Neuro2a cells 24 h following CAC-717 treatment. (B) Representative photomicrographic images of Neuro2a cells 24 h following CAC-717 treatment. CT, 100 μl/ml negative control solution; 25, 25 μl/ml CAC-717 solution; 50, 50 μl/ml CAC-717 solution; 100, 100 μl/ml CAC-717 solution. (C) Western blots showing the levels of soluble Tau (Sol-Tau), insoluble Tau (Ins-Tau), and β-actin in Neuro2a cells 48 h after plasmid transfection. Cells were treated with CAC-717 for the last 24 h. (D) Bar graphs showing the relative protein levels of both Sol-Tau and Ins-Tau in Neuro2a cells 48 h after plasmid transfection. Cells were treated with CAC-717 for the last 24 h. Tau levels were normalized to β-actin levels. Values are means ± SEM; N=6. *p < 0.05 versus control cells (CT).

lysosome inhibitors, which prevent the degradation of autophagosomes. In this study, Neuro2a cells were treated with a combination of inhibitors targeting different types of cathepsins, specifically pepstain A, E64d, and leupeptin (PEL). Western blot analyses indicated that CAC-717 treatment resulted in a significant decrease in both soluble and insoluble Tau levels, despite the presence of PEL (Fig. 3A, B). We conducted immunocytochemical analyses and found that numerous autophagosomes were visibly accumulated in Neuro2a cells with PEL treatment (Fig. 3C). Immunocytochemistry showed that CAC-717 treatment significantly decreased intracellular Tau levels in Neuro2a cells, even when combined with PEL treatment (Fig. 3C).

CAC-717 treatment improves neurodegeneration caused by R406W Tau mutation in a transgenic fly model

Finally, we evaluated whether CAC-717 could decrease insoluble Tau levels and improve Tau-induced neurodegenerative effects *in vivo*. We employed *Drosophila melanogaster*, which exhibits human mutant *TauR406W* expression in the neuron, since this particular fly model can induce neurodegeneration accompanied by the accumulation of insoluble Tau[29]. We confirmed that CAC-717 treatment had no impact on climbing efficiency and the survival rate for flies that only expressed *GFP* (GFP-CT vs. GFP-CAC, Fig. 4A, B). Noteworthy, CAC-717 treatment greatly improved climbing ability of *TauR406W*-expressed flies (R406W-CT vs. R406W-CAC, Fig. 4A). Furthermore, CAC-717 treatment significantly improved the survival rate of *TauR406W*-expressed flies up to 26 days (R406W-CT vs. R406W-CAC, Fig. 4B). Western blot analyses confirmed that CAC-717 treatment decreased both soluble and insoluble Tau levels in fly brain

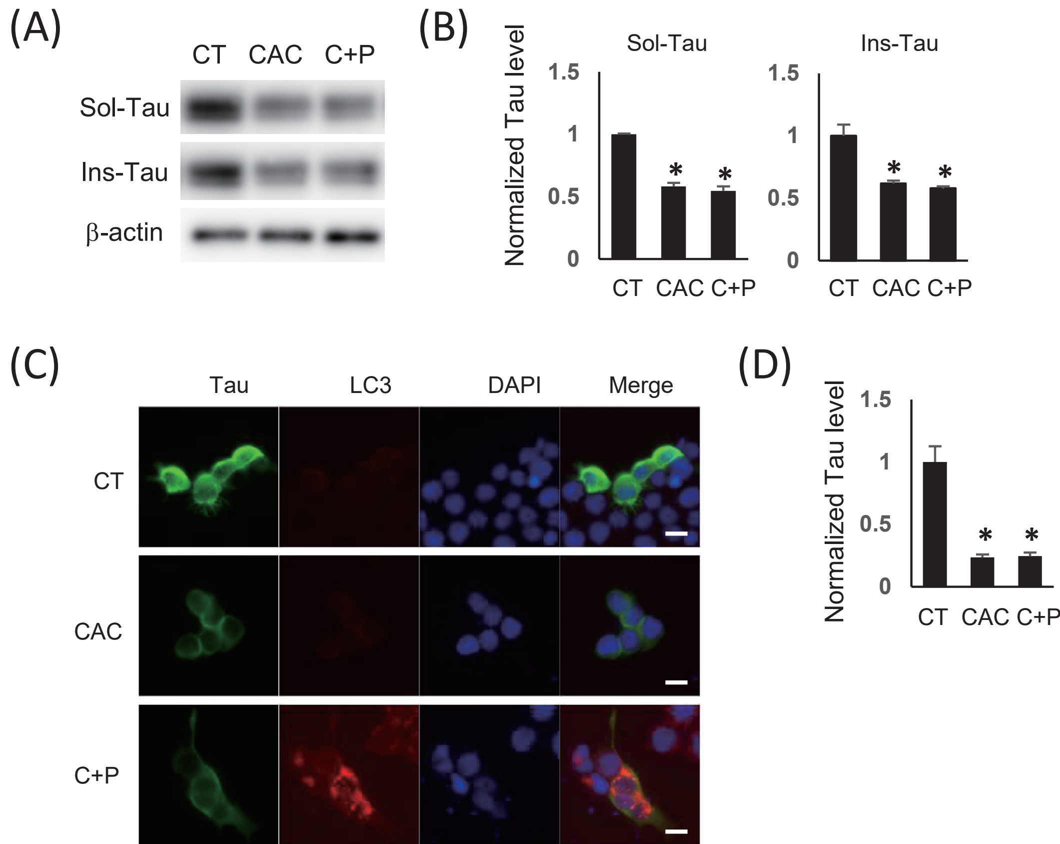

Fig. 3. CAC-717 treatment breaks down exogenous human Tau without relying on lysosomal degradation processes.
(A) Western blots showing the levels of Sol-Tau, Ins-Tau, and β-actin in Neuro2a cells 48 h after plasmid transfection. Cells were treated with 100 μl/ml CAC-717 and a mixture of cathepsin inhibitors (PEL) for last 24 h. CT, control; CAC; CAC-717 treatment; C+P, a combined treatment of CAC-717 and PEL. (B) Bar graphs showing the relative protein levels of both soluble and insoluble Tau in Neuro2a cells. Tau levels were normalized to β-actin levels. Values are means ± SEM; N=6. *p < 0.05 versus CT. (C) Representative photomicrographic images of Neuro2a cells. PEL treatment induced intracellular accumulation of LC3-II positive autophagosomes. CAC-717 treatment (100 μl/ml) decreased the levels of Tau-positive immunofluorescence, even in the presence of PEL. Scale bars = 40 μm. (D) Bar graphs showing the relative Tau-immunopositive area in photomicrographic images of Neuro2a cells. Values are means ± SEM; N=6. *p < 0.05 versus CT.

expressing *TauR406W* (Fig. 4C). Another *in vivo* study involving flies that expresses *TauR406W* in photoreceptor cells was also examined. Overexpression of *Tau R406W* induces the degeneration of photoreceptor cells (white arrows, Fig. 4D). CAC-717 treatment significantly eased the degeneration of photoreceptor cells caused by *TauR406W* (Fig. 4D, E).

Discussion

Our previous studies demonstrated that CAC-717 prominently decreased the levels of aggregated PrP^{sc} and Aβ[7,8,27]. The current investigation showed that CAC-717 significantly decreased the levels of high-molecular weight bands of human mutant Tau as well as Aβ42 (Fig. 1). The results indicate that CAC-717 can break down the aggregated Tau protein. Surprisingly, we found that the bands corresponding monomeric Tau were also significantly reduced in the presence of CAC-717 (Fig. 1B). Previous study showed that CAC-717 diminished the immunoreactive bands of PrP^{sc} without altering the levels of normal endogenous prion protein in Neuro2a cells[7]. Although further studies are required, CAC-717 could potentially identify and disassem-

ble abnormal form of proteins down to peptide level regardless of their species.

Tau pathology is a characteristic intracellular pathology, and mutant Tau can accelerate its fibril formation more rapidly than wild-type Tau[34]. Therefore, we examined whether CAC-717 could influence the intracellular pathological form of Tau in Neuro2a cells expressing human mutant Tau. Significantly, the CAC-717 treatment decreased both soluble and insoluble human mutant Tau in Neuro2a cells while leaving endogenous β-actin levels unchanged (Fig. 2C, D). The results of this study suggest that CAC-717 is capable of dissociating the intracellular pathological form of Tau. While insoluble Tau aggregates are hallmark features of Tau pathology, accumulating evidence suggests that soluble Tau oligomers play a pivotal role in Tau-related neurotoxicity, including synaptic dysfunction and subsequent cognitive impairment[40,41]. Therefore, CAC-717 may not only inhibit the formation of Tau pathology but also prevent the progression of neuronal deficits induced by soluble Tau oligomer. In this study, we noted that the addition of CAC-717 did not alter the color of culture medium (data not shown), indicating that CAC-717 exerts its effects within a physiological (neutral) pH range.

Previous studies showed that initiating the autophagy-lysosomal pathway can improve Tau pathology-related dysfunction in mice[42-44], while growing evidence indicates that lysosomal malfunction plays a role in the development of neurodegenerative diseases such as Tauopathy[45]. In this study, we examined chemical treatment study to assess whether lysosomal dysfunction would interfere with the effect of CAC-717. Western blot analyses demonstrated that CAC-717 was able to significantly decrease both soluble and insoluble Tau levels in Neuro2a cells, even when PEL, a combination of cathepsin inhibitors, was present (Fig. 3A, B). Immunocytochemical analyses verified that PEL treatment led to an intracellular build-up of autophagosomes in Neuro2a cells, indicating that PEL treatment effectively disrupted the clearance of autophagosomes (Fig. 3C). CAC-717 was able to significantly decrease Tau levels in these cells, suggesting that CAC-717 can break down intracellular human mutant Tau independently of lysosomal degradation pathway (Fig. 3C).

It is noteworthy that CAC-717 treatment succeeded to ameliorate neurodegeneration *in vivo* (Fig. 4). Feeding CAC-717 to transgenic flies that expressed a mutant form of human Tau in their brains substantially improved their climbing ability and survival rate (Fig. 4A, B), and western blot analyses revealed that both soluble and insoluble human Tau levels were significantly lowered by CAC-717 (Fig. 4C). This is the first study to demonstrate that the feeding of CAC-717 could alleviate intracellular accumulation of insoluble Tau *in vivo*. Since flies has open circulatory system, CAC-717 absorbed from the intestinal tract would be effectively transferred to brain. That may be why CAC-717 could strongly decreased Tau levels in the fly brain (Fig. 4C). A previous study showed that gut microbial load and immune activation were altered in transgenic flies which pan-neuronally express TauR406W[46]. Therefore, CAC-717 has the potential to improve not only neural but also non-neural malfunctioning, leading to enhanced performance in the climbing assay (Fig. 4A). On the other hand, CAC-717 treatment failed to improve the survival rate of flies after 26 days (Fig. 4B). Although protein levels were effectively lowered by CAC-717 treatment (Fig. 4C), TauR406W would reduce fly viability through an unidentified pathway. We also confirmed that CAC-717 treatment succeeded to rescue the degeneration of photoreceptor cells which expressing *TauR406W*. Although we have to assess whether CAC-717 can cross the blood-brain barrier in mammals, our present study implies that the CAC-717 solution could potentially serve as a medication to break down abnormal aggregated proteins in the brain (Fig. 4).

Taken together, this study demonstrates that CAC-717 can break down and inhibit the intracellular accumulation of pathological form of Tau even in fly brain. Although further research is necessary to fully understand how CAC-717 break down aggregated pathological proteins, our present

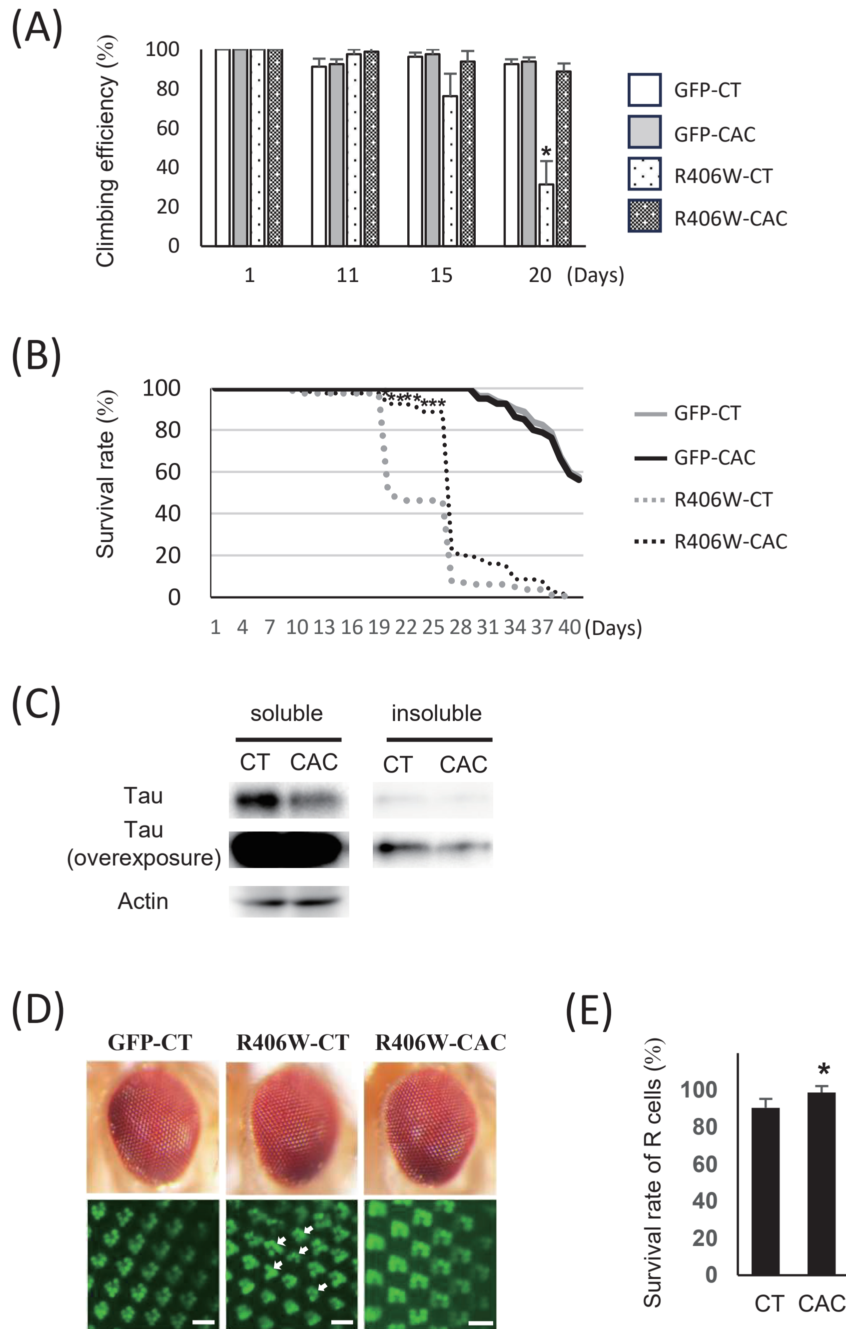

Fig. 4. Feeding of CAC-717 improves neurodegeneration and decreases both soluble and insoluble Tau levels in flies expressing human mutant Tau.
(A) The climbing efficiency of transgenic flies examined in this study. Feeding of CAC-717 significantly improved climbing ability of flies expressing human *TauR406W* in the neuron (*nSyb-Gal4/Y*; *UAS-tauR406W/tub-gal80ts*). GFP, *nSyb-Gal4*; *UAS-mCD8-GFP/tub-Gal80t*; R406W, *nSyb-Gal4*; *UAS-tauR406W/tub-Gal80ts*; CT, control; CAC, CAC-717 treatment. Values are means ± SEM; N=80. *p < 0.01 versus GFP-CT. (B) The survival rate of transgenic flies used in this study. CAC-717 feeding significantly enhanced the survival rate of R406W flies (N=20). *p < 0.01 versus R406W-CT. (C) Immunoblot showing the levels of Sol-Tau, Ins-Tau, and β-actin in R406W fly brain. (D) Representative photomicrographic images of fly eyes and photoreceptor cells. Overexpression of *TauR406W* induced degeneration of photoreceptor cells (white arrows). Feeding of CAC-717 halted the degeneration of photoreceptor cells. GFP, *GMR-Gal4*; *P{ninaE.GFP}1/tub-Gal80ts*; R406W, *GMR-Gal4*; *UAS-tauR406W/tub-Gal80ts*; CT, control; CAC, CAC-717 treatment. (E) Bar graphs showing the survival rate of photoreceptor cells in *TauR406W* flies. Values are means ± SEM. *p < 0.01 versus CT.

study demonstrated that administration of CAC-717 orally can lower levels of both soluble and insoluble Tau proteins in the fly brain (Fig. 4). Therefore, CAC-717 could potentially be a novel therapeutic agent to ameliorate soluble Tau oligomers-induced neuronal dysfunction, or at least, halt the progression of AD pathology in conjunction with anti-A β disease modifying drugs.

Acknowledgements: This work was partially supported by a Grant-in-Aid for Challenging Research (Exploratory) (grant no. 22K19762) the Japan Society for the Promotion of Science.

Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

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