Tannic Acid Is a Natural β-Secretase Inhibitor That Prevents Cognitive Impairment and Mitigates Alzheimer-like Pathology in Transgenic Mice*

Received for publication, August 15, 2011, and in revised form, December 20, 2011. Published, JBC Papers in Press, January 4, 2012, DOI 10.1074/jbc.M111.294025

Takashi Mori,1* Kavon Rezai-Zadeh,2 Naoki Koyama,2 Gary W. Arendash,3,5 Haruyasu Yamaguchi,7 Nobuto Kakuda,9 Yuko Horikoshi-Sakuraba,9 Jun Tan,6 and Terrence Town6,7,8

From the Departments of Biomedical Sciences and Pathology, Saitama Medical Center and University, Kawagoe, Saitama 350-8550, Japan, the Department of Biomedical Sciences and Regenerative Medicine Institute Neural Program and the Department of Neurosurgery, Maxine Dunitz Neurosurgical Institute, Cedars-Sinai Medical Center, Los Angeles, California 90048, the Florida Alzheimer’s Disease Research Center and the Department of Cell Biology, Microbiology, and Molecular Biology, University of South Florida, Tampa, Florida 33620, the Gunma University School of Health Sciences, Maebashi, Gunma 371-8514, Japan, the Immuno-Biological Laboratories Co., Ltd., Fujikura, Gunma 375-0005, Japan, the Rashid Laboratory for Developmental Neurobiology, Silver Child Development Center and the Department of Psychiatry and Neurosciences, College of Medicine, University of South Florida, Tampa, Florida 33613, and the Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, California 90048

Background: Recent focus has been given to anti-amyloidogenic naturally occurring polyphenols known as flavonoids. Results: The polyphenol tannic acid prevented behavioral impairment and mitigated Alzheimer disease-like pathology. Conclusion: Tannic acid may be prophylactic for Alzheimer disease by inhibiting β-secretase activity and mitigating brain pathology. Significance: This nutraceutical approach offers a new class of drug for inhibiting β-secretase activity with few if any side effects.

Amyloid precursor protein (APP) proteolysis is essential for production of amyloid-β (Aβ) peptides that form β-amyloid plaques in brains of Alzheimer disease (AD) patients. Recent focus has been directed toward a group of naturally occurring anti-amyloidogenic polyphenols known as flavonoids. We orally administered the flavonoid tannic acid (TA) to the transgenic PSAPP mouse model of cerebral amyloidosis (bearing mutant human APP and presenilin-1 transgenes) and evaluated cognitive function and AD-like pathology. Consumption of TA for 6 months prevented transgene-associated behavioral impairment including hyperactivity, decreased object recognition, and defective spatial reference memory, but did not alter nontransgenic mouse behavior. Accordingly, brain parenchymal and cerebral vascular β-amyloid deposits and abundance of various Aβ species including oligomers were mitigated in TA-treated PSAPP mice. These effects occurred with decreased cleavage of the β-carboxyl-terminal APP fragment, lowered soluble APP-BP production, reduced β-site APP cleaving enzyme 1 protein stability and activity, and attenuated neuroinflammation. As in vitro validation, we treated well characterized mutant human APP-overexpressing murine neuron-like cells with TA and found significantly reduced Aβ production associated with less amyloidogenic APP proteolysis. Taken together, these results raise the possibility that dietary supplementation with TA may be prophylactic for AD by inhibiting β-secretase activity and neuroinflammation and thereby mitigating AD pathology.

Alzheimer disease (AD) is the most common dementia and is a growing worldwide public health concern (1). AD neuro-pathological hallmarks include extracellular deposits of amyloid-β (Aβ) peptides, intracellular neurofibrillary tangles, neuronal and synaptic degeneration/loss, and neuroinflammation (2). Brain Aβ deposition likely results from increased peptide accumulation/reduced clearance, endorsing toxic events that drive AD pathogenesis (3, 4). Aβ is produced from sequential endoproteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretases (5–9), and enters a dynamic equilibrium between soluble and deposited forms (10). In recent years, much attention has been directed toward soluble multimeric forms of Aβ peptides as the toxic species. These so-called “Aβ oligomers” disrupt synaptic function and induce neurotoxicity in vivo (11–13).

*This work was supported, in whole or in part, by National Institutes of Health Grants 5R00AG029726-04, 3R00AG029726-0451, and 1R01NS076794-01 from the NIA and the NINDS (to T. T.), Grant-in-aid for Scientific Research (C) 22500320 (to T. M.) from the Japan Society for the Promotion of Science and Grant-in-Aid for Scientific Research (B) 19300122 (to H. Y.) from the Ministry of Education, Culture, Sports, Science and Technology, Alzheimer’s Association Zenith Fellows Award ZEN-10-174633 (to T. T.), and American Federation of Aging Research/Ellison Medical Foundation Julie Martin Mid-Career Award in Aging Research M11472 (to T. T.).

1 To whom correspondence may be addressed. Tel.: 81-49-228-3592; E-mail: t_mori@saitama-med.ac.jp.

2 Inaugural holder of the Ben Winters Endowed Chair in Regenerative Medicine. To whom correspondence may be addressed: Regenerative Medicine Institute, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Steven Spielberg Building, Rm. 361, Los Angeles, CA 90048. Tel.: 310-248-8581; E-mail: terrence.town@cshs.org.

3 The abbreviations used are: AD, Alzheimer disease; CTF, carboxyl-terminal fragment; APP, amyloid precursor protein; Aβ, amyloid-β; BACE1, β-site APP cleaving enzyme 1; CAA, cerebral amyloid angiopathy; EGCG, (-)-epigallocatechin-3-gallate; TAN, tannic acid; Iba1, ionized calcium-binding adapter molecule 1; GFAP, glial fibrillary acidic protein; QPCR, quantitative real-time PCR; ANOVA, analysis of variance; CC, cingulate cortex; H, hippocampus; EC, entorhinal cortex; sAPP, soluble APP.

Published in the U.S.A.
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Rooted in the “amyloid cascade hypothesis” of AD, which purports that cerebral Aβ accumulation sets a toxic downstream cascade into motion (2–4), much focus has been directed toward anti-amyloid therapies. Specific approaches include reducing cerebral Aβ production or enhancing Aβ clearance (14–19). Although synthetic drugs have been anti-amyloid agents of choice, these compounds can have significant undesirable side effects, especially when given long-term in a disease prevention paradigm. For example, the ADAPT trial to test nonsteroidal anti-inflammatory drugs for primary AD prevention was prematurely halted due to nonsteroidal anti-inflammatory drug-associated cardiotoxicity (20, 21). Naturally occurring dietary compounds, or “nutraceuticals,” represent an alternative class of molecules that typically have fewer side effects than designer drugs (22).

Others and we have previously reported that nutraceuticals including the green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) (23, 24), the citrus bioflavonoid luteolin (25), grape-derived polyphenols (26, 27), and caffeine (28) have anti-amyloidogenic properties. Based on our findings that EGCG enhances α-secretase APP cleavage and mitigates cerebral amyloidosis in the Tg2576 mouse model of cerebral amyloidosis (23, 24), we sought to investigate a structurally related compound, tannic acid (TA). TA is a plant-derived hydrolyzable tannin polyphenol (29) that is a gallic acid polymer glucoside (C_{76}H_{52}O_{46}; Fig. 1). In addition to structural similarity between TA and EGCG (both contain gallate moieties), both compounds inhibit/destabilize Aβ fibrils in vitro (30–32). To explore whether TA impacted AD-like features, we orally administered the compound for 6 months to the doubly transgenic (APPswe) plus Presenilin 1 exon 9 deleted (PS1ΔE9) B6C3-Tg 85Dbo/J mice on a C57BL/6xC3H background (designated PSAPP mice) were obtained from the Jackson Laboratory (Bar Harbor, ME) and were bred with female C57BL/6 mice to yield mutant PSAPP (APPswe + PS1ΔE9) and wild-type (WT) offspring. PSAPP mice overproduce human Aβ1–40 and Aβ1–42 peptides and develop progressive cerebral β-amyloid deposits and learning and memory impairment (33–36). All mice were characterized by PCR genotyping for mutant human APP and PS1 transgenes as described elsewhere (35). We strictly used PSAPP and WT littermates obtained from this breeding strategy for all analyses. Thus, all mice used in this study are genetically comparable.

TA was obtained from Sigma, resuspended in distilled water, and orally administered to 16 PSAPP mice (PSAPP-TA mice; 8 males and 8 females). As a vehicle control, 16 additional PSAPP mice received distilled water (PSAPP-V mice; 8 males and 8 females). In addition, 32 WT littermates received TA (WT-TA mice; 8 males and 8 females) or distilled water (WT-V mice, 8 males and 8 females). Beginning at 6 months of age, animals were gavaged with TA (30 mg/kg) or vehicle once daily for 6 months. In parallel, to examine if PSAPP mice were cognitively impaired at the initiation of dosing and whether TA treatment prevented delayed kinetics of disease progression, 12 untreated PSAPP mice (PSAPP-6M, 6 males and 6 females) and 12 untreated WT mice (WT-6M, 6 males and 6 females) at 6 months of age were included for analyses of behavior, β-amyloid pathology, and neuroinflammation. Mice were housed in a specific pathogen-free barrier facility under a 12/12-h light-dark cycle, with ad libitum access to food and water. All experiments were performed in accordance with the guidelines of the Animal Use Ethics Committee of the Saitama Medical University and of the NIH.

Behavioral Analyses—Two weeks prior to sacrifice, a battery of behavioral tests was conducted to assess exploratory activity, novel-object recognition and memory retention, and spatial learning and memory in the six groups of mice detailed above. Exploratory activity was evaluated by individually placing mice into a novel environment (the left corner of a white polycarbonate chamber; 54 × 39 × 20 cm). Their activity was recorded for 20 min by an overhead video camera (BL-C131, Panasonic, Fukuoka, Japan) connected to a Windows PC, and horizontal locomotion and rearing scores were counted for each 2-min time bin (37, 38). The next day, novel-object recognition and memory retention were assessed as described (39). Briefly, each mouse was habituated in a cage for 4 h, and then two different shaped objects were concurrently provided to the mouse for 10 min. The number of times that the mouse explored the object (defined as number of instances where a mouse directed its nose 2 cm or less distance from the object) that was later replaced by a novel object was counted for the initial 5 min of exposure (training phase). To test memory retention on the following day, one of the original objects was replaced with a different shaped novel object, and then the number of explorations of the novel object was counted for 5 min (retention test). The recognition index, taken as an index of memory, is reported as frequency (%) of explorations of the novel versus original objects.

Subsequently, Morris water maze testing was performed essentially as previously described (40, 41). The water maze consisted of a circular pool (80 cm diameter) filled with water maintained at 23–26 °C. For the purpose of post hoc analyses, the pool was divided into quadrants (Q1 to Q4), and a 6-cm diameter plexiglass platform was located 1 cm above the water surface in the center of Q2. After a minimum of 20 min habituation to the room, mice naïve to the test were placed in the pool and allowed to search for the platform for 60 s. On the first 2 days (four trials were conducted per day with a 20-min inter-

FIGURE 1. Chemical structure of tannic acid (CAS 1401–55–4, C_{76}H_{52}O_{46}). TA consists of a glucose core, which covalently connects to 3–5 gallic acid (3,4,5-trihydroxy benzoic acid) residues through ester bonds. Each gallate residue can covalently link to other gallic acid molecules. Thus, TA is referred to as a glucoside polymer of gallic acid.
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trial interval), a visible cue was placed on the platform and its location was randomly varied among four possible locations (counterbalanced across mice). The trial ended when a mouse climbed the platform, or in the allocated 60 s, whichever came first. After finding and climbing on the platform, each mouse was allowed to remain there for 20 s, and was then returned to its cage. Animals that did not locate the platform within 60 s were guided to it and allowed to remain there for 20 s before being returned to their cages. On the third day, submerged platform testing was conducted for five consecutive days (learning phase; four trials per day with a 20-min inter-trial interval). The location of the indiscernible platform remained in Q2, 1 cm below the water surface, and mice were placed into the pool in one of seven randomly selected locations (excluding the position immediately adjacent to the platform). One day after the conclusion of the learning phase, memory retention was determined in a single 60-s probe trial. The submerged platform was removed from the water maze, and mice were placed and released opposite the site where the platform had been located and time spent in each quadrant was recorded for the probe trial. All behavioral tests were performed in a room (6 m × 4.5 m) with indirect lighting and multiple visible cues on the walls. The examiner determined the time of swimming until the mouse reached the platform (latency) using a stopwatch. In addition, trials were recorded using an overhead video camera and were analyzed using customized macro software in Microsoft Excel. All trials were performed at the same time of day (±1 h), during the animals’ light phase. So as not to interfere with behavioral testing, TA or vehicle treatment was carried out 1 h after behavioral testing.

Tissue Preparation—Tissue was processed according to our previously described methods (16, 18, 42, 43). At 12 months of age, animals were anesthetized with sodium pentobarbital (50 mg/kg) and euthanized by transcardial perfusion with ice-cold physiological saline containing heparin (10 units/ml). Brains were isolated and quartered (sagittally at the level of the longitudinal fissure of the cerebrum, and then coronally at the level of the anterior commissure) using a mouse brain slicer (Muro-machi Kikai, Tokyo, Japan). Right anterior cerebral quarters were weighed and snap-frozen at −80 °C for α- or β-secretase activity analyses. Right posterior cerebral quarters were further divided into two pieces, and weighed and snap-frozen at −80 °C. One-half was sequentially extracted in Tris-buffered saline (TBS; 25 mM Tris-HCl, pH 7.4, 150 mM NaCl), 2% SDS-, and guanidine-soluble fractions for Aβ sandwich enzyme-linked immunosorbent assays (ELISAs). The other half was used for holo-APP, β-site APP cleaving enzyme 1 (BACE1), and β-carboxyl-terminal fragment (β-CTF: phospho-C99 and C99) Western blots. Left anterior cerebral quarters were weighed and immersed in RNA stabilization solution (RNAlater®, Applied Biosystems, Foster City, CA) and then snap-frozen at −80 °C for proinflammatory cytokine and BACE1 quantitative real-time PCR (QPCR) analyses. Left posterior cerebral quarters were immersion fixed in 4% paraformaldehyde in 0.1 m phosphate buffer at 4 °C overnight, and routinely processed in paraffin for immunohistochemical analyses.

Immunohistochemistry—For paraffin blocks, we sectioned five coronal sections (per set) with a 100 μm interval and a thickness of 5 μm for each brain region (for cingulate cortex (CC), bregma −0.10 to −0.82 mm; for hippocampus (H) and entorhinal cortex (EC), bregma −2.92 to −3.64 mm) (44). We prepared three sets of five sections in each separate region for analyses of Aβ deposits/β-amyloid plaques (for burden, plaque number, and maximum diameter morphometry) as well as ionized calcium-binding adapter molecule 1 (Iba1, to mark reactive microglia) and glial fibrillary acidic protein (GFAP, an astrocytosis marker) burdens. Immunohistochemical staining was conducted according to the manufacturer’s protocol using a Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA) coupled with the diaminobenzidine reaction, except that the biotinylated secondary antibody step was omitted for Aβ immunohistochemical staining. The following primary antibodies were used: a biotinylated human Aβ monoclonal antibody (4G8; 1:200, Covance Research Products, Emeryville, CA), Iba1 polyclonal antibody (1:1,000, Wako, Osaka, Japan), and GFAP polyclonal antibody (1:500, Dako, Carpinteria, CA). Using additional sets of five sections, normal mouse or rabbit serum (isotype control) or phosphate-buffered saline (0.1 m PBS, pH 7.4) was used instead of primary or secondary antibody or ABC reagent as negative controls.

Image Analysis—Quantitative image analysis was done based on previously validated methods (16, 18, 42, 43). Images were acquired as digitized tagged-image format files to retain maximum resolution using a BX60 microscope with an attached CCD camera system (DP-70, Olympus, Tokyo, Japan), and digital images were routed into a Windows PC for quantitative analyses using SimplePCI software (Hamamatsu Photonics, Hamamatsu, Shizuoka, Japan). We captured images of five 5-μm sections through each anatomic region of interest (CC, EC, and H) based on anatomical criteria defined by Franklin and Paxinos (44), and obtained a threshold optical density that discriminated staining from background. Each anatomic region of interest was manually edited to eliminate artifacts. For Aβ, Iba1 (microgliosis), and GFAP (astrocytosis) burden analyses, data are reported as the percentage of labeled area captured (positive pixels) divided by the full area captured (total pixels). Selection bias was controlled for by analyzing each region of interest in its entirety.

For β-amyloid plaque morphometric analyses, diameters (based on maximum length) of β-amyloid plaques were measured, and numbers of β-amyloid plaques falling into three mutually exclusive diameter categories (<25, 25–50, or >50 μm) were tabulated. Results are presented as mean plaque number per mouse in each region examined. For cerebral amyloid angiopathy (CAA) morphometric analysis, we counted numbers of Aβ antibody-stained cerebral vessels in each anatomic region of interest based on our previous methods (43); those data are shown as mean CAA deposit number per mouse.

Cell Culture—The N2a cell line that stably overexpresses human “Swedish”-mutated APP-695 (SweAPP N2a cells) was kindly provided by Dr. Gopal Thinakaran (Department of Neurobiology, University of Chicago). SweAPP N2a cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml of penicillin, 0.1 μg/ml of streptomycin, and 200 μg/ml of G418 sulfate according to previously described methods (16, 23, 25).
SweAPP N2a cells were seeded in 24-well tissue culture plates at $1 \times 10^5$ cells per well. Cultured cells were differentiated into neuron-like cells by 2 h pre-treatment with neurobasal media containing 300 $\mu$M dibutyryl cAMP and then treated with TA (1.563, 3.125, 6.25, 12.5, or 25 $\mu$M) or 0.1 mM PBS (pH 7.4; as vehicle control) for 12 h in the same media prior to analyses.

**Lactate Dehydrogenase Release Assay**—SweAPP N2a cells were seeded in 24-well tissue culture plates at $1 \times 10^5$ cells per well. Culture cells were differentiated into neuron-like cells by 2 h pre-treatment with neurobasal media containing 300 $\mu$M dibutyryl cAMP and then treated with TA (3.125, 6.25, 12.5, or 25 $\mu$M) or 0.1 mM PBS (pH 7.4; vehicle control) for 12 h in the same media. Culture wells were then assayed for cell death by a lactate dehydrogenase release assay (Promega) as described (45).

**Cell-free BACE1 Activity Assay**—To directly test the effect of TA on BACE1 activity, we used available kits based on secretase-specific peptides conjugated to DABCYL/EDANS fluorogenic reporter molecules (Cayman Chemical, Ann Arbor, MI) in accordance with the manufacturer’s instructions. Briefly, BACE1 enzyme was incubated with various concentrations of TA (1.563, 3.125, 6.25, 12.5, or 25 $\mu$M) or BACE1 inhibitor II (1.25 $\mu$M; as a positive control) in the presence of $1 \times$ reaction buffer for 40 min prior to reading fluorescence values on a FLUOstar Omega (BMG LABTECH, San Diego, CA) fluorescent microplate reader.

**ELISA**—We separately quantified Aβ$_{1-40}$ and Aβ$_{1-42}$ in brain homogenates and cultured SweAPP N2a cell supernatants by sandwich ELISAs. Brain Aβ$_{1-40}$ and Aβ$_{1-42}$ species were detected by a three-step extraction protocol according to previously published methods (46, 47). Briefly, we homogenized brains using TissueLyser LT (Qiagen, Valencia, CA; two times for 1 min at 50 Hz) in TBS solution containing protease inhibitor mixture (Sigma), centrifuged homogenates at 18,800 $\times$ g for 60 min at 4 °C, and removed the supernatants (TBS-soluble fraction). Resulting pellets were treated with 2% SDS in H$_2$O with the same protease inhibitors and homogenized using TissueLyser LT (one time for 1 min at 50 Hz). We then centrifuged the homogenates at 18,800 $\times$ g for 60 min at 4 °C and collected supernatants (2% SDS-soluble fraction). Finally, the remaining pellets were treated with 5 $\mu$g guanidine HCl and dissolved by occasional mixing on ice for 30 min, then centrifuged at 18,800 $\times$ g for 60 min at 4 °C, and supernatants were collected representing the guanidine HCl-soluble fraction.

Aβ$_{1-40}$ and Aβ$_{1-42}$ species were separately quantified in individual samples in duplicate using ELISA kits (catalogue number 27718 for Aβ$_{1-40}$ and number 27712 for Aβ$_{1-42}$; IBL, Fujioka, Gunma, Japan) in accordance with the manufacturer’s instructions (48). We also quantified Aβ oligomers in the 2% SDS-soluble fraction in duplicate individual samples by Aβ oligomer ELISA (catalogue number 27725; IBL) according to the manufacturer’s instructions (49). All samples fell within the linear range of the standard curve. Aβ$_{1-40}$ and Aβ$_{1-42}$ ELISA values are reported as picograms of Aβ$_{1-x}$/wet mg of brain, and the Aβ oligomer concentration is reported as picomolar.

**Western Blot**—Cultured SweAPP N2a cells were treated with various doses of TA (1.563, 3.125, 6.25, 12.5, or 25 $\mu$M) or 0.1 mM PBS (pH 7.4; as a vehicle control) for 12 h. Cultured cells were then lysed in ice-cold lysis buffer (containing 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na$_2$EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na$_3$VO$_4$, 1 μg/ml of leupeptin, and 1 mM PMSF). Mouse brain homogenates were lysed in TBS solution containing protease inhibitor mixture (Sigma) followed by TNE buffer (10 mM Tris-HCl, 1% NP-40, 1 mM EDTA, and 150 mM NaCl), and aliquots corresponding to 10 μg of total protein were electrophoretically separated using 10% or 15% Tris glycine gels based on target protein molecular weights. Electrophoresed proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad) that were subsequently blocked in blocking buffer (1% (w/v) nonfat dry milk in TBS containing 0.1% (v/v) Tween 20) for 1 h at ambient temperature. After blocking, membranes were hybridized for 1 h at ambient temperature with primary antibodies: amino-terminal APP polyclonal antibody (1:400, IBL), carboxyl-terminal soluble APP-α (sAPP-α) monoclonal antibody (2B3; 1:100, IBL) directed against amino acids DAERFRDGYEVHHQK, carboxyl-terminal soluble APP-β (sAPP-β) monoclonal antibody that recognizes Swedish mutant (ISEVNL) protein (6A1; 1:100, IBL), carboxyl-terminal BACE1 polyclonal antibody (1:400, IBL), amino-terminal Aβ monoclonal antibody (82E1; 1:150, IBL), carboxyl-terminal APP polyclonal antibody (1:1,000, Merck Millipore, Billerica, MA), or actin polyclonal antibody as a loading control (1:500, Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were then rinsed three times for 30 min each in TBS containing 0.1% (v/v) Tween 20 and incubated for 1 h at ambient temperature with appropriate horseradish peroxidase-conjugated secondary antibodies. After additional rinsing as above, membranes were incubated for 5 min at ambient temperature with enhanced chemiluminescence substrate (SuperSignal West Dura Extended Duration Substrate, Thermo Fisher Scientific, Waltham, MA), exposed to film, and developed.

**Secretase Activity Assays**—For α- and β-secretase activity analyses in brain homogenates, we used available kits based on secretase-specific peptides conjugated to fluorogenic reporter molecules (DABCYL/EDANS; R & D Systems, Minneapolis, MN) according to our published methods (23, 43). Briefly, brains were lysed in ice-cold 1 × cell extraction buffer for 10 min and centrifuged at 18,800 $\times$ g for 1 min. Supernatants were collected and kept on ice. Appropriate amounts of brain homogenate, reaction buffer, and fluorogenic substrate were added in duplicate to a 96-well plate and incubated in the dark at $37$ °C for various periods of time. Following incubation, fluorescence was monitored (335 nm excitation and 495 nm emission) at $25$ °C using a SH-9000 microplate fluorimeter with SF6 software (CORONA ELECTRIC, Hitachinaka, Ibaraki, Japan). Background was determined from negative controls (omission of brain homogenate or fluorogenic substrate).

**QPCR**—We quantified tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), BACE1, and β-actin mRNA levels by QPCR. Total RNA was extracted using the RNeasy Mini Kit (Qiagen), and first strand cDNA synthesis was carried out using the QuantiTect Reverse Transcription Kit (Qiagen) in accordance with the manufacturer’s instructions. We diluted cDNA 1:1 in H$_2$O and carried out QPCR for all genes of interest using...
cDNA-specific TaqMan primer/probe sets (TaqMan Gene Expression Assays, Applied Biosystems) on an ABI 7500 Fast Real-time PCR instrument (Applied Biosystems). Each 20-μl reaction mixture contained 2 μl of cDNA with 1 μl of TaqMan Gene Expression Assay reagent, 10 μl of TaqMan Fast Universal PCR Master Mix (Applied Biosystems), and 7 μl of H2O.

Thermocycler conditions consisted of: 95 °C for 15 s, followed by 40 cycles of 95 °C for 1 s and 60 °C for 20 s. TaqMan probe/primer sets were as follows: mouse TNF-α (catalogue number Mm00443258_m1), mouse IL-1β (number Mm00434228_m1), mouse BACE1 (number Mm00478664_m1), and mouse β-actin (number Mm00607939_s1; used as an internal reference control) (Applied Biosystems). Samples that were not subjected to reverse transcription were run in parallel as negative controls to rule out genomic DNA contamination (data not shown). A “no template control” was also included for each primer set (data not shown).

The cycle threshold number (CT) method (50) was used to determine relative amounts of initial target cDNA in each sample. Results for BACE1 expression are expressed relative to vehicle-treated WT mice, whereas TNF-α and IL-1β expression values are normalized to WT-6M littermates.

Statistical Analysis—All experiments were performed by an examiner blinded to sample identities, and code was not broken until the analyses were completed. Data are presented as the mean ± 1 S.E. A hierarchical analysis strategy was used for time-dependent behavioral data in which the first step was a repeated-measures analysis of variance (ANOVA) to assess the significance of the main effects and interactive terms. If significant, post hoc testing was done with Tukey’s HSD or Dunnett’s T3 methods, and appropriate p values are reported based on adjustment according to Levene’s test for equality of the variance. For all other data, in instances of single mean comparisons, Levene’s test followed by t test for independent samples was performed. In instances of multiple mean comparisons, one-way ANOVA was used, followed by post hoc comparison of the means using Bonferroni’s or Dunnett’s T3 methods (where appropriateness was determined using Levene’s test). A p value of less than 0.05 was considered to be significant. All analyses were performed using the Statistical Package for the Social Sciences, release IBM SPSS 19.0 (IBM, Armonk, NY).

RESULTS

Oral Tannic Acid Treatment Mitigates Hyperactivity and Cognitive Impairment in PSAPP Mice—We began by orally administering TA or vehicle to PSAPP or WT mice starting at 6 months of age for a period of 6 months and subsequently conducted a behavioral testing battery. In addition, to examine cognitive status when dosing started, untreated PSAPP and WT mice at 6 months of age were included for analyses of behavior. When placed into a novel environment, PSAPP-V mice were hyperactive as measured by higher locomotion and rearing scores compared with the other 5 groups of mice (Fig. 2A). This behavioral phenotype has been observed in mouse models of cerebral amyloidosis (e.g. Tg2576 or PSAPP) (18, 34, 51), and may be associated with disinhibition resulting from cortical and/or hippocampal injury (38). Overall ANOVA showed main effects of time (p < 0.001), genotype (p < 0.001), and treatment (p < 0.001), and post hoc comparisons showed statistically significant differences between PSAPP-V mice and the other 5 mouse groups at each time point for locomotion scores (Fig. 2A, *, p < 0.05 for PSAPP-V versus PSAPP-TA, WT-V, WT-TA, PSAPP-6M, or WT-6M mice) and for rearing scores (Fig. 2A, *, p < 0.05 for PSAPP-V versus PSAPP-TA, WT-V, WT-TA, PSAPP-6M, or WT-6M mice). Hyperactivity was fully prevented in PSAPP-TA mice, as they did not statistically differ from WT-V WT-TA, PSAPP-6M, or WT-6M mice (p > 0.05).

We then tested learning and memory in the same cohort of mice by a novel object recognition assay. If mice remember an initial encounter with a novel object, they tend to preferentially explore the new versus familiar object, typically operationalized as “recognition index” (39). Although all groups performed similarly during the training phase of the test, in the retention phase, one-way ANOVA followed by post hoc comparison showed statistically significant differences on recognition index between PSAPP-V mice and the other 5 mouse groups as indicated (Fig. 2B, *, p < 0.05 for PSAPP-V versus PSAPP-TA, WT-V, WT-TA, PSAPP-6M, or WT-6M mice). Importantly, PSAPP-TA mice had significantly increased novel object exploration frequency versus PSAPP-V animals (Fig. 2B), but did not significantly differ from WT-V, WT-TA, PSAPP-6M, or WT-6M groups (p > 0.05), showing that TA also prevented novel object recognition impairment associated with PSAPP transgene expression.

We further tested animals in the Morris water maze, a widely accepted assay of spatial reference learning and memory in rodents (40, 41). For the learning phase of the test, overall ANOVA showed main effects of time (p < 0.001) and genotype (p < 0.001), and post hoc comparison revealed statistically significant differences between PSAPP-V mice and the other 5 mouse groups as indicated (Fig. 2C, *, p < 0.05 for PSAPP-V versus PSAPP-TA, WT-V, WT-TA, PSAPP-6M, or WT-6M mice). PSAPP-V mice had greater latency to reach the platform location after training than the other 5 mouse groups, whereas PSAPP-TA mice showed significant improvement, indicating that oral TA treatment inhibited PSAPP transgene-associated impaired spatial reference learning. For the probe trial (day 6 of testing), the invisible platform was removed from the pool and platform location memory was evaluated. When considering Q2 (goal quadrant) data, one-way ANOVA and post hoc testing showed statistically significant differences between PSAPP-V mice and the other 5 mouse groups as indicated (Fig. 2D, *, p < 0.05 for PSAPP-V versus PSAPP-TA, WT-V, WT-TA, PSAPP-6M, or WT-6M mice). PSAPP-TA mice swam in the goal quadrant significant longer than PSAPP-V mice, and their behavior did not significantly differ from WT-V, WT-TA, PSAPP-6M, or WT-6M mice, showing that TA treatment prevents PSAPP transgene-associated spatial memory impairment.

It is unlikely that behavioral differences in the Morris water maze were due to motivational issues or to locomotor impairment, as there were no significant between group differences (p > 0.05) on swim speed during either the learning or probe trial phases of the test. Moreover, it is important to note that the degree of thigmotaxis could indicate levels of anxiety and impact interpretation of Morris water maze results. In this regard, we did not observe evidence of thigmotaxis, operationalized as prolonged movement of the mice along the pool cir-
cumference, in any mice examined during either the learning or probe trial phases of the test. Furthermore, untreated PSAPP mice at 6 months of age (PSAPP-6M) did not clearly show cognitive impairment as compared with WT mice at the same age or to WT-V or WT-TA mice at 12 months of age by open field, object recognition, or either the learning or probe trial phases of Morris water maze testing. Importantly, behavioral testing performance in PSAPP-TA mice was not significantly different from PSAPP-6M animals in each of the tests conducted. This result can be interpreted as prevention of cognitive impairment by a 6-month treatment regimen of TA. Finally, for all of the behavioral tests conducted, we used multiple ANOVA models with gender as a categorical covariate, but did not detect significant gender main effects or interactive terms (p > 0.05). We also stratified by gender and found a similar pattern of results as above in both males and females (data not shown).

**Tannic Acid Treatment Ameliorates Aβ Pathology in an Accelerated Mouse Model of Cerebral Amyloidosis**—We next evaluated Aβ/β-amyloid pathology by three strategies: Aβ antibody immunoreactivity (conventional β-amyloid “burden” analysis), β-amyloid morphometric analysis, and separate Aβ_{1-40} and Aβ_{1-42} sandwich ELISAs. PSAPP-V mice showed typical β-amyloid deposition (33, 36), which was significantly reduced by 51–58% in CC, EC, and H regions of PSAPP-TA mouse brains (Fig. 3, A and B, ***, p < 0.001). Of note, PSAPP-TA plaques were not completely attenuated versus PSAPP-6M mice (Fig. 3, A and B), indicating that TA mitigated as opposed to completely prevented cerebral amyloid deposition. TA reduction in β-amyloid deposits was independent of gender, being evident in both male and female PSAPP-TA versus PSAPP-V mice (data not shown). To assess whether reduced β-amyloid burden was specific to a particular plaque size subset or occurred more generally, we performed morphometric analysis of β-amyloid plaques in PSAPP-V and PSAPP-TA mice. According to previously described methods (18, 16, 42, 43), plaques were assigned to one of three mutually exclusive categories according to maximum diameter: small (< 25 μm), medium (between 25 and 50 μm), or large (>50 μm). Data showed that all three subsets of plaques were significantly reduced in PSAPP-TA versus PSAPP-V mice across all three brain regions examined (Fig. 3, A and C, ***, p < 0.001, % reduc-
tion for: small, 39–44%; medium, 44–52%; large, 54–66% plaque subsets). We stratified by gender and observed the same pattern of results in both males and females (data not shown).

Untreated PSAPP-6M mice had quantitatively minor (0.5–0.7%) cerebral $\beta$-amyloid burden (Fig. 3, A and B), and the majority of these deposits were seed-like dots $\leq 25 \mu m$ in size, with only a few plaques between 25 and 50 $\mu m$. Because of this, we were unable to perform morphometric analysis of $\beta$-amyloid deposits for the PSAPP-6M group.

In addition to brain parenchymal $\beta$-amyloid as senile plaques, 83% of AD patients present with $\beta$-amyloid deposits in cerebral vessels, known as CAA (52). PSAPP mice also develop vascular $\beta$-amyloid deposits with age (36), and we found almost no evidence for CAA in young PSAPP-6M mice (data not shown). Having shown that TA treatment led to reduced brain parenchymal $\beta$-amyloid plaques in PSAPP mice, we wondered if cerebral vascular $\beta$-amyloid deposits might also be altered. In both PSAPP mouse groups, cerebral vascular $\beta$-amyloid deposits were predominantly detected within penetrating arteries at the pial surface in CC and EC regions and within small arteries at the hippocampal fissure. We scored $\beta$-amyloid antibody (4G8)-stained cerebral vascular deposits in PSAPP-V and PSAPP-TA mice and found reductions in PSAPP-TA mouse brains that reached statistical significance in all three brain regions examined (Fig. 4, A and B, **, $p < 0.01$).

In agreement with histological observations, biochemical analysis of $\beta$ species in brain homogenates revealed significant reductions in TBS-soluble $\beta_{1-40}$ and in both $\beta_{1-40}$ and $\beta_{1-42}$ abundance in the detergent-soluble fraction from PSAPP-TA versus PSAPP-V mice (Fig. 4C, 25–33% depending on the particular $\beta$ species; *, $p < 0.05$). Similarly, extraction of the insoluble pellet in guanidine HCl revealed significant reductions in PSAPP-TA mice for both $\beta_{1-40}$ and $\beta_{1-42}$ levels (27–28%) by biochemical analysis (Fig. 4C, ***, $p < 0.001$).

**FIGURE 3.** Cerebral parenchymal $\beta$-amyloid deposits are mitigated in tannic acid-treated PSAPP mice. Data were obtained from PSAPP mice treated with vehicle (PSAPP-V, $n = 16$) or TA (PSAPP-TA, $n = 16$) for 6 months commencing at 6 months of age (mouse age = 12 months at sacrifice). To examine if the TA treatment delayed versus prevented disease progression, untreated PSAPP mice (PSAPP-6M, $n = 12$) at 6 months of age were also included in these analyses. A, representative photomicrographs of 4G8 immunohistochemistry are shown for cerebral $\beta$-amyloid plaques in PSAPP-V, PSAPP-TA, and PSAPP-6M mice. Brain regions shown include: cingulate cortex (CC, top), hippocampus (H, middle), and entorhinal cortex (EC, bottom). Scale bar denotes 50 $\mu m$. B, quantitative image analysis for $\beta$ burden is shown, and each brain region is indicated on the x axis. C, morphometric analysis of cerebral parenchymal $\beta$-amyloid plaques is shown in PSAPP-V and PSAPP-TA mice. Brain sections were stained with 4G8 antibody, and plaques were counted based on maximum diameter and assigned to one of three mutually exclusive categories: small ($\leq 25 \mu m$; top), medium (between 25 and 50 $\mu m$; middle), or large ($> 50 \mu m$; bottom). Mean plaque subset number per mouse is shown on the y axis, and each brain region is represented on the x axis. The statistical comparison for B is within the brain region and versus PSAPP-TA mice, and for C, between PSAPP-V and PSAPP-TA mice.
By contrast, Aβ1–42 levels in the TBS-soluble fraction were similar between PSAPP-TA and PSAPP-V mice. We also analyzed PSAPP-6M mice by Aβ biochemistry, but found low abundance of Aβ species that was below the threshold of ELISA detection in certain fractions (data not shown). Collectively, these data show that cerebral amyloidosis, including brain parenchymal and cerebral vascular β-amyloid deposits and Aβ peptide abundance, is delayed but not completely prevented by oral treatment with TA in PSAPP mice.

**Inhibition of Cerebral Amyloidogenic APP Metabolism in Tannic Acid-treated PSAPP Mice**—Mitigated cerebral amyloidosis in PSAPP-TA mice could be due to 1) increased brain-to-blood Aβ efflux (10), 2) reduced expression of APP or PS1 transgenes, or 3) attenuated amyloidogenic APP metabolism. We obtained peripheral blood samples from PSAPP-V and PSAPP-TA mice at the time of sacrifice and assayed plasma Aβ1–40 and Aβ1–42 species, but did not detect differences between groups (data not shown). To examine if TA-attenuated cerebral amyloidosis could be due to decreased expression of transgene-derived APP or PS1, we probed brain homogenates from PSAPP-V and PSAPP-TA mice using amino-terminal APP polyclonal or carboxyl-terminal PS1 monoclonal antibodies and found no change in APP or PS1 holoprotein levels (Fig. 5A and data not shown).

To determine whether APP metabolites including sAPP-α and sAPP-β were affected by TA treatment, brain homogenates were probed with monoclonal antibody 2B3 that detects the carboxyl terminus of human sAPP-α and with monoclonal antibody 6A1 that recognizes the carboxyl terminus of human Swedish mutant sAPP-β. Western blot and densitometry data showed significantly reduced expression of sAPP-β in PSAPP-TA mouse brains, whereas sAPP-α abundance did not differ between groups (Fig. 5, A–C, ***, p < 0.001). To further determine whether amyloidogenic APP processing was inhibited by TA treatment, we probed brain homogenates with a carboxyl-terminal BACE1 polyclonal antibody. Densitometry showed that BACE1 protein abundance was significantly decreased in PSAPP-TA mice (Fig. 5, A and D, *, p < 0.05). To assess whether this effect was due to TA attenuation of BACE1 transcription, relative expression levels of BACE1 mRNA were assayed in each mouse group by QPCR. However, there were no detectable differences between groups (Fig. 5E), suggesting a post-transcriptional mode of TA action on BACE1.
To further address steady-state APP metabolism, we probed brain homogenates with monoclonal antibody 82E1 that recognizes amino acids 1–16 of human Aβ/H9252, and detects the amyloidogenic/Aβ-CTF (C-99) and phospho-Aβ-CTF (P-C99) and monomeric plus oligomeric Aβ species. Densitometry confirmed that APP metabolism to phospho-C99 and C99 was significantly deceased in PSAPP-TA mice (Fig. 6, A and B, **, p < 0.01). These effects were accompanied by reduced abundance of the Aβ species between 25 and 75 kDa (presumed Aβ oligomers) and monomeric Aβ in PSAPP-TA mice (Fig. 6 C). To confirm that Aβ oligomers were attenuated by TA treatment, we quantified them in the detergent-soluble fraction by sandwich ELISA and found significant reduction in PSAPP-TA versus PSAPP-V mice (Fig. 6 D, *, p < 0.05). Collectively, these data indicate that TA works at the protein level to inhibit BACE1 expression. Consistently, β-secretase activity was significantly decreased, whereas α-secretase activity was unaltered in brain homogenates from PSAPP-TA versus PSAPP-V mice (Fig. 6 D, ***, p < 0.001 at each time point). These data support the notion that TA affords prophylaxis against cerebral amyloidosis by inhibiting amyloidogenic APP metabolism as a consequence of decreasing BACE1 expression at the protein (but not mRNA) level and reducing β-secretase activity.

**Tannic Acid Reduces Aβ Production and Inhibits Amyloidogenic APP Metabolism in Neuron-like Cells**—Mitigated cerebral amyloidosis and reduced amyloidogenic APP metabolism in PSAPP-TA mice could be due to a direct, neuron cell autonomous affect or to an indirect mode of TA action. To determine whether TA could directly modulate APP metabolism in neuron-like cells, we cultured SweAPP N2a cells, which stably express human APP bearing the Swedish mutation, and treated them with a dose-range of TA. As shown in Fig. 7 A, TA dose dependently inhibited both Aβ1–40 and Aβ1–42 release into the media by separate sandwich ELISAs. Although the optimal effect occurred at 12.5 μM, significant reduction for both Aβ peptides was evident even at the lowest dose (3.125 μM) of TA used (*, p < 0.05; **, p < 0.01 for each dose versus PBS control). We moved on to investigate whether these effects were because of reduced amyloidogenic APP metabolism, and performed Western blots with a carboxyl-terminal APP polyclonal antibody that simultaneously detects both amyloidogenic C99 and nonamyloidogenic C83 (α-carboxyl-terminal fragment, α-CTF) (Fig. 7 B). Results qualitatively showed less abundance of C99 with increasing doses of TA. Quantitative densitometry followed by one-way ANOVA revealed a significant main effect of dose (p < 0.01), and post hoc testing revealed significant
differences when comparing control (PBS-treated) SweAPP N2a cells to cells that were treated with 12.5 to 25 μM TA (Fig. 7C, *, p < 0.05). To determine whether BACE1 protein levels were reduced by TA treatment, we probed brain homogenates with a carboxyl-terminal BACE1 polyclonal antibody. Results showed TA dose-dependent inhibition of BACE1 protein (Fig. 7D).

To determine whether TA directly or indirectly inhibited BACE1 activity, we developed a BACE1 activity assay consisting of combining a dose range of TA in a cell-free system with BACE1 enzyme and fluorogenic reporters. The result was positive, as one-way ANOVA revealed a significant main effect of TA dose (p < 0.001) and post hoc testing revealed significant differences when comparing BACE1 enzyme alone (positive control) to samples that contained from 1.563 to 25 μM TA. Of note, significant reduction for BACE1 activity was evident even at the lowest dose (1.563 μM) of TA used (Fig. 7E, **, p < 0.01).

BACE1 inhibitor II treatment (used as a BACE1 inhibitor control; IC50 = 0.97 μM) at 1.25 μM revealed an inhibitory effect for BACE1 (497.3 ± 8.8 relative fluorescence units) as compared with 100% BACE1 enzyme activity (646.3 ± 25.4 relative fluorescence units). Interestingly, this effect was quantitatively more minor than even the lowest dose of TA used in this system.

Finally, to determine whether escalating doses of TA were toxic to SweAPP N2a cells, we performed a lactate dehydrogenase release assay, but did not observe evidence of TA toxicity (Fig. 7F). When taken together, these data show that TA is capable of exerting a direct effect on reducing Aβ secretion and amyloidogenic APP metabolism in neuron-like cells.

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**FIGURE 6. Anti-amyloidogenic APP processing in PSAPP mice treated with tannic acid.** A, Western blots using an amino-terminal Aβ monoclonal antibody (mAb 82E1) are shown. This antibody detects various fragments generated by amyloidogenic APP cleavage, including Aβ oligomers and monomer as well as phospho-C99 (P-C99) and non-phospho-C99 (C99). Actin is shown as a loading control. B, densitometry analysis is shown for ratios of C-99 or P-C99 to actin. C, the levels of Aβ oligomers in the 2% SDS-soluble brain homogenate fraction were measured by sandwich ELISA. D, α-secretase and β-secretase activity assays are shown. Relative fluorescence units are shown on the y axis, and reaction time is represented on the x axis. Data for A–D were obtained from PSAPP mice treated with vehicle (PSAPP-V, n = 16) or with TA (PSAPP-TA, n = 16) for 6 months beginning at 6 months of age. The statistical comparisons for B–D are between PSAPP-V and PSAPP-TA mice.
(conventional microgliosis and astrogliosis burden analyses) and quantified brain expression of the proinflammatory cytokines TNF-α and IL-1β. PSAPP-V mice demonstrated elevated β-amyloid plaque-associated reactive gliosis (microgliosis and astrogliosis), as evidenced by increased expression of Iba1 and GFAP in glial somata and processes. Yet, microgliosis and astrocytosis were significantly reduced in PSAPP-TA mice compared with PSAPP-V animals (Figs. 8, A and B, and 9, A and B, ***, p < 0.001). These effects were gender-independent, as a similar pattern of statistically significant results was observed in both male and female PSAPP-TA mice (data not shown). When considering brain mRNA abundance of TNF-α and IL-1β, a similar pattern of statistically significant results was found (Fig. 9C, **, p < 0.01). Neuroinflammation in PSAPP-TA mice was not reduced to levels of untreated PSAPP-6M mice, indicating mitigation but not complete prevention of this pathology (Figs. 8, A and B, and 9, A–C).

Phagocytic microglia have been detected in the aged APP23 cerebral amyloidosis mouse model in small numbers (58) and in increased abundance in aged Tg2576 mice bearing a CD11c promoter-driven dominant-negative transforming growth factor-β receptor type II transgene (18) as well as in the ischemic core after cerebral ischemia in the rat brain (59). However, reactive microglia in both groups of mice reported here had ramified thin and long cell processes and did not appear to be phagocytic based on morphological criteria (amoeboid structure, puffy cytoplasm, few or no processes resembling brain-infiltrating macrophages) (60).

**DISCUSSION**

Nutraceuticals are naturally occurring compounds that, because of their existence throughout evolution, tend to have fewer side effects than designer pharmaceuticals. The purpose of the present study was to evaluate the effects of TA, a plant-derived flavonoid nutraceutical, on behavioral impairment, AD-like pathology, and APP metabolism in vivo and in vitro. Collectively, our results demonstrate that oral TA prevents behavioral impairment, mitigates cerebral amyloidosis, and promotes nonamyloidogenic APP processing by inhibiting BACE1 expression and β-secretase activity without altering BACE1 mRNA abundance in an accelerated mouse model of
cerebral amyloid deposition. In addition, TA dose-dependently inhibited Aβ1–40 and Aβ1–42 production and β-CTF cleavage in human SweAPP-expressing neuron-like cells in vitro. Finally, TA attenuated neuroinflammation in PSAPP mice, including β-amyloid plaque-associated gliosis and expression of the pro-inflammatory cytokines TNF-α and IL-1β.

TA is a member of the tannin category of plant-derived polyphenols, and is found in numerous herbaceous and woody plants. Tannins are chemically divided into four groups based on the structure of the monomer: hydrolysable tannins, condensed tannins (proanthocyanidins), phlorotannins, and complex tannins. Among these, hydrolyzable tannins (including TA) are derivatives of gallic acid (3,4,5-trihydroxyl benzoic acid), characterized by a variable number of gallate moieties esterified to a core phenol (29). Similar to other polyphenols, tannins have antioxidant/free radical scavenging, antiviral/bacterial, anticarcinogenic, antimutagenic, and anti-inflammatory properties (29, 61–63). Given these pleiotropic biological activities, TA has yielded promising clinical results against cancer (61), myocardial infarction (64), and renal failure (65). Given these pleiotropic biological activities, TA has yielded promising clinical results against cancer (61), myocardial infarction (64), and renal failure (65).

In this study, we orally administered TA to mice at 30 mg/kg/day via gavage, as this treatment strategy more precisely delivers the targeted amount of agent compared with ad libitum access in drinking water or chow. TA is well tolerated in rodents, with a lethal dose resulting in 50% mortality as high as 2,260 mg/kg in the rat (66), and the dose that we administered to mice is orders of magnitude lower. Similarly, the human tolerable daily intake of TA is 13.6 g/60 kg (67). Unwanted side effects are always a concern in the clinic, and it is important to note that we did not observe evidence for adverse events associated with TA treatment in mice, including abnormal behavior, altered body weight or food intake, or mortality. Likewise, we did not detect pathological features in major organs such as the brain, lung, heart, liver, digestive tract, and kidney upon postmortem examination of TA-treated PSAPP or WT mice. These findings reinforce the notion that the dose of TA used in our study is safe; of course, our results are limited to mice. Because BACE1 likely has important physiologic functions, and TA may have long-term toxic effects due to inhibition of BACE1, possible side effects of TA would need to be properly investigated in humans.

It is important to consider how TA exerts its biological effects. The compound is a high molecular weight molecule that does not easily penetrate the cell or freely cross the blood-
brain barrier. Nonetheless, TA is metabolized into much lower molecular weight, absorbable tannins that are biologically active in various organs including the brain (68). For example, hydrolysable tannins are degraded to gallic acid, pyrogallol, phloroglucinol, and finally to acetate and butyrate via sequential enzymatic action (29). In particular, oral administration of gallic acid led to intestinal absorption over a period of 60 min in rodents (69), which equated to 76 min in humans (70). Flavanoids including gallate have also been shown to cross into the brain in rodents (68, 71, 72). Thus, the gallate moiety is a likely candidate for mediating the bioactivity of TA in our system; yet, future study is warranted to characterize which chemical structure in TA plays a pivotal role on mitigating Aβ pathology.

Our in vivo and in vitro results show that TA shifts APP metabolism toward the nonamyloidogenic pathway. Specifically, TA lowered BACE1 expression (without any change in BACE1 mRNA expression) and β-secretase activity (but had no effect on α-secretase activity), leading to reduced abundance of the amyloidogenic C99 and phospho-C99 APP CTFs, attenuated sAPP-β abundance, and lower levels of Aβ peptides. Interestingly, TA exerts its effects on BACE1 both at the post-translational level and also by directly attenuating enzymatic activity, as demonstrated in a cell-free BACE1 activity assay. Although Aβ is generally regarded as the pathogenic species in both mouse models of cerebral amyloidosis and in human AD, it remains possible that TA reduction of any combination of amyloidogenic APP metabolites may be responsible for bringing about reduced behavioral impairment in PSAPP mice.

It is generally recognized that APP is a limiting reagent in the cell, and it has been postulated that α- and β-secretases compete for APP proteolysis (73). Reduced β-secretase activity could therefore be responsible for mitigated cerebral Aβ pathology in TA-treated PSAPP mice and attenuated Aβ secretion by SweAPP N2a cells. In this regard, we previously demonstrated that another polyphenol, EGCG (a bioactive flavonoid ingredient in green tea), was also able to promote nonamyloidogenic APP metabolism in the Tg2576 cerebral amyloidosis mouse model and in SweAPP N2a cells (23). Interestingly, the mechanism for the beneficial effects of EGCG on nonamyloidogenic APP processing relied on promoting activity

**FIGURE 9. Tannic acid-treated PSAPP mice have reduced astrocytosis.** Data for A and B were obtained from PSAPP mice treated with vehicle (PSAPP-V, n = 16) or with TA (PSAPP-TA, n = 16) for 6 months beginning at 6 months of age. In addition, 6-month-old untreated PSAPP mice (PSAPP-6M, n = 12) and wild-type (WT-6M, n = 12) mice were included for C. A, representative photomicrographs of GFAP immunohistochemistry, taken from each brain region indicated on the left (CC, top; H, middle; EC, bottom), for β-amyloid plaque-associated astrocytosis are shown for PSAPP-V and PSAPP-TA mice at 12 months of age and for untreated PSAPP-6M mice. Scale bar denotes 50 μm. B, quantitative image analysis for GFAP burden is shown for each brain region indicated on the x axis. C, expression of brain proinflammatory TNF-α and IL-1β cytokine mRNAs is attenuated by TA treatment in PSAPP mice. Data are expressed as relative fold over WT-6M mice, and all statistical comparisons are versus PSAPP-TA mice.
of the candidate α-secretase, a disintegrin and metalloprotease 10 (23, 24). Given their complementary modes of action then, a combined TA/EGCG approach may make sense, at least in principle, as an Aβ lowering strategy.

It is generally accepted that newly produced Aβ exists in dynamic equilibrium between soluble and deposited forms in the brain, with continual transport of soluble Aβ out of the brain and into the circulation (10). In this regard, it has been reported that TA inhibits Aβ fibrillogenesis, and destabilizes preformed Aβ fibrils in vitro (30). We tested the impact of TA on various pools of Aβ peptides in vivo, and observed that TA treatment nonselectively reduced Aβ in TBS-, SDS-, and guanidine HCl-soluble brain homogenate fractions from PSAPP mice. One interesting exception was TBS-soluble Aβ1-42, which remained unaltered by oral TA treatment. Aβ1-42 is typically thought to have a higher propensity to form oligomers and higher molecular weight aggregates than Aβ1-40 (11, 74), and we went on to probe whether TA impacted Aβ oligomers. Data showed a reduced abundance of both monomeric and oligomeric Aβ by Western blot and reduced Aβ oligomers by sandwich ELISA in brain homogenates from TA-treated PSAPP mice. These results may be because of a combination of reduced APP metabolism to Aβ and reduced microgliosis, astrocytosis, and expression of the hallmark (53, 54), in PSAPP mice. Interestingly, oral TA treatment reduced neuroinflammation, an important AD pathoetiologic feature (76, 77), and we went on to determine whether the compound impacted neuroinflammation. The present nutraceutical approach offers a new class of drug for inhibiting β-secretase activity with few if any side effects in PSAPP mice or in humans, and provides proof-of-concept that BACE1 is indeed still a “drug-able target.”

In conclusion, our data demonstrate that the plant-derived polyphenol, TA, opposes behavioral impairment and AD-like pathology in PSAPP mice. These beneficial effects occur with reduction in: cerebral Aβ pathology, cleavage of β-CTF, sAPP-β, BACE1 protein expression and activity, and neuroinflammation. If Aβ pathology in these transgenic models is representative of the clinical syndrome, then our data raise the possibility that dietary supplementation with TA may represent a potentially safe and effective AD prophylaxis.

Acknowledgments—We thank Dr. Joshua J. Breunig for helpful discussion of the manuscript, and Dr. Gopal Thinakaran for generously gifting the SweAPP N2a cells.

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