Cotinine Reduces Amyloid-β Aggregation and Improves Memory in Alzheimer’s Disease Mice

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Abstract. Alzheimer’s disease (AD) affects millions of people worldwide and new effective and safe therapies are needed. Cotinine, the main metabolite of nicotine, has a long half-life and does not have cardiovascular or addictive side effects in humans. We studied the effect of cotinine on amyloid-β (Aβ) aggregation as well as addressed its impact on working and reference memories. Cotinine reduced Aβ deposition, improved working and reference memories, and inhibited Aβ oligomerization in the brains of transgenic (Tg) 6799 AD mice. In vitro studies confirmed the inhibitory effect of cotinine on Aβ1-42 aggregation. Cotinine stimulated Akt signaling, including the inhibition of glycogen synthase kinase 3β (GSK3β), which promotes neuronal survival and the synaptic plasticity processes underlying learning and memory in the hippocampus and cortex of wild type and Tg mice. Simulation of the cotinine–Aβ1-42 complex using molecular dynamics showed that cotinine may interact with key histidine residues of Aβ1-42, altering its structure and inhibiting its aggregation. The good safety profile in humans and its beneficial effects suggest that cotinine may be an excellent therapeutic candidate for the treatment of AD.

Keywords: Alzheimer’s disease, amyloid-β, cotinine, neurodegeneration, oligomerization

INTRODUCTION

Alzheimer’s disease (AD) is the main cause of dementia in the elderly [1]. This devastating disease is characterized by memory loss, synaptic dysfunction, and neuropathological changes, such as the presence of plaques of aggregated amyloid-β (Aβ) peptide, amyloid angiopathy, and neurofibrillary tangles of phosphorylated tau protein in the brain [2, 3]. At least some of the loss of cognitive abilities has been attributed to deterioration of the cholinergic system induced by the toxic forms of Aβ [1, 4, 5]. The currently available treatments for AD include the use of...
acetylcholinesterase inhibitors (i.e., donepezil, galan-tamine, or tacrine) [6–9] and the N-methyl-D-aspartate (NMDA) antagonist, memantine. Unfortunately, these drugs only marginally ameliorate the cognitive deficits and have mostly short-term positive effects [6, 10–12]. Epidemiological studies have shown a negative correlation between tobacco consumption and the development of AD [13]. It has also been found post-mortem that the levels of soluble and insoluble Aβ peptides were significantly decreased in the brains of smoking AD patients compared to non-smokers with the disease [14]. The putative beneficial effect of tobacco has been mainly attributed to nicotine, which has been reported to improve cognitive abilities and reduce plaques in a mouse model of AD [15]. Since nicotinic receptors play an important role in attention, learning, and memory, the positive effects of nicotine on memory were mostly credited to the activation of these receptors. Unfortunately, due to its toxicity, addictive properties, and side effects, nicotine has not been considered an attractive therapeutic agent against AD [16–18].

In mammals, more than 80% of nicotine is metabolized into cotinine, a metabolite with a longer half-life (nicotine, 2–3 h; cotinine, 19–24 h) and much lower toxicity [19]. For these reasons, we speculate that the cotinine–Aβ interaction by phosphorylation [30, 31]. Finally, to understand the effect of cotinine on Aβ plaque deposition and cognitive impairment, we investigated the effects of cotinine on Aβ aggregation in vitro and model the interaction of cotinine with Aβ1–42. We found that cotinine reduced cerebral Aβ deposition and ameliorated cognitive impairment in Tg mice. These beneficial actions may result from its capacity to reduce Aβ aggregation. Computational modeling of the cotinine–Aβ1–42 complex suggests that cotinine’s inhibition of Aβ aggregation may be related to its ability to bind to amino acid residues that participate in the aggregation of the peptide. The implications of these findings for developing a new therapy for AD are discussed.

MATERIALS AND METHODS

Drugs

Cotinine was purchased from Sigma-Aldrich Corporation (St. Louis, MO).

Mice

We used the Tg6799 mice, which express the human AβPP and PS1 genes containing five FAD mutations [25], including three FAD mutations in AβPP (Swedish mutation: K670N, M671L; Florida mutation: I716 V; London mutation: V717I); and PS1 (M146L, L286 V) (The Jackson Laboratories, Bar Harbor, ME) [26]. The Tg6799 lines were maintained as hemizygous on a B6SJL hybrid background. Male mice were used as heterozygotes with respect to the transgene and non-Tg (NT) wild type littermate mice served as controls. All behavioral analyses were performed between 5.5 and 6.5 months of age by investigators blind to the genotype and treatment of mice. Mice were maintained on a 12-h dark and 12-h light cycle with ad libitum access to food and water. All protocols were previously approved by the Institutional Animal Care and Use Committees of the University of South Florida and Bay Pines Veterans Affairs Healthcare System.

Cotinine treatment

At 2 months of age, Tg mice were started on daily treatment with cotinine (2.5 mg/kg) dissolved in PBS or vehicle alone orally via gavage. Treatment was administered for 3.5 months, as well as for the ensuing one-month period of behavioral testing and subsequently for two weeks until euthanasia (total of 5 months of treatment).
Behavioral testing

A one-month battery of sensorimotor, anxiety, and cognitive-based tasks was performed beginning at 3.5 months into treatment (5.5 months of age) [32, 33]. In the order of their performance, the tasks administered were: sensorimotor (open field, balance beam, string agility, and Y-maze), anxiety (elevated plus maze), and cognition-based [Morris water maze (MWM), circular platform, platform recognition, radial arm water maze (RAWM)] [32], and cognitive interference [33]. Since there were no transgene or treatment effects on any of the sensorimotor or anxiety tasks, and there were no cognitive effects of treatment observed in NT or Tg mice in RAWM or platform recognition, we only describe the three cognitive tasks that did exhibit significant effects of treatment (circular platform, RAWM, and cognitive interference) as detailed below.

Circular platform (spatial reference learning/memory)

A walled 69 cm circular platform, with 16 equidistantly spaced holes along its periphery, was encircled by a black curtain [34]. Visual cues, located on the black curtain and platform walls can be used by the animal to find the one hole through which it can escape the platform surface to avoid the aversive stimuli of bright lights and fan wind. During a single 5 min maximum daily trial, the total number of errors (head poke into non-escape holes) and latency to find the escape hole were recorded. Although the escape hole remained constant for any given animal over the 8 days of testing, it was relocated after each animal’s trial to control for olfactory cues. To minimize interference from olfactory cues, the maze was cleaned with a dilute vinegar solution following each animal’s trial. Performance for four 2-day blocks of testing was analyzed statistically with Analysis of Variance (ANOVA), followed by post hoc comparisons done with the Fisher’s least significant difference test.

Radial arm water maze (working memory)

For the RAWM task of spatial working memory, an aluminum insert was placed into a 100 cm circular pool to create 6 radially distributed swim arms emanating from a central circular swim area [32]. An assortment of 2-D and 3-D visual cues surrounded the pool. The latency and number of errors prior to locating which one of the 6 swim arms contained a submerged escape platform (9 cm diameter) was determined for 5 trials/day over 8 days of testing, with statistical analysis involving performance over all 8 days of RAWM performance. There was a 30-min time delay between the 4th trial (T4; final acquisition trial) and 5th trial (T5; memory retention trial). The platform location was changed daily to a different arm, with different start arms for each of the 5 trials (randomly selected from the remaining 5 swim arms). During each trial (60-s maximum), the mouse was returned to that trial’s start arm upon swimming into an incorrect arm and the number of seconds required to locate the submerged platform was recorded. If the mouse did not find the platform within a 60-s trial, it was guided to the platform for the 30-s step. The latency and number of errors during T4 and T5 are both considered indices of working memory and are temporally similar to the standard registration/recall testing of specific items used clinically in evaluating AD patients.

Cognitive interference task (proactive/retroactive interference)

We designed this task based on a cognitive task used to discriminate normal aged, mild cognitive impairment, and AD patients from one another [35]. Our interference task for mice involves two RAWM set-ups in two different rooms, each with different sets of visual cues [33]. The task requires animals to remember a set of visual cues, so that following interference with a different set of cues, the initial set of cues can be recalled to successfully solve the RAWM task. A set of four behavioral measures was examined. Behavioral measures were: A3 (the last of three recall trials performed in RAWM “A”), “B” (proactive interference measure attained from a single trial in RAWM “B”), A4 (retroactive interference measure attained during a single trial in RAWM “A”), and “A5” (delayed-recall measure attained from a single trial in RAWM “A” following a 20-min delay between A4 and A5). As a distraction between trials, animals are placed in a Y-maze and allowed to explore for 60 s between successive trials of the three-trial recall task, as well as during the proactive interference task. As with the standard RAWM task, this interference task involves the platform location being changed daily to a different arm for both of the RAWM set-ups utilized, and different start arms for each day of testing for both RAWM set-ups. For A1 and B trials, the animal was initially allowed one min to find the platform on their own before they were guided to the platform. The actual trial was then performed in each case. As with the standard RAWM task, animals were given 60 s to find the escape platform for each trial, with the number
of errors and escape latency recorded for each trial. Given the very close correspondence between error and latency scores in individual animals for both the RAWM and cognitive interference tasks, only latency scores are presented in this report. Animals were tested for cognitive interference performance on two successive days, with statistical analysis performed on the resultant 2-day block.

Brain tissue preparation

Following behavioral testing of seven-month-old mice, animals were euthanized and perfused with a cold physiological saline. The left frontal half of each brain was placed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) overnight, wherein tissues remained until the paraffin embedding process for Aβ immunohistochemical analyses. The remaining left portion of the brain, as well as the entire right side of the brain, was dissected out into regions (e.g., hippocampus and cortex), quickly frozen, and stored at −80°C for later neurochemical analyses.

Western blot analysis of tissue extracts

Tissues were analyzed by Western blot as previously described [33]. Briefly, brains were rapidly removed and tissues dissected and disrupted by sonication in radiomimunoprecipitation assay (RIPA) buffer (Tris 50 mM pH 7.4, NaCl 150 mM; SDS 0.1%; NaOAc-Cholate 0.5%; Triton X-100 1%; Cell Signaling Technology, Danvers, MA) with a complete protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN). Brain extracts were incubated on ice for 30 min and centrifuged at 20,000 × g for 30 min at 4°C. Equal amounts of protein from the supernatant were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using either 10–20% Tris-Tricine gel (Bio-Rad Laboratories, Inc., Hercules, CA) for Aβ/PP or 4–20% Tris-Glycine gel (Thermo Fisher Scientific Inc., Rockford, IL) for Akt and β-tubulin analyses and transferred to nitrocellulose membranes. The membranes were blocked in TBS with 0.05% Tween 20 (TBST; Bio-Rad Laboratories, Inc.) containing 10% dry skim milk and incubated in TBST with primary antibodies overnight at 4°C and secondary antibodies for 1h at RT. Rabbit monoclonal antibodies directed against Akt phosphorylated at serine 473 (pAkt [Ser473]) (1 : 500) and total Akt (1 : 1,000) were obtained from Cell Signaling Technology. A rabbit polyclonal antibody directed against phospho-GSK3β (Serine 9) and total GSK3β were obtained from Cell Signaling Technology. A mouse monoclonal antibody directed against Aβ (6E10) (1 : 5,000; Covance, Emeryville, CA) that also recognizes AβPP and several forms of the peptide was used to detect Aβ oligomers. A monoclonal mouse antibody against β-tubulin (1 : 10,000; Promega, Madison, WI) was used to control protein sample loading and transfer efficiency. The monoclonal antibody against total AβPP (clone 22C11) (1 : 2,000; Millipore, Temecula, CA) was used to confirm the immunoreactivity of total AβPP. Membranes were washed with TBST and incubated with LI-COR's goat anti-mouse IRDye secondary antibodies (LI-COR Biosciences, Lincoln, NE) for 1 h, washed with TBST and TBS, and images were acquired using an Odyssey Infrared Imaging System (LI-COR Biosciences) or the Kodak Image Station 440CF (Eastman Kodak Company, Rochester, NY) using a Molecular Imaging Software version 4.0 (Eastman Kodak Company), and analyzed using an NIH Image J software.

Analysis of cotinine levels in the brain

The levels of cotinine in brain tissues of mice were estimated using ELISA. Briefly, after treatments and behavioral testing, mice were euthanized, and brains were removed, dissected, and disrupted by sonication in RIPA buffer (Cell Signaling Technology) containing a complete protease inhibitor cocktail (Roche Molecular Biochemicals). Cortical extracts were incubated on ice for 30 min and centrifuged at 20,000 × g for 30 min at 4°C. The resultant supernatants were used to determine cotinine levels according to the manufacturer’s instructions using a commercial ELISA kit (Calbiochem, San Diego, CA). In this solid phase competitive ELISA, the cotinine present in the samples competes with a cotinine-enzyme conjugate for the binding to a plate coated with anti-cotinine antibody. Upon the addition of the substrate for the conjugated enzyme (horseradish peroxidase), the concentration of the cotinine in the samples is inversely proportional to the intensity of the color developed in the wells.

Analysis of Aβ levels

The levels of Aβ40 and Aβ42 were quantified in brain tissues by ELISA. To determine soluble Aβ levels, brain tissues were homogenized in RIPA buffer, centrifuged at 20,000 × g for 20 min at 4°C and the supernatants were stored at −80°C until use. To mea-
sure insoluble Aβ levels, brain tissues were prepared
by sonication of the samples in a solution of 5 M guani-
dine HCl (Sigma-Aldrich Corporation) (pH 8.0). After
sonication, samples were incubated for 3 h at RT and
centrifuged at 20,000 × g for 20 min at 4 °C. The super-
natants were stored at −80 °C until use or immediately
diluted using PBS with 5% bovine serum albumin
(Sigma-Aldrich Corporation) and 0.03% Tween 20
supplemented with 1× protease inhibitor cocktail
(Roche). Then samples were analyzed for Aβ levels
using an ELISA kit (Invitrogen Corporation, Carlsbad,
CA), according to the manufacturer’s recommenda-
tions.

Aβ plaque analysis

For brain Aβ immunohistochemical staining and
analysis, we used our well-established protocol as
previously described [32]. Briefly, the frontal cortex
from the left side of the brain was dissected. At the
level of cingulate and motor cortex, three 15-μm sec-
tions (150 μm apart) were made from each mouse
brain using a sliding microtome. Immunohistochem-
ical staining was performed using a Vectastain ABC
Elite kit (Vector Laboratories, Burlingame, CA) cou-
pled with the diaminobenzidine reaction, except that
the biotinylated secondary antibody step was omitted
for Aβ immunohistochemical staining. A biotinylated
human Aβ monoclonal antibody (clone 4G8; 1 : 200,
Covance Research Products, Emeryville, CA) was
incubated for 1 h at RT. PBS (pH 7.4) or normal rab-
bbit serum was used instead of primary antibody or
ABC reagent as a negative control. Brain sections
were treated with 70% formic acid prior to the pre-
blocking step. Quantitative image analysis utilized
previous methods with modifications [36,37]. Images
were acquired using an Olympus BX51 microscope
with an attached digital camera system (DP70, Olym-
pus, Tokyo, Japan), and the digital image was routed
into a Windows PC for quantitative analysis using a
Molecular Imaging Software version 4.0 (Eastman Kodak
Company), and scanned on the Kodak Image Station 440CF (East-
man Kodak Company) using a Molecular Imaging
System (LI-COR Biosciences) and analyzed with
an NIH Image J software.

Western blot analysis of Aβ oligomers

The Western blot analysis was performed as des-
cribed previously [38]. Briefly, hippocampal tissues
were disaggregated with a pellet in RIPA buffer and
centrifuged at 20,000 × g for 30 min. Aliquots of hippocampal extract super-
natants (2 μg protein) were applied onto nitrocellulose
membranes and allowed to dry, after which the mem-
branes were blocked for 1 h at RT with LI-COR
blocking buffer, washed, and incubated with A11 anti-
body overnight at 4°C. After washing, membranes were
incubated with LI-COR’s IRDye secondary antibod-
ies for 1 h and then washed. The immunoreactive dots
were then visualized using an Odyssey Infrared Imag-
ing System (LI-COR Biosciences) and analyzed with
an NIH Image J software.

Dot blot analysis of Aβ oligomers in brain tissues
of Tg6799 mice

To investigate the effect of cotinine on the for-
mation of soluble Aβ oligomers, we performed dot
blot analysis using the A11 antibody (1 : 1,000; Invit-
rogen Corporation), which is highly specific for the
 oligomeric forms of Aβ and does not recognize the
monomeric or fibrillar forms of the peptide. Briefly,
hippocampal tissues were disaggregated using a pellet
in RIPA buffer and centrifuged at 20,000 × g for 30 min. Aliquots of hippocampal extract super-
natants (2 μg protein) were also applied onto nitrocellulose
membranes and allowed to dry, after which the mem-
branes were blocked for 1 h at RT with LI-COR
blocking buffer, washed, and incubated with A11 anti-
body overnight at 4°C. After washing, membranes were
incubated with LI-COR’s IRDye secondary antibod-
ies for 1 h and then washed. The immunoreactive dots
were then visualized using an Odyssey Infrared Imag-
ing System (LI-COR Biosciences) and analyzed with
an NIH Image J software.
The first step of this protocol consists in dissolving 
\( \text{A} \beta_{1-42} \) in (1,1,1,3,3,3-hexafluoroisopropanol) (HFIP; 
Sigma-Aldrich Corporation) to obtain a starting solu-
tion containing only \( \text{A} \beta_{1-42} \) monomers [39]. Briefly, 
lyophilized \( \text{A} \beta_{1-42} \) (American Peptide) was dissolved 
in HFIP; evaporated, re-dissolved in dimethyl sulfox-
die (DMSO) (Sigma-Aldrich Corporation), and then 
diluted in PBS (pH 7.4) alone or plus cotinine. Peptide 
solutions containing \( \text{A} \beta_{1-42} \) (1 mM) with and without 
cotinine (2 mM) were incubated for 10 days at RT and 
the formation of oligomers and fibers was examined 
by AFM.

AFM analysis

20-μl aliquots of \( \text{A} \beta_{1-42} \) solutions were deposited on 
freshly cleaned and dried silicon wafers (approxi-
mately 1-mm thick). After waiting for 10 min, non-
adsorbed portions of the samples were washed with 
de-ionized water (2 ml). The wet surface of the silici-
on wafer was then dried using a gentle flow of air.
The AFM analysis was performed using an AFM 
apparatus (AFM, J.A multimode SPM, Model no. 
920-006-101, Veeco, Plainview, NY) that permits the 
aquisition of images using a tapping mode approach. 
This approach allows intermittent contact of the tip 
with the sample and minimizes the chances of deforma-
tion of the peptide samples. The cantilever and the tip 
were made of silicon and the cantilever force constant 
was approximately 20–100 N/m with the resonance 
frequency between 200 and 400 kHz. The scan rate 
was 1.0 Hz. The analysis of fibrils and oligomers was 
performed using the Nanoscope Control software (ver-
sion 5.30) (Veeco). The analysis of the height of the 
aggregates was performed using Pico Image software 
(PicoView version 1.6.4) from Agilent Technologies 
(Mississauga, ON, Canada).

Molecular modeling

The chemical structure of cotinine is similar to that 
of nicotine, and also possesses two different enan-
tomers (i.e., S and R forms). As the exact binding 
site of cotinine on \( \text{A} \beta_{1-42} \) is not known, the binding 
site of nicotine on \( \text{A} \beta_{1-42} \) was used to develop starting 
models for the molecular dynamics (MD) simula-
tions. Because S-cotinine was used in the experiments 
reported in this study, we utilized this form of coti-
nine in the simulations on the cotinine–\( \text{A} \beta_{1-42} \) complex. 
The molecular modeling of the interaction between 
cotinine and the \( \text{A} \beta_{1-42} \) monomer was performed in 
the following two steps.

Molecular docking

Cotinine was docked near the His13 and His14 
residues of the full-length \( \text{A} \beta_{1-42} \) peptide using the 
AutoDock program (version 4.0). The Scripps Research 
Institute, La Jolla) [40]. The most representative struc-
ture obtained from the previous 50-ns MD simulations 
on full-length \( \text{A} \beta_{1-42} \) in aqueous solution was used in the docking procedure [41]. The reported bind-
ing sites of nicotine on \( \text{A} \beta_{1-42} \) were utilized in this 
process [42–44]. The AutoDock program performed 
the rapid energy evaluation through a pre-calculated 
grid and found the suitable binding position of coti-
nine on \( \text{A} \beta_{1-42} \). Polar hydrogens were added using the 
hydrogen module in the AutoDock tools for the pep-
tide and the Kollman united atom partial charges were 
assigned. The grid was calculated using the Auto Grid 
protocol. It was chosen to include all the His residues 
(His6, His13, and His14) of \( \text{A} \beta_{1-42} \). The dimension 
of the grid was set to 50 x 50 x 50 Å with a spac-
ing of 0.375 Å between the two consecutive grids. In 
the docking process, \( \text{A} \beta_{1-42} \) was kept rigid and coti-
nine was allowed to form all the possible torsional 
bonds. The AutoDock Lamarckian genetic algorithm 
using the standard protocol with 150 randomly placed 
individual initial populations was applied. In total, 50 
independent docking runs were performed. The low-
est energy conformer taken from the docked complex 
was utilized to perform 50-ns MD simulations on the 
cotinine–\( \text{A} \beta_{1-42} \) complex in aqueous solution.

Molecular dynamics simulations

All MD simulations were performed using a GRO-
MACS software package (General Public License), 
utilizing the GROMACS force field [45]. Dundee Pro 
Drug Server was used for generating the topology of 
the cotinine molecule for the MD simulation and the 
partial charges were also calculated using this server 
[46]. The cotinine–\( \text{A} \beta_{1-42} \) complex was placed in the 
center of a box with dimensions 4.9 x 4.2 x 4.6 nm. 
The box contained over 2,852 single point charge water 
molecules. Some water molecules were replaced by 
sodium and chloride ions to neutralize the system and 
to simulate an experimentally used ion concentration 
of 150 mM. The starting structure was subsequently 
energy minimized with a steepest descent method for 
2,000 steps. The results of these minimizations pro-
duced the initial structure for the MD simulations. The 
MD simulations were then carried out with a constant 
number of particles, pressure, and temperature. The 
SETTLE algorithm was used to constrain the bond 
length and angle of the water molecules [47], while the 
LINCS algorithm was used to constrain the bond
length of the peptide [48]. The long range electrostatic interactions were calculated by the particle-mesh Ewald method [49, 50]. A constant pressure of 1 bar was applied with a coupling constant of 1.0 ps; peptide, water molecules, and ions were coupled separately to a bath at 300 K with a coupling constant of 0.1 ps. The periodic boundary conditions were applied and the equation of motion was integrated at time-steps of 2 fs. The secondary structure analyses were performed by employing the defined secondary structures of proteins protocol [51]. The contact maps and similarity factor of the most representative structures obtained from a cluster analysis have also been employed as structural descriptors. A contact for a pair of amino acid side chains is considered to form when a minimal distance between any pair of their atoms is less than 0.5 nm. In the cluster analysis, the trajectories were analyzed by grouping structurally similar frames [root-mean-square-deviation (RMSD) cutoff = 0.30 nm [52]], and the frame with the largest number of neighbors is denoted as a “middle” structure, which represents that particular cluster.

RESULTS

Cotinine improves cognitive performance in Tg6799 mice

In a one-month battery of cognitive tasks administered between 5.5 and 6.5 months of age, there were no transgene or cotinine treatment effects on sensorimotor or anxiety function. There were also no effects of cotinine on several basic cognitive tasks (MWM and Platform recognition). However, significant effects of cotinine were detected in three other tasks (Fig. 1A–C). In the circular platform task of spatial reference memory, Tg controls were severely impaired in performance, as indicated by their much higher number of errors and latencies during the final
block of testing (Fig. 1A). In sharp contrast, Tg mice that had been chronically treated with cotinine exhibited error and latency scores that were significantly lower and no different from NT controls. Cotinine treatment did not affect performance of NT mice in this any task of the entire test battery.

In the RAWM task of working memory, a modest beneficial effect of cotinine treatment was present in Tg mice for working memory trial T4 (Fig. 1B). Across all 8 days of testing, Tg6799 controls were significantly impaired (versus NT groups) during trial T4, while cotinine-treated Tg mice were significantly better than Tg6799 controls. Nonetheless, cotinine-treated Tg mice were not at the performance level of NT controls on T4. For delayed working memory trial T5, both Tg groups were impaired in performance.

As a more challenging variant of the RAWM task, the cognitive interference task evaluates not only working memory, but proactive and retroactive interference as well. Over both days of cognitive interference testing, Tg mice were impaired in all four measures evaluated in comparison to the excellent performance of NT controls. By contrast, Tg mice chronically treated with cotinine were no different from NT controls for both the final recall trial (A3) and the proactive interference trial (B) (Fig. 1C). As well, the performance of cotinine-treated Tg mice on these trials tended to be significantly different from Tg controls at $p = 0.08$ and 0.13, respectively. For the remaining two measures of cognitive interference (i.e., proactive interference and delayed recall), cotinine treatment did not provide any cognitive benefit, with performance of treated Tg6799 mice being impaired and no different from control Tg mice.

Thus, long-term cotinine treatment to Tg mice provided complete protection from spatial reference memory impairment in the circular platform task, while also improving the performance of Tg mice to the level of NT controls in two working memory and cognitive interference tasks.

**Effect of cotinine on Akt phosphorylation**

We investigated the effect of cotinine on the activation of Akt by phosphorylation in the hippocampus and cortex of Tg and NT mice. The results show that cotinine stimulates Akt in both tissues. For vehicle-treated Tg mice, the levels of the active form of Akt (pAkt [Ser473]) in the hippocampus and cortex were similar to the values found in the vehicle-treated NT mice, considered as 100% immunoreactivity (Fig. 2A and B). By contrast, the treatment of Tg mice with cotinine (2.5 mg/kg) significantly increased the levels of pAkt [Ser473] in the hippocampus and cortex by +51% ($p = 0.023$) and +189% ($p = 0.019$, respectively, as normalized to total Akt levels (Fig. 2A and B). For NT mice, cotinine also induced a significant increase in the levels of pAkt [Ser473] in the hippocampus (+74%, $p = 0.023$) and cortex (+295%, $p = 0.033$) (Fig. 2C and D). When normalized against tubulin, no significant changes in the levels of total Akt were observed in the cotinine-treated Tg mice when compared to untreated Tg mice (Fig. 2A and B). However, we observed a highly significant increase in total levels of Akt as normalized against tubulin in the Tg mice when compared to NT mice in the hippocampus (+49%, $p = 0.0043$) (Fig. 2A). This increase was not observed in the frontal cortex of the same mice (Fig. 2B). We did not find changes in total Akt in cotinine-treated NT mice when compared to untreated NT mice in hippocampus and cortex (Fig. 2C and D).

**Effect of cotinine on the inhibition of GSK3β by phosphorylation**

Over-activation of GSK3β has been associated with tau hyperphosphorylation and neuronal cell death in AD brains [53]. It is well-known that Akt inactivates GSK3β by phosphorylation at Ser 9. Thus, based on our previous finding that cotinine activated Akt, we investigated the effect of cotinine on GSK3β phosphorylation in the brains of Tg6799 mice. First, we found no significant changes in the levels of pGSK3β [Ser9] normalized to total GSK3β in both the hippocampus and cortex of control Tg mice compared to control NT mice (Fig. 3A and B). However, cotinine treatment resulted in a significant increase in the levels of pGSK3β [Ser9] in the hippocampus of Tg mice (+42%, $p = 0.035$) with respect to vehicle-treated Tg mice (Fig. 3A). Also, a significant increase in the levels of pGSK3β [Ser9] was observed in the hippocampus of cotinine-treated NT mice compared to NT controls (+56%, $p = 0.048$) (Fig. 3C). Similarly, in the cotinine-treated Tg mice we observed a trend of increase in the levels of pGSK3β [Ser9] in the frontal cortex when compared to vehicle-treated Tg mice, but the differences did not reach significance (+54%, $p = 0.059$) (Fig. 3B). However, cotinine treatment significantly increased the levels of pGSK3β [Ser9] in the cortex of NT mice compared to untreated otherwise identical mice (+295%, $p = 0.0056$) (Fig. 3D). No significant differences in the levels of total GSK3β were found in the hippocampus or cortex among groups of NT mice (data not shown).
Fig. 2. Cotinine increased the active form of Akt in the brains of Tg6799 and non-transgenic (NT) control littermate mice. Tg and NT control littermate mice were treated with saline or cotinine (2.5 mg/kg) for 5 months and RIPA-soluble hippocampal and cortical protein extracts were analyzed by Western blot using antibodies against β-tubulin as well as total and phosphorylated Akt. The plots represent the immunoreactivity values expressed as percentage of control of saline-treated NT mice. Levels of normalized phospho-Akt [Ser473] immunoreactivity against total Akt levels in hippocampal (A, C) and cortical extracts (B, D) are shown. There was a clear activation of the Akt in the hippocampus and cortex of both Tg (hippocampus, n=7–8; cortex, n=7–9) and NT (hippocampus, n=8–9; cortex, n=4–6) mice. Dividing lines separate immunoreactive bands from different parts of the same membrane. The data are expressed as the mean ± SEM. Student’s t-test was used to compare the mean of the values between groups. ns, non-significant change; *p<0.05; **p<0.01. pAkt, phospho-Akt [Ser473].

Effect of cotinine on Aβ levels in the brains of Tg6799 mice

To investigate the effect of cotinine on Aβ levels, we analyzed the levels of Aβ in the RIPA-soluble and insoluble fractions of cortex and hippocampus tissues of Tg mice treated with cotinine or vehicle for 5 months. It has been shown that in AD Tg mice, the Swedish mutation increases the synthesis of total Aβ, whereas AβPP London, Florida, and PS1 mutations promote the synthesis of Aβ42 [25]. In coherence with this previous evidence, we found that the levels of Aβ42 were substantially higher than the levels of Aβ40 in both the hippocampus and cortex of 7-month-old Tg6799 mice (Table 1). The insoluble Aβ42 levels were significantly decreased in the cortex (but not the hippocampus) of cotinine-treated Tg mice relative to control Tg mice (−26%, p=0.031). However, for both brain areas, no differences were observed for soluble levels of Aβ42 and Aβ40 in the cotinine-treated Tg mice when compared with untreated Tg mice. Although no differences in the levels of insoluble Aβ40 were observed in the cortex, they showed a significant increase (+31%, p=0.019) in the hip-
Fig. 3. Cotinine inhibits GSK3β by phosphorylation at serine 9 in the brains of Tg6799 and non-transgenic (NT) control littermate mice. Mice were treated with saline or cotinine (2.5 mg/kg) for 5 months and RIPA-soluble and RIPA-insoluble hippocampal and cortical extracts were analyzed by Western blot using antibodies against β-tubulin as well as anti-GSK3β and phosphorylated GSK3β. The plots represent the levels of immunoreactivity for phospho-GSK3β (Ser9) expressed as percentage of the saline-treated NT mice and normalized to total GSK3β levels. Levels of phospho-GSK3β (Ser9) in the hippocampus (A, C) and cortex (B, D) of Tg and NT mice are shown. Cotinine increased the levels of phospho-GSK3β (Ser9) in the hippocampus of both Tg (n = 8–10) and NT mice (n = 8–10) (A, C), as well as in the cortex of NT mice (n = 4–6) (D). After the cotinine treatment, the levels of phospho-GSK3β (Ser9) also showed a trend of increase (p = 0.059) in the cortex of Tg mice (n = 8–9) (B). Dividing lines separate immunoreactive bands from different parts of the same membrane. The data are expressed as the mean ± SEM. Student’s t-test was used to compare the mean of the values between groups. *p < 0.05; **p < 0.01.

Table 1

<table>
<thead>
<tr>
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<th>Soluble (pg/mg)</th>
<th>Insoluble (ng/mg)</th>
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</thead>
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<tr>
<td></td>
<td>Aβ(42)</td>
<td>Aβ(40)</td>
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<tr>
<td><strong>Cortex</strong></td>
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<td></td>
</tr>
<tr>
<td>Vehicle control</td>
<td>249 ± 54</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Cotinine</td>
<td>222 ± 46</td>
<td>7 ± 1</td>
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<tr>
<td><strong>Hippocampus</strong></td>
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<tr>
<td>Vehicle control</td>
<td>5829 ± 988</td>
<td>35 ± 8</td>
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<tr>
<td>Cotinine</td>
<td>6942 ± 718</td>
<td>37 ± 5</td>
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</tbody>
</table>

The data are expressed as the mean ± SEM from 8–10 mice for the insoluble levels and 5–10 for soluble levels of the Aβ peptides. Student’s t-test was used to compare the mean of the values between groups. *p < 0.05; **p < 0.01.
pocampus of cotinine-treated Tg mice relative to Tg controls. The insoluble ratio of Aβ_{42/40} was decreased by cotinine treatment in the cortex (–25%, p = 0.006) and hippocampus (–28%, p = 0.030). No significant changes were observed in the soluble ratio of Aβ_{42/40} in these same brain regions.

**Effect of cotinine on amyloid burden in the brains of Tg6799 mice**

As expected from previous reports [54, 55], cotinine crossed the blood-brain barrier as evidenced using ELISA analysis of cotinine levels in the brain tissues. On average, the levels of cotinine in the RIPA-soluble fraction of the cortex of the NT and Tg mice fluctuated between 500 and 700 ng/mg protein.

To investigate whether the cotinine treatment reduces amyloid plaque deposition, Tg mice were evaluated for forebrain Aβ burden at 7 months of age (following completion of behavioral testing). Tg6799 mice present Aβ deposition detectable as early as 2 months of age [25]. As shown in Fig. 4, control Tg mice had robust amyloid burdens of around 3% in both cingulate and motor cortices. Compared to these Tg controls, Tg mice that had been given 5 months of cotinine treatment exhibited a significant decrease in amyloid burden within both cingulate cortex (–26%, p = 0.034) and motor cortex (–17%, p = 0.048) (Fig. 4A and B, respectively).

**Effect of cotinine on the levels of Aβ oligomers in the hippocampus and cortex of Tg6799 mice**

Growing evidence supports the view that Aβ oligomers are the main neurotoxic form of the peptide [56, 57]. Thus, we investigated whether cotinine affected the levels of Aβ oligomers in the brains of the Tg mice, using the highly specific anti-oligomeric Aβ antibody A11, which does not recognize Aβ monomers or AβPP. The results indicate that cotinine induced an 18–20% reduction in the immunoreactivity for A11 in the hippocampus (Fig. 5A and B) and cortex (data not shown) of Tg mice. No significant differences in 6E10 immunoreactivity were found between cotinine-treated Tg mice and Tg controls in both the hippocampus and cortex (data not shown).

Furthermore, Western blot analyses of the RIPA-soluble fractions from Tg mice untreated or treated with cotinine (2.5 mg/kg) showed a reduction in the...
levels of soluble Aβ oligomers (Fig. 5C and D). We found a significant reduction in the levels of high molecular weight oligomers (i.e., ~20-mers) (~53%, \( p = 0.018 \)) (Fig. 5C and D). No changes in the levels of low molecular weight oligomers (<50 kDa) (Fig. 5C and D) or the levels of AβPP induced by cotinine were observed in the hippocampus of Tg mice as determined with the antibodies directed against Aβ (6E10) and total AβPP (22C11), respectively (Fig. 5C and D).

**Effect of cotinine on Aβ aggregation**

To investigate whether cotinine affects fibril formation, we used AFM to analyze Aβ aggregation in the presence and absence of cotinine. The solutions were prepared by dissolving Aβ in HFIP and after evaporation, in PBS alone or containing cotinine, then incubated at 37°C for 10 days.

Figure 6 shows representative AFM images (900 × 900 nm field) of aggregated forms of Aβ formed in either the absence (Fig. 6A and B) or presence (Fig. 6C) of cotinine. The analysis of Aβ fibril formation at day 0 showed no presence of aggregated Aβ in the initial solutions (Fig. 6A). After 10 days of incubation at 37°C, the height of the Aβ aggregates used for the AFM studies fluctuated approximately between 1 and 15 nm (data not shown), indicating that the solutions contained a mixture of oligomers, protofibrils and fibrils. The pre-incubation of the peptide in the presence of cotinine significantly reduced the length of Aβ fibrils. The average length of Aβ fibrils incubated in the presence of cotinine was significantly lower (239 ± 34 nm) than the average fibril length in the absence of cotinine (486 ± 157 nm, Student’s \( t \)-test, \( p = 0.026 \)) (Fig. 6D).

**Molecular modeling of cotinine–Aβ1-42 interaction**

A previous analysis of the interaction of Aβ with nicotine by NMR preceded and facilitated the molecular modeling of cotinine [42, 43]. These NMR studies suggested that nicotine binds to the segment of Aβ between amino acids 1–28 when folded in an α-helical conformation. According to the proposed mechanism, nicotine inhibits the conformational change from α-helix to the amyloidogenic β-sheet conformation [42]. Here, we used molecular modeling to analyze the interaction of cotinine with Aβ1-42 and define a putative mechanism that could explain the effect of
cotinine on Aβ aggregation. The RMSD of the MD simulation confirmed that the complex is thermodynamically equilibrated only after 30 ns (Fig. 7A). The most representative structure derived from the simulation indicates that cotinine interacts with His6, Tyr10, and His14 residues of the Aβ1-42 peptide (Fig. 7B). As shown in the figure, the pyridine ring of cotinine is positioned between the imidazole ring of His6 and the phenyl ring of Tyr10. It interacts with these residues through strong π–π interactions that are indicated by the distances of 4.3 Å and 4.1 Å between cotinine–His6 and cotinine–Tyr10 aromatic rings, respectively. In the equilibrated region, the distance between the center of the aromatic ring of Tyr10 and the pyridine ring of cotinine remains around 4.0 Å (Fig. 7C). On the other hand, cotinine interacts with His14 via C–H–π interaction ([cotinine–C]–H–His14 = 3.2 Å). As discussed below, the interactions of cotinine with His6, Tyr10, and His14 residues of Aβ1-42 introduce significant changes in the secondary structure of the peptide (Fig. 7D).

In the free Aβ1-42 peptide, the first 38 ns, the Phe20–Val24 region is dominated by bend-and-turn conformations with sporadic helical structures (Fig. 7D, top). It is then transformed into bend and coil structures. However, in the cotinine-bound structure, for the first 25 ns this region exists in helical conformation but it is later converted into the stable turn structure (shown by the yellow color in Fig. 7D, bottom). In the free peptide, the loop region (24–28, VGSNK) is quite unstable and undergoes a large dynamical transformation between bend and turn. In the presence of cotinine, in a marked difference, initially (for the first 22 ns) this segment exists in the helical form but later it adopts stable bend and coil conformations.

**DISCUSSION**

The progressive deterioration of working memory is one of the main characteristics of AD, and effective therapies targeting memory loss in AD have been elusive. Here, we investigated the actions of cotinine as an anti-Aβ aggregation and memory-enhancing agent. More specifically, we studied the effect of cotinine on the deterioration of cognitive abilities, plaque formation, Aβ aggregation, and the activation of Akt/GSK3β pathway in the brains of the Tg6799 mice. We found that chronic treatment with cotinine improved working memory and reference memories and reduced both Aβ1-42 oligomerization and plaque burden in Tg6799 mice. Furthermore, we found that cotinine stimulated the activation of Akt and the inhibition of GSK3β by phosphorylation in the hippocampus and cortex of both Tg and NT littermate mice. We also discovered that cotinine inhibited Aβ1-42 aggregation into oligomers and fibrils in vitro. Furthermore, using MD we modeled the cotinine–Aβ1-42 interaction and elucidated a mechanism by which cotinine may interfere with Aβ aggregation.
In the search for new therapeutic agents against AD, cotinine, the main metabolite of nicotine, attracted our attention due to its unique pharmacological characteristics and safety profile. Specifically, cotinine is a poor agonist of the nAChRs [20–22], crosses the blood-brain barrier [54], is much less prone to induce respiratory arrest than nicotine, and has minimal toxic side-effects in humans [23, 58, 59]. We found that long-term treatment of Tg6799 mice with cotinine beginning in young adulthood (2 months of age) protected their cognitive abilities in multiple tasks and cognitive domains when tested between 5.5 and 6.5 months of age. It has been reported that at the age we started cotinine treatment, senile plaques in Tg6799 mice are beginning to appear with a large increase occurring thereafter. Thus, it is likely that plaque formation when the treatment started. Regarding behavioral characterization of the Tg6799 mice, this group reported initial cognitive impairment (in Y-maze and Morris maze acquisition/retention) to occur at 4–6 months of age [25, 26], with impairment in trace fear conditioning present by 5–6 months of age [26, 27].

In the present study, we found several cognitive domains that were impaired in Tg6799 mice between 5.5 and 6.5 months of age. In the tasks testing reference (circular platform) and working memory (RAWM, cognitive interference), treatment with cotinine administered from early adulthood provided significant protection against otherwise certain cognitive impairment. We did not observe changes in the Morris maze likely due to the lower sensitivity of this test to detect cognitive changes in AD mice. The tasks
Reviewing cotinine did enhance performance are more sensitive tasks (i.e., radial arm water maze, cognitive interference tasks) and/or analyze different cognitive abilities (circular platform). Indeed, we have previously reported that on AD therapeutics some drugs have effects in these tasks without being effective in the Morris water maze [60].

Overall, we hypothesize that cotinine may be useful in preventing cognitive deterioration when administered to individuals not yet exhibiting AD cognitive impairment or those with mild cognitive impairment at early stages of the disease.

Consistent with a positive effect of cotinine on brain homeostasis and function, we have found that cotinine treatment stimulated Akt in the hippocampus and cortex of both Tg and NT mice. The multiple effects of the activation of Akt in the brain, including the enhancement of brain plasticity and neuronal survival as well as the inhibition of GSK3β, may be critical to mediate the positive effect of cotinine on memory [61].

GSK3β is a proline-directed serine/threonine kinase considered to be a key protein in both sporadic and genetic forms of AD. According to this view, the over-activation of GSK3β leads to an increase in Aβ production, tau hyperphosphorylation, Neuroinflammation, and consequently memory impairment [62].

Hyperphosphorylated tau is the main component of paired-helical filaments forming the neurofibrillary tangles (NFT), one of the main pathological hallmarks in AD brains. GSK3β phosphorylates tau at several sites [63] and its levels positively correlate with the presence of NFT in AD brains [64]. Furthermore, the over-expression of GSK3β in forebrain regions is associated with neurodegeneration [53]. Interestingly, the activation of the Akt/GSK3β pathway is also induced by other drugs currently used for the treatment of AD such as physostigmine and memantine [30].

We also investigated the effect of long-term cotinine treatment on plaque deposition in Tg6799 mice. Treatment with cotinine significantly reduced the extent of Aβ deposition into plaques in the cingulate and motor cortices and the levels of insoluble Aβ/42 in the cortex. This decrease in Aβ deposition was most likely the result of a reduction in Aβ aggregation, although an enhancement of Aβ clearance by cotinine as an additional mechanism cannot be ruled out at the present time.

More importantly, we observed that cotinine treatment induced a clear decrease in the ratio Aβ/42/Aβ/40 in the hippocampus and cortex of transgenic mice. The decrease in the Aβ/42/Aβ/40 ratio can explain at least in part its beneficial actions, as this ratio has been shown to be important for the development of AD, by determining the fibrillogenesis and toxicity of Aβ [65, 66].

For example, it has been reported that Aβ42 affects the Aβ42 fibrillation, decreasing its conversion to mature fibrils [65]. In another study, it was found that the age-of-onset of the pathology in individuals carrying PS1-linked FAD mutations inversely correlated with Aβ42/Aβ40 and absolute levels of Aβ42, but directly with Aβ40 levels [67]. Furthermore, clinical studies have demonstrated that this ratio can also determine the distribution of Aβ (e.g., parahippocampal or vascular Aβ deposition) in AD brains [65, 68]. Coherent with these previous findings, in conjunction with a decrease in the Aβ42/Aβ40 ratio we observed an increase in insoluble Aβ42 in the hippocampus of cotinine-treated Tg mice, which can have a further beneficial effect in inhibiting Aβ fibrillogenesis.

Cotinine did not change the total levels of soluble Aβ peptides. However, it decreased the levels of Aβ42 oligomers in the brains of the transgenic mice. Two other potential therapeutics, which suppress Aβ aggregation and also provide clear cognitive benefit to AD TG mice such as melatonin and electromagnetic fields, induced deposited/insoluble Aβ without affecting the soluble Aβ [69]. These studies, along with our present findings, suggest that a small reduction in oligomers without changes in total Aβ levels may be enough to attain cognitive benefits in affected individuals.

The soluble aggregated forms of Aβ, including oligomers and protofibrils, have been proposed as the main pathological species in AD brains, as their accumulation is sufficient to induce synaptic and cognitive deficits in vivo [2, 57, 70–74]. The aggregation of Aβ is required for its toxicity; in fact, a physiological function for the monomeric form of the peptide stimulating synaptic plasticity has been suggested [39, 75]. Thus, compared to other therapeutic approaches, a distinct advantage of anti-Aβ aggregation agents like cotinine is that they can target the toxic forms of Aβ without disrupting a possible normal function of the monomeric form of the peptides.

To investigate whether the cotinine-induced reduction of Aβ aggregates in the brain was at least in part due to an inhibition of the peptide aggregation, we examined the effect of cotinine on Aβ aggregation in vitro. It was previously shown using NMR and circular dichroism techniques in vitro that cotinine binds to Aβ with high affinity, inhibiting its aggregation into fibrils [42, 75, 76]. Using X-ray fiber diffraction, we have previously found evidence suggesting that cotinine may reduce the extent of hydrogen-bonding and fiber growth for the fibrillogenic peptide Aβ12-28 [77].
Additionally, we studied the effect of cotinine on Aβ1-42 aggregation using AFM. Previous studies showed that in vitro Aβ monomers can associate into soluble high molecular weight (HMW) oligomers with a diameter between 15 and 25 nm, which after further aggregation transform into structures of greater dimensions. It has been reported that for Aβ oligomers, the average height is 2–5 nm [39, 75] and for fibrils, the height fluctuates between 3 and 9 nm [75, 76]. The analysis of the height of the Aβ aggregates indicated that the fibrillation mixtures contained a heterogeneous mix of oligomeric, protofibrillar, and fibrillar forms of the peptide. Notably, even under extreme conditions that promoted fibrillation such as high concentrations of Aβ, high temperature, and long-incubation time, cotinine inhibited the growth of Aβ fibrils as evidenced by the significant decrease in the average length of the Aβ aggregates. Overall, these findings support the view that the reduction in Aβ oligomers and plaques in the brains of cotinine-treated Tg mice was the result of an inhibition of Aβ aggregation.

The effect of cotinine over Aβ aggregation may be explained by the nature of the molecular interactions of cotinine with the peptide. The analysis of MD simulations of cotinine–Aβ1-42 interactions suggests that in the thermodynamically-equilibrated region, cotinine may interact with His6, Tyr10, and His14 residues of the Aβ1-42 Peptide, with His6 and Tyr10 residues through π–π interactions and with His14 via C=−H–π interaction. The interaction of cotinine with these residues introduced significant changes in the secondary structure of Aβ1-42.

According to the data, the cotinine-bound structure is different from the free form of the Aβ1-42 peptide. The secondary structural analysis of MD simulations also showed that the interaction with cotinine significantly influences the Phe20–Met35 region of the full-length monomeric form of the Aβ1-42 peptide. This segment contains both the loop (24–28, VGSNK) and the second hydrophobic domain (29–35, GALGLM) regions, which have been proposed to play important roles in the aggregation process [78]. It is noteworthy that the binding of cotinine does not affect the stable helical conformation of its immediate binding site (His6, Tyr10, and His14 residues containing the Asp1–Phe19 region). Altogether, these results suggest that cotinine binds to Aβ1-42, inducing important structural changes that may play a critical role in inhibiting aggregation of the peptide.

The investigation of compounds that inhibit Aβ aggregation has shown that numerous compounds affect Aβ fibrillation [38], with a small number of compounds affecting oligomerization such as, β-cyclodextrin derivatives [79], curcumin [80], Ginkgo biloba [81], and o-vanillin [82]. From the agents that inhibit Aβ oligomerization, few (e.g., Ginkgo biloba) have been tested in vivo [83, 84]. To our best knowledge, this study provides the first evidence that cotinine reduces Aβ aggregation in the brain, prevents memory loss, and stimulates the Akt/GSK3β pathway in vivo. Thus, the multiple-action drug cotinine has therapeutic benefits against AD-like pathology in an animal model of the disease. The unique pharmacological properties, including its good safety profile in humans, makes cotinine an attractive candidate for the treatment of AD.

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REFERENCES


