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Reduction of Amyloid-β Deposition and Attenuation of Memory Deficits by Tolfenamic Acid

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Abstract. We have previously reported that tolfenamic acid treatment decreases the amyloidogenic proteins in C57BL/6 and in old hemizygous R1.40 transgenic mice via the degradation of the transcription factor specificity 1 protein (Sp1). The lowering of amyloid-β protein precursor (AβPP) and amyloid-β (Aβ) in hemizygous R1.40 transgenic mice was accompanied by reversal of the identified spatial reference and working memory deficits observed in the mouse model. In this study, we examined the ability of tolfenamic acid to reduce the amyloid plaque burden, as well as to ameliorate spatial learning and memory deficits in homozygous R1.40 mice. Results from immunohistochemical analysis indicated that tolfenamic acid treatment resulted in a profound decrease in cerebral Aβ plaque burden that was accompanied by improvements in spatial working memory assessed by spontaneous alternation ratio in the Y-maze. These results provide further evidence that tolfenamic acid could be utilized as a repurposed drug to modify Alzheimer's disease pathogenesis.

Keywords: AD transgenic mouse model, Alzheimer's disease, amyloid-β plaque burden, immunohistochemistry, learning and memory, Morris water maze, tolfenamic acid, Y-maze

INTRODUCTION

Alzheimer's disease (AD) patients suffer from profound general memory loss and dementia until death and the disease is known as the most prevalent neurodegenerative disorder [1]. Even though Alois Alzheimer first described AD more than a century ago, no cure has been discovered yet [2, 3]. Different brain regions, especially in the cerebral cortex, hippocampus, subcortical nuclei, and amygdala, exhibit extracellular senile plaques and intraneuronal neurofibrillary tangles that characterize the neuropathological deposits identified in AD [2, 4]. In addition, the neuritic plaques induce the proliferation of astrocytes and microglia with altered morphology leading to inflammatory responses that contribute to the brain degeneration observed in the disease [5, 6].

Senile plaques are mainly composed of aggregated amyloid-β (Aβ) peptides, which are 38–43 amino acids long, that are generated by the cleavage of the amyloid-β protein precursor (AβPP) by the β-site AβPP cleaving enzyme 1 (BACE1) and γ-secretase [7, 8]. The formation of insoluble Aβ deposits depends on the rate of Aβ production and the rate of its elimination. Thus, the amyloid cascade hypothesis of AD was developed suggesting that amyloid plaque aggregates

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formed by the amyloidogenic breakdown of AβPP cause the neurodegeneration and dementia seen in AD [9].

Current FDA approved medications for AD are not disease-modifying therapies and do not interrupt the progression of AD [10]. Tolfenamic acid, a non-steroidal anti-inflammatory drug (NSAID), induces the degradation of the transcription factor specificity protein 1 (Sp1) [11]. Sp1 regulates the expression of several AD-related genes, and its expression is elevated in the frontal cortex of human AD brains as well as in the brains of transgenic mouse models of AD [12, 13].

We have previously provided proof-of-concept that tolfenamic acid interrupts the *de novo* synthesis of AβPP and consequently, alters the downstream levels of Aβ [14]. Treatment of wild type C57BL/6 mice with tolfenamic acid lowered the levels of Sp1 protein (SP1) and the expression of AD-related Sp1 target genes including AβPP [14], tau, and BACE1 (unpublished observations), and was supported by measurements which demonstrated the presence of tolfenamic acid in the brain following IV administration [15]. In addition, data from our lab showed that tolfenamic acid reversed the cognitive deficits in the hemizygous R1.40 transgenic mouse model of AD and lowered the levels of SP1, AβPP, and soluble and insoluble $A\beta_{40}$ and $A\beta_{42}$ [16].

The purpose of the current study was to investigate the ability of tolfenamic acid to lower Aβ plaque load in old homozygous R1.40 mice that exhibit fibrillar plaque depositions between the ages of 14 and 15 months [17, 18]. Furthermore, we investigated if tolfenamic acid would ameliorate the cognitive impairments in these mice long after plaque deposition has begun in homozygous R1.40 mice.

METHODS

Animal model

The genomic-based transgenic mouse model, R1.40, was utilized for this study. R1.40 mouse model utilizes a mutant human AβPP gene that is driven by its endogenous human promoter. The human promoter region has numerous CpG boxes that SP1 binds to while activating gene expression. Since tolfenamic acid lowers SP1, R1.40 mouse model was identified as an ideal mouse model to conduct our experiments on, especially that there is no available double or triple transgenic animal models of AD, whose mutant genes are driven by the endogenous human promoter region. R1.40 mouse model utilizes a yeast artificial chromosome (YAC) that includes the full 400 kb human AβPP gene and the flanking sequence of approximately 250 kb to harbor the Swedish mutation AβPPK670N/M671L and human transcriptional regulatory elements, allowing for proper spatial and temporal expression [19–21]. The mnemonic deficits in homozygous R1.40 are similar to those observed in AD and the model shows a great increase in AβPP and Aβ production with Aβ deposition occurring at 14-15 months of age [22, 23].

The transgenic mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Mice colonies were established in-house at the University of Rhode Island (URI) animal quarter facility. Mice were bred and genotyped using two genotyping techniques including standard PCR followed by gel electrophoresis, and was confirmed using the TaqMan® allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). Mice of different genotypes were housed in standard mouse cages in rooms with temperature maintained at $22 \pm 2^{\circ}C$ with humidity levels of $55 \pm 5\%$. The rooms were set for 12:12 h light-dark cycle (light on 6:00 AM, light off at 6:00 PM) and the animals were provided with food and water *ad libitum*. A URI veterinarian continuously supervised the animals during the entire period of the study and assisted in drug administration. The URI Institutional Animal Care and Use Committee (IACUC) approved all the protocols including breeding and genotyping techniques.

Animal exposure to tolfenamic acid

Tolfenamic acid was obtained from Sigma Aldrich (St. Louis, MO, USA) and homozygous R1.40 AβPP YAC transgenic mice of mixed gender aging between 19–24 months were used to examine the ability of this drug to reduce the amyloidogenic plaque load and to improve learning and memory deficits observed in these mice. The animals were divided into three groups of similar age and gender variation and were administered tolfenamic acid daily via oral gavage for 34 days. The first group was administered 5 mg/kg/day tolfenamic acid in corn oil ($n = 7$); the second group was administered 50 mg/kg/day tolfenamic acid in corn oil ($n = 6$), and the third group was administered corn oil, the vehicle, ($n = 7$).

Tissue preparation

Following 34 days of daily administration of tolfenamic acid and on Day 35, homozygous R1.40

mice were deeply anesthetized with an intraperitoneal injection of 0.1 ml/10g of xylazine-ketamine mixture (100 mg/ml-10 mg/ml) and were perfused transcardially with 100 ml of perfusion wash that consisted of 0.8% sodium chloride, 0.8% sucrose, 0.4% dextrose, 0.034% anhydrous sodium cacodylate, and 0.023% calcium chloride. After that, mice were perfused with 100 ml of perfusion fix that consisted of 4% paraformaldehyde, 4% sucrose, and 1.07% anhydrous sodium cacodylate, and their brains were removed.

The extracted brains were post-fixed in the perfusion fix solution overnight and then they were cryopreserved in 30% sucrose solution. Fixed brains were subject to coronal sectioning (40 μm) and collection using the MultiBrain® Technology (NeuroScience Associates, Knoxville, TN, USA). Sections were stored in preservative fix at −20°C. MultiBrain® Technology allows brains from different groups to be embedded together in the solid matrix and to be processed as a single unit. Different brain sections under the same conditions can be processed simultaneously providing more built-in quality control leading to uniform staining results.

Immunohistochemistry

To identify and quantify amyloid plaques in brain sections from different groups, free-floating sections were taken from the preservative solution and were washed with distilled water. That was followed by washing with 1X phosphate buffer saline (PBS) three times, three min each. After that, sections were immersed in a solution of 3% hydrogen peroxide (H_2O_2) for 30 min to quench the endogenous peroxidase activity. Sections were rinsed three times with PBS for three min each and then incubated in 70% formic acid solution for 30 min at room temperature. The sections were washed with PBS three times, three min each, and were incubated in 3% bovine serum albumin (BSA) and 0.1% Triton X-100 for 30 min. After rinsing with PBS, sections were incubated overnight at 4°C with primary antibody against Aβ (6E10, Sig-39320, Covance, Dedham, MA, USA) with dilution of 1:200. That was followed by incubation with the appropriate species-specific biotinylated secondary antibody (Vector Labs, Burlingame, CA, USA) of 1:500 dilution for 30 min and then the sections were incubated with horseradish peroxidase-conjugated streptavidin (Vector Labs, Burlingame, CA, USA) for 30 min. After that, sections were washed with PBS and the immunoreactivity was detected and visualized with the substrate 3-3, diaminobenzidine (DAB) (Vector Labs, Burlingame, CA, USA). Brain sections were counterstained with hematoxylin. The sections were mounted on microscope slides according to the procedure provided by NeuroScience Associates and coverslips were mounted with permanent mounting medium (Vector Labs, Burlingame, CA, USA).

Quantification of Aβ plaques

Evaluation of the extracellular Aβ plaque load in the cerebral cortex was performed using a Nikon Eclipse E600 microscope (Nikon, Melville, NY, USA) attached with a Diagnostic Instruments digital camera and using SPOT Diagnostic Instruments software (Diagnostic Instruments, Sterling Heights, MI, USA). Serial images of 10X magnification were captured on four sections per animal that were 80 μm apart from each other. Data were analyzed using ImageJ software from NIH (Bethesda, MD, USA). Using ImageJ software for plaque quantification has been documented and validated in several publications [24–26]. In brief, regions of interests (ROIs) of the cortex were manually delineated and the images were binarized to 16-bit grey scale with a threshold value to yield the boundaries that corresponds to the observed plaques boundaries and to distinguish the aggregates from background. The minimum and maximum size was set to exclude objects in the image that were clearly not of interest and data were collected including size, number and area of the plaques. The same settings and threshold values were used for all the analyzed sections. Amyloid burden was expressed as the percent area stained positive for Aβ to the total area analyzed (detected plaque area/ROI area × 100).

Assessment of cognitive functions

Memory and cognitive functions in homozygous R1.40 transgenics were characterized through behavioral testing in mazes that examine the integrity of the hippocampus and the brain cortex including the Morris water maze (MWM) and spontaneous alternations in the Y-maze. After 14 days of daily dosing, testing began in the MWM and on Day 33 mice were tested for spontaneous alternation in the Y-maze.

MWM

Mice were tested in the hidden version of the MWM task, where they had to locate a hidden platform by

learning multiple spatial relationships between the platform and the distal extra-maze cues [27–29]. The apparatus consisted of a white 48" diameter pool that is 30" in height and was filled with water to a depth of 14". Non-toxic washable liquid paint was added to the water to keep it opaque. Distinct fixed visual cues surrounded the pool that the animals used for navigation while trying to reach the escape platform. A clear Plexiglas platform square of 10 cm was kept submerged 0.5 cm below the surface of the water. The temperature of the water was always kept around $25 \pm 2^{\circ}C$ during all procedures in MWM.

Habituation trials in which mice were allowed to swim freely for 60 s to acclimate to the procedure were conducted on Day 15 of drug administration. On the following day and for a total of 8 days, mice received training sessions of three trials daily. For each trial, the starting position was randomly assigned between the four possible positions while the platform position was kept the same for the entire training sessions.

Each animal was allowed to swim until it found the immersed hidden platform or for a maximum duration of 60 s. A mouse that failed to locate the platform in the first 60 s would be gently guided to sit on the platform for a maximum duration of 30 s. Upon a successful trial, the mouse would be left to sit on the platform for a maximum of 10 s. Following the eighth acquisition sessions, probe trials for up to 60 s on Day 1 and Day 11 after the last day of training were performed to assess long-term memory retention. This was achieved by studying the preference of the mice to swim in the correct quadrant that previously contained the hidden platform. All trials including the swim paths and latencies to locate the platform were videotaped and tracked with a computerized video tracking system (ObjectScan, Clever Sys. Inc., Reston, VA, USA) and the resultant data were analyzed.

Spontaneous alternation in the Y-maze

The spontaneous alternation ratio, defined as the percentage of the number of arm entries, different from the previous two entries, divided by the total arm entries minus two was measured [23, 30]. Testing trials were conducted in a white Y-maze with arms that were 12" (long) by 3" (wide) with 8" height walls. Mice were placed into one of the Y-maze arms and were left to explore the maze freely for 5 min. After each trial, the maze was cleaned with 70% ethanol. Y-maze trials were videotaped and tracked with a computerized video tracking system (ObjectScan, Clever Sys. Inc., Reston, VA, USA) and the data were analyzed.

Statistical analysis

The significance of difference between different treatment groups was determined by repeated measure analysis of variance (ANOVA) and Tukey-Kramer multiple comparison a *posteriori* analysis. Data is expressed as the mean ± the standard error of the mean (SEM). All statistical analyses were conducted using GraphPad InStat 3 software (GraphPad Software, La Jolla, CA, USA) and probability (p) value of <0.05 was considered acceptable for statistical significance.

RESULTS

Overall toxicity and safety observations

Tolfenamic acid administration did not produce overt toxic effects in treated mice. Compared to non-treated animals, there were no significant alterations in body weight or any abnormal behavior in the animals treated with tolfenamic acid. In Europe, tolfenamic acid has been in use for decades safely in humans for the treatment of rheumatoid arthritis and migraine headaches. Even though the safety profile is established for tolfenamic acid, our collaborators conducted recent toxicity studies. The results showed that chronic administration of 50 mg/kg tolfenamic acid three times a week for 6 weeks did not produce any changes in hemoglobin or hematocrit in tolfenamic acid treated mice. Additionally, acute and chronic administration of the drug did not alter gastric or intestinal epithelia as evidenced by histopathological analyses [31].

Tolfenamic acid reduces Aβ amyloid deposition in aged homozygous R1.40 mice

As described in the literature, the homozygous R1.40 mouse model develops extracellular Aβ plaques that manifest between the ages of 14 and 15 months [17, 18]. Tolfenamic acid treatment with both doses greatly reduced amyloid plaque pathology in multiple cerebral cortical regions such as the primary somatosensory cortex, parietal association cortex, auditory cortex and insular cortex, compared to non-treated mice (Fig. 1). Quantitative immunohistochemistry analysis revealed that tolfenamic acid treatment for 34 days significantly lowered Aβ plaque deposition $F(2,14) = 6.685$, $p = 0.009$) (Fig. 2). The Tukey-Kramer HSD *post-hoc* test indicated that 5 and 50 mg/kg/day tolfenamic acid doses lowered amyloid deposition significantly ($HSD = 3.779$, $p < 0.05$ and $HSD = 5.012$ $p < 0.01$, respectively) (Fig. 2).

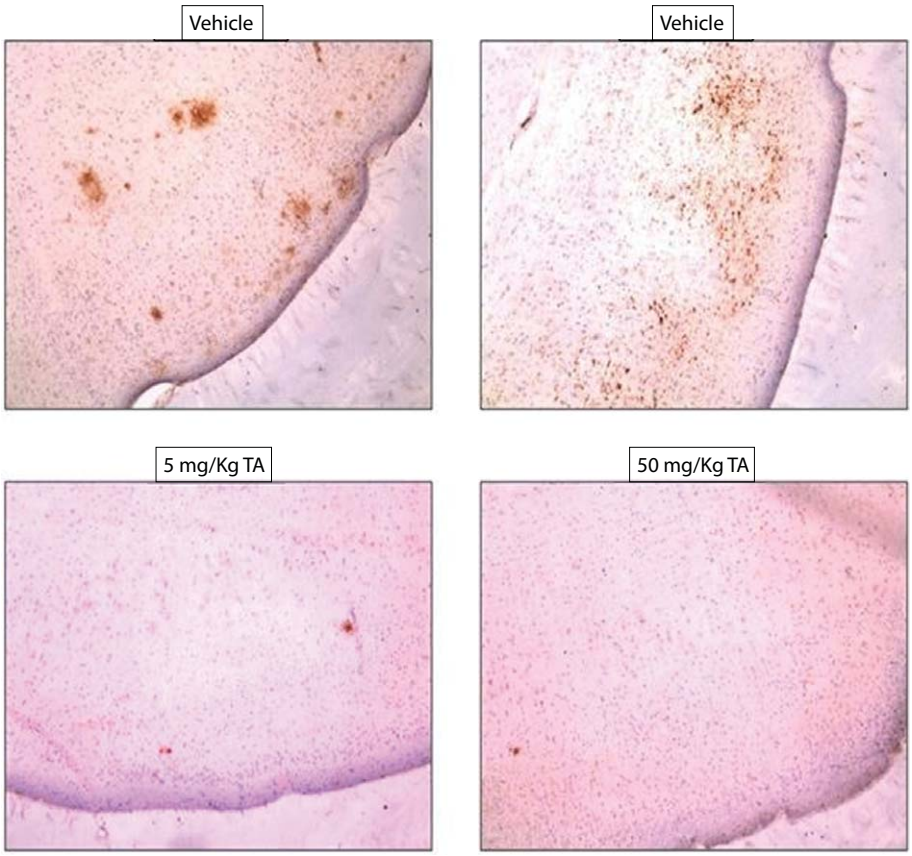

Fig. 1. Reduction of amyloid pathology in homozygous R1.40 following treatment with tolfenamic acid. Tolfenamic acid was administered by oral gavage daily for 34 days. See the methods section for details. Images represent Aβ plaque morphology and density in the cerebral cortex in different treatment groups after immunohistochemical staining against Aβ using 6E10 antibody.

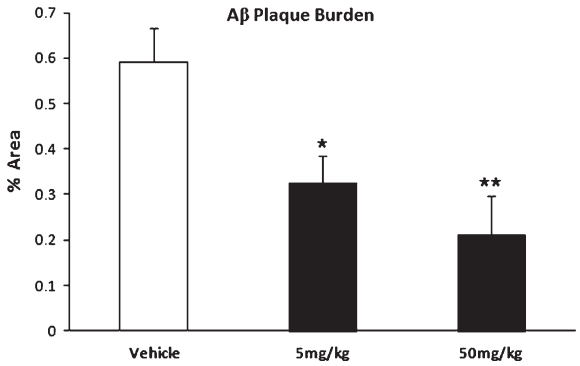

Fig. 2. Treatment with tolfenamic acid reduces cortical Aβ plaque burden in homozygous R1.40 mice. Tolfenamic acid was administered by oral gavage daily for 34 days. See the methods section for details. The figure represents quantification of total Aβ plaque area in different treatment groups. * indicates that values are significantly different from control, as determined by one-way ANOVA analysis with Tukey-Kramer *post-hoc* test to compare all pairs of columns (*p<0.05, **p<0.01), obtained using GraphPad InStat 3 software. Vehicle $n = 5$; 5 mg/kg/day $n = 7$; 50 mg/kg/day $n = 5$.

Tolfenamic acid attenuates cognitive deficits in homozygous R1.40 mice

We examined the effects of tolfenamic acid treatment with 5 and 50 mg/kg/day on learning and memory in groups of old homozygous R1.40 transgenic mice aging between 19–24 months. There was a significant effect of training as ANOVA analysis showed that the difference in escape latency between the first and last day of training sessions was statistically significant ($F(7,152) = 7.293, p < 0.0001$). The results showed that in the MWM, the difference in the escape latency during the training phase between different experimental groups was not statistically significant. However, the tolfenamic acid treated groups showed a trend of better performance than the control vehicle group through days 4–8 of the training sessions (Fig. 3A).

Analysis of probe trials on Day 1 and Day 11 indicated that while both tolfenamic acid treatment groups scored higher percentages in the correct quadrant than

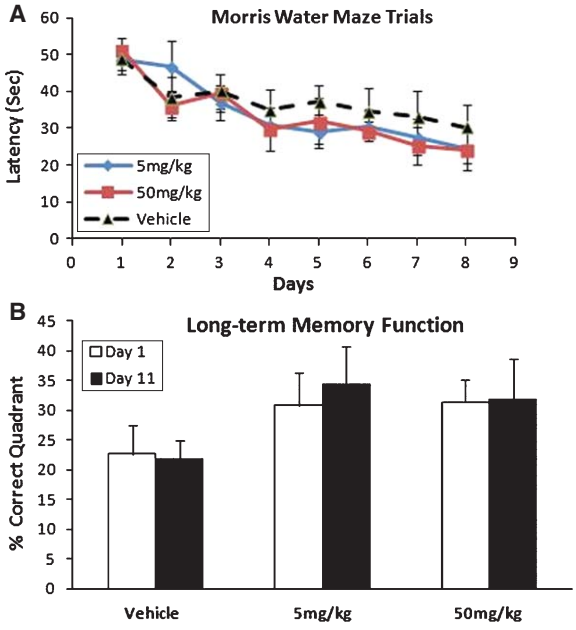

Fig. 3. Effects of tolfenamic acid on spatial reference memory in homozygous R1.40 mice. Tolfenamic acid was administered by oral gavage daily for 34 days and behavioral testing was conducted in MWM. See the methods section for details. A) Acquisition patterns during daily training trials for the 5 mg/kg/day, 50 mg/kg/day and vehicle groups; B) Probe trials assessing the long-term memory retention on Days 1 and 11 following acquisition training trials. Data were analyzed by one-way ANOVA with Tukey-Kramer *post-hoc* test to compare all pairs of columns, obtained using GraphPad InStat 3 software. Vehicle $n=7$; 5 mg/kg/day $n=7$; 50 mg/kg/day $n=6$.

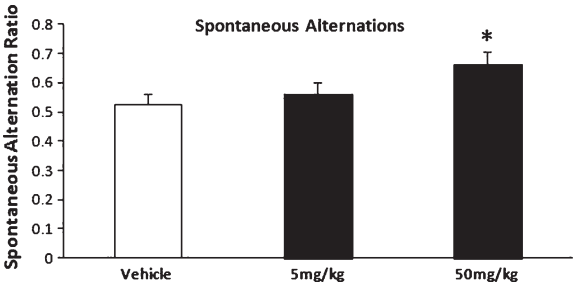

Fig. 4. Working memory improvement in homozygous R1.40 transgenic mice after administration of tolfenamic acid. Tolfenamic acid was administered by oral gavage daily for 34 days. Working memory was assessed by spontaneous alternations in the Y-maze. See the methods section for details. * indicates that values are significantly different from control vehicle, as determined by one-way ANOVA analysis with Tukey-Kramer *post-hoc* test to compare all pairs of columns ($p<0.05$), obtained using GraphPad InStat 3 software. Vehicle $n=7$; 5 mg/kg/day $n=7$; 50 mg/kg/day $n=6$.

the control group in both probe trials, the difference was not statistically significant ($p \geq 0.05$) (Fig. 3B). Nevertheless, results from repeated measures ANOVA analysis of the spontaneous alternation ratio in the Y-maze showed a significantly improved working memory function ($F(2,14)=4.495$, $p=0.0311$) (Fig. 4). The Tukey-Kramer HSD *post-hoc* test indicated a significant effect of 50 mg/kg/day tolfenamic acid treatment in improving spatial working memory (HSD = 3.702, $p<0.05$), while the treatment with 5 mg/kg/day did not reach significance (HSD = 0.91, $p \geq 0.05$) (Fig. 4).

DISCUSSION

Certain NSAIDs have been shown to alter AD pathology and attenuate learning and memory deficits in murine mouse models of AD by mechanisms other than their classic cyclooxygenase (COX) inhibition pathway and have led researchers to consider such NSAIDs as potential disease-modifying agents for AD [32–34]. Tolfenamic acid, of all other NSAIDs, has the unique mechanism of inducing the proteasome-dependent degradation of SP transcription factors including SP1, SP3, and SP4 and thereby, decreasing the expression of vascular endothelial growth factor, which is involved in tumor progression and metastases [11]. Elevated levels of Sp1 have been found in the frontal cortex of the brains of AD patients as well as in the brains of transgenic mouse models of AD [12, 13]. Interplay between inflammatory processes and Sp1-driven gene expression has been suggested, as the elevation of Sp1 was accompanied by an increase in COX-2 expression, an Sp1 target gene [12].

Sp1 induces the expression of genes involved in AD pathology which include AβPP, BACE1, and tau as their promoter regions are rich with 5'-GGGGCGGGGC- sequence to which Sp1 binds [12, 35–38]. Thus, we hypothesized and showed that tolfenamic acid could interrupt the *de novo* synthesis of AβPP and alter the downstream levels of Aβ in C57BL/6 mice [14] and hemizygous R1.40 transgenics leading to improvements in learning and memory profiles [16].

As a genomic-based transgenic mouse model of AD, the R1.40 line represents a valuable tool for assessing the effects that potential AD therapeutics have in reducing amyloidogenic pathology and in improving learning and memory profiles. At 14-15 months of age, homozygous R1.40 mice exhibit depositions of extracellular Aβ plaques accompanied by cognitive deficits [17, 18, 23]. The mice demonstrate the ability to learn the location of the hidden platform during daily training session trials in the MWM; however, they exhibit a

decline in long-term memory retention compared to non-transgenic mice as assessed by probe trials. In addition, when these mice were assessed for the spontaneous alternation ratio in the Y-maze, impairments in the working memory functions were observed [23].

The present study provides the first evidence of the ability of a relatively short-term tolfenamic acid treatment to reduce Aβ plaque burden drastically in homozygous R1.40 compared to longer treatments for 6–9 months in other transgenic models with ibuprofen, another NSAID [39, 40]. Tolfenamic acid treatment resulted in a greater lowering of Aβ peptide levels in wild type C57BL/6 and hemizygous R1.40 mice compared to the reduction of AβPP [14, 16], indicating that additional mechanisms responsible for plaque degradation and clearance of Aβ are involved.

We also examined the ability of tolfenamic acid treatment to attenuate the spatial memory deficits observed in old homozygous R1.40 mice aging between 19–24 months with Aβ plaque deposition reported to start as early as 14 months of age [22]. We found that both doses of 5 and 50 mg/kg/day resulted in a trend of shorter latency during daily MWM training trials and higher percentage in the correct quadrant during both probe trials compared to the vehicle treated group. However, those differences from the vehicle treated group did not reach statistical significance.

The animals were treated with tolfenamic acid at senescence (19–24 months of age) while the combined effect of age and the mutant transgene persisted. Homozygous R1.40 starts to develop fibrillar amyloid deposits at the age of 14 months [22] and reports indicated that they show cognitive deficits at the age of 16 months [23]. Thus, lowering the amyloidogenic pathology may not have been sufficient to retrieve enough spatial reference memory capabilities to perform significantly well in the MWM compared to the non-treated group. At the onset of tolfenamic acid exposure, the extensive plaque pathology in different cortical regions has been in effect for a long period of time. It is well known that Aβ plaques are toxic, affect synaptic plasticity, and stimulate the production of reactive oxygen species leading to the elevation of oxidative stress and neuronal cell death. It is possible that the damage by Aβ deposition on memory networks was extensive and irreversible by the time treatment started, even though tolfenamic acid treatment reduced the plaque burden significantly.

Also, epigenetics plays an important role in AD, since it is mainly a sporadic disease in nature. Such hypothesis has now several supporting evidence from our lab and others [41–44]. Epigenetics pathways such as DNA methylation and histone acetylation are important for learning and memory [45, 46]. A correlation has been established between the decline in learning and memory abilities and reductions in DNA methylation [47]. DNA methylation, for example, is catalyzed by DNA methyltransferases (DNMTs) such DNMT1, DNMT2, DNMT3a, and DNMT3b, which are important in memory formation [48]. In this regard, the action of tolfenamic acid on Sp1 may lie at the intersection of its transcriptional role in regulating AD-related genes as well as a gene subject to regulation by epigenetic pathways.

Alternatively, there may be a lesser connection between spatial memory functions and the plaque burden. The previous explanation is supported by the fact that tolfenamic acid significantly improved mice performance in the spontaneous alternations in the Y-maze. The spontaneous alternation task is mainly dependent on the hippocampus and the prefrontal cortex [49], and as described in the literature, R1.40 mice only show scattered Aβ plaque deposition in hippocampal formation [17, 23] which is consistent with our observations (data not shown).

Similar to our prior findings in hemizygous R1.40 mice [16], the present study indicates that tolfenamic acid is effective in lowering the amyloidogenic proteins and Aβ plaques with both doses, with dose-dependent improvements in cognitive deficits. We still have to study the effects of extended periods of tolfenamic acid exposure with low doses as it may result in significant improvements in cognitive functions. It is essential to mention that compared to other FDA approved AD drugs and NSAIDs, tolfenamic acid significantly decreased plaque pathology and produced significant behavioral improvements in a relatively short duration of treatment. For example, 0.58 mg/kg/day donepezil, an FDA-approved anticholinesterase drug for AD, improved performance in MWM after two months of treatment in AβPP23 transgenic mice [50]. Another FDA-approved drug for use in moderate to severe AD, memantine, improved cognition in 3xTg-AD mice after three months of treatment [51]. In addition, ibuprofen was able to produce improvement in cognitive functions in triple transgenic mice (AβPPswe, PS1M146V, and tauP301L) following its administration for six months [52].

As we have discussed before in a previous publication [16], several anti-amyloid agents that do not lower SP1, such as γ-secretase inhibitors, anti-Aβ monoclonal antibodies, certain NSAIDs and BACE1 inhibitors, failed clinical trials [10, 53]. Reasons for such disappointing results were either due to the ensu-

ing of adverse effects or the lack of efficacy. In addition, the targets of those drugs were end stage proteins of the amyloidogenic pathway. There may be better promise for success with tolfenamic acid as its effects are not dependent on those end stage proteins. Rather, tolfenamic acid impacts the downstream events as it acts at upstream pathways through altering the transcriptional pathways associated with AD-related genes.

In conclusion, we report that tolfenamic acid treatment drastically reduces Aβ plaque pathology accompanied by improvement in spatial learning and memory in a murine mouse model of AD. This occurs within a period that is much shorter than that observed with other NSAIDs. The data provide further evidence of the ability of tolfenamic acid to disrupt the development and the progression of the pathological processes of AD. Thus, tolfenamic acid, as a repurposed AD drug, could be a promising disease modifying therapeutic agent acting through an alternative mechanism that maybe further established after its scheduled clinical trials are complete.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=2385).

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