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Featured Article

Translational inhibition of APP by Posiphen: Efficacy, pharmacodynamics, and pharmacokinetics in the APP/PS1 mouse

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Abstract

Introduction: Translational inhibition of amyloid precursor protein (APP) by Posiphen has been shown to reduce APP and its fragments in cell culture, animal models, and mildly cognitively impaired patients, making it a promising drug candidate for the treatment of Alzheimer's disease.
Methods: We used a mouse model of Alzheimer's disease (APP/presenilin-1) to examine Posiphen's efficacy, pharmacodynamics, and pharmacokinetics.
Results: Posiphen treatment normalized impairments in spatial working memory, contextual fear learning, and synaptic function in APP/presenilin-1 mice, without affecting their visual acuity, motor skills, or motivation and without affecting wild-type mice. Posiphen had a prolonged effect in reducing APP and all related peptides for at least 9 hours after the last dose. Its concentration was higher in the brain than in plasma, and the most abundant metabolite was N^8-norPosiphen.
Discussion: This is the first study demonstrating the therapeutic efficacy of inhibiting the translation of APP and its fragments in an Alzheimer's disease model.

Keywords: Alzheimer's disease; Posiphen; APP; $A\beta_{42}$; CTFα; CTFβ; APP/PS1; Cognition; Long-term potentiation

1. Background

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. The hallmarks of AD at the microscopic level are senile plaques and neurofibrillary tangles (NFTs), formed by aggregation of amyloid β (Aβ) peptide, mostly of 42-aa length, and hyperphosphorylated tau protein, respectively [1]. The Aβ precursor, amyloid precursor protein (APP), expressed in many tissues but concentrated at the synapse of neurons, is alternatively processed to generate amyloidogenic and nonamyloidogenic products. In the former case, APP is cleaved by β-secretase to carboxy-terminal fragment (CTF) β of 99 aa, which is further processed by γ-secretase to Aβ and APP intracellular domain (a.k.a. CTFγ/ε) of 50 aa [2]. Most secreted Aβ is 40-aa ($A\beta_{40}$) long, but its 42-residue form ($A\beta_{42}$) is more prone to aggregation to create oligomers that are particularly neurotoxic. The major processing pathway uses α-secretase to cleave APP within the Aβ sequence to generate CTFα and the secreted derivative, soluble APPα (sAPPα). Other fragments of APP have additionally been implicated in AD pathology. Specifically, the N-terminal fragment N-APP has been shown to bind to death receptor 6 and activate caspases 3 and 6, leading to the

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initiation of apoptotic pathways [3]. Likewise, CTFs containing the last 31-residues, including C31 and APP intracellular domain, have been described as neurotoxic [4–6]. The microtubule-associated tau protein physiologically stabilizes the microtubules that support the structure and transport of molecules along axons to optimize neuronal function. However, the hyperphosphorylated version cannot perform this function and, instead, aggregates into paired helical filaments that deposit intracellularly to form NFTs, leading to neurodegeneration [7]. Familial AD mutations in APP and presenilin-1 (PS1) and presenilin-2 (PS2) increase the yield of $A\beta_{42}$ in cell culture and induce dementia with the entire spectrum of AD pathology, whereas mutations in tau induce NFTs and dementias without simultaneously increasing senile plaque formation [8]. Notably, it has been reported that the presence of extracellular Aβ leads to the activation of the kinases that phosphorylate tau, and that Aβ deposition precedes NFT pathology [2]. Moreover, Down syndrome [9] and familial AD-linked APP duplication are reported to be sufficient to trigger early-onset familial AD [10]; in this light, APP represents a promising therapeutic target, especially if regulated at the expression level, so that the production of all toxic fragments can be reduced [11].

Posiphen, (+)-phenserine tartrate, is the optically pure positive enantiomer of (−)-phenserine, an acetyl-cholinesterase inhibitor (AChEI) [12]. Posiphen lacks AChEI activity but is a translational inhibitor reported to reduce the levels of APP and $A\beta_{42}$ in human neuroblastoma cell cultures, rodent primary neurons, and in the brain of wild-type (WT) mice [13,14]. It also reduced levels of $A\beta_{42}$ in the cerebral cortex of transgenic mice overexpressing the human *APP* gene with the Swedish mutation K670N/M671L (APP_{SWE}), a model of early-onset AD [15].

Secondary beneficial effects due to decreased APP and $A\beta_{42}$ levels have also been described. First, Posiphen treatment resulted in an increase in hippocampal neurogenesis in both young and aged APP_{SWE} mice [15]. Additional studies showed that Posiphen treatment is neurotrophic and neuroprotective in neural cell cultures under conditions that mimic AD [16]. Posiphen has also been used in two studies in combination with transplantation of human neural stem cells in the brain of APP_{SWE} transgenic mice, albeit with mixed results [17,18]. Salehi et al. studied the effect of Posiphen on Ts65Dn mice, which contain three copies of chromosome 16, encoding *App* and corresponding to the syntenic Down syndrome critical region on human chromosome 21. It is a Down syndrome model that over expresses APP. Treatment with 50 mg/kg Posiphen normalized APP levels in the hippocampus of Ts65Dn mice [19]. Furthermore, in a phase I clinical trial, Posiphen treatment proved well tolerated and reduced the level of sAPP fragments, $A\beta_{42}$, and tau in the CSF of mildly cognitively impaired patients [20].

Posiphen lowers APP levels by inhibiting its translation via a mechanism that involves the 5′-untranslated region of the *APP* mRNA [21,22]. The *APP* 5′-untranslated region contains an iron-response element (IRE) that allows endogenous control of APP expression levels. For example, high iron levels upregulate APP expression, while iron regulatory protein-1 binds to the IRE and prevents the association of the mRNA with the ribosome, thus downregulating translation. The IRE was therefore proposed as the element affected by Posiphen [21,23,24]. This hypothesis is supported by the fact that Posiphen also inhibits the translation of α-synuclein mRNA, which also contains an IRE in its 5′-untranslated region [14,25,26].

Maccecchini et al. [17] studied the pharmacokinetics of Posiphen in humans and rats. Concentrations of Posiphen were higher in the brain as opposed to plasma, in agreement with the compound's high lipophilicity. Its primary metabolites were N^1-norPosiphen, N^8-norPosiphen, and N^1, N^8-bisnorPosiphen [20]. These metabolites have, likewise, been shown to reduce the levels of APP and α-synuclein in primary neuron cultures. However, whereas Posiphen and N^8-norPosiphen have no AChEI, N^1-norPosiphen and N^1, N^8-bisnorPosiphen possess some AChEI activity and may be the metabolites that determine its maximum tolerated dose [14,27].

Although several effects of Posiphen have been examined at the protein and cellular level, its efficacy in ameliorating the symptoms of AD in vivo has yet to be studied. Here, we used the APP_{SWE}/PS1 mouse model of AD to examine the efficacy of Posiphen in treating AD. This model is characterized by high levels of $A\beta_{42}$, early senile plaque formation, and cognitive deficits [28]. The functional outcome of Posiphen treatment was examined using radial arm water maze (RAWM) and fear conditioning (FC) tests, to evaluate effects in spatial and associative memory, respectively, as well as electrophysiology of brain slices to examine long-term potentiation (LTP). We also examined Posiphen effects on motor function, visual acuity, motivation, and sensitivity to electric shock to rule out confounding effects on the behavior of treated mice. In addition, the distribution of Posiphen and its primary metabolites was appraised in the mouse brain and plasma, to draw a comparison to previous results from rats and humans [20].

Finally, to elucidate the pharmacodynamic actions of Posiphen, we quantified levels of APP in the hippocampus of treated animals. To evaluate the duration of the inhibition of protein translation, we treated a parallel series of animals with Posiphen and measured the levels of APP, the C-terminal fragments—CTFα and CTFβ—of APP, $A\beta_{42}$, and $A\beta_{40}$ within the hippocampus at various time points following the last Posiphen dose.

2. Methods

2.1. Animals

All experiments were performed with approval of the Columbia University Institutional Animal Care and Use Committee and the Medical University of South Carolina Institutional Animal Care and Use Committee. The APP_{SWE}/PS1 animals at Columbia University were double transgenic mice expressing the human APP mutation (K670N, M671L) and the human PS1 mutation (M146L)

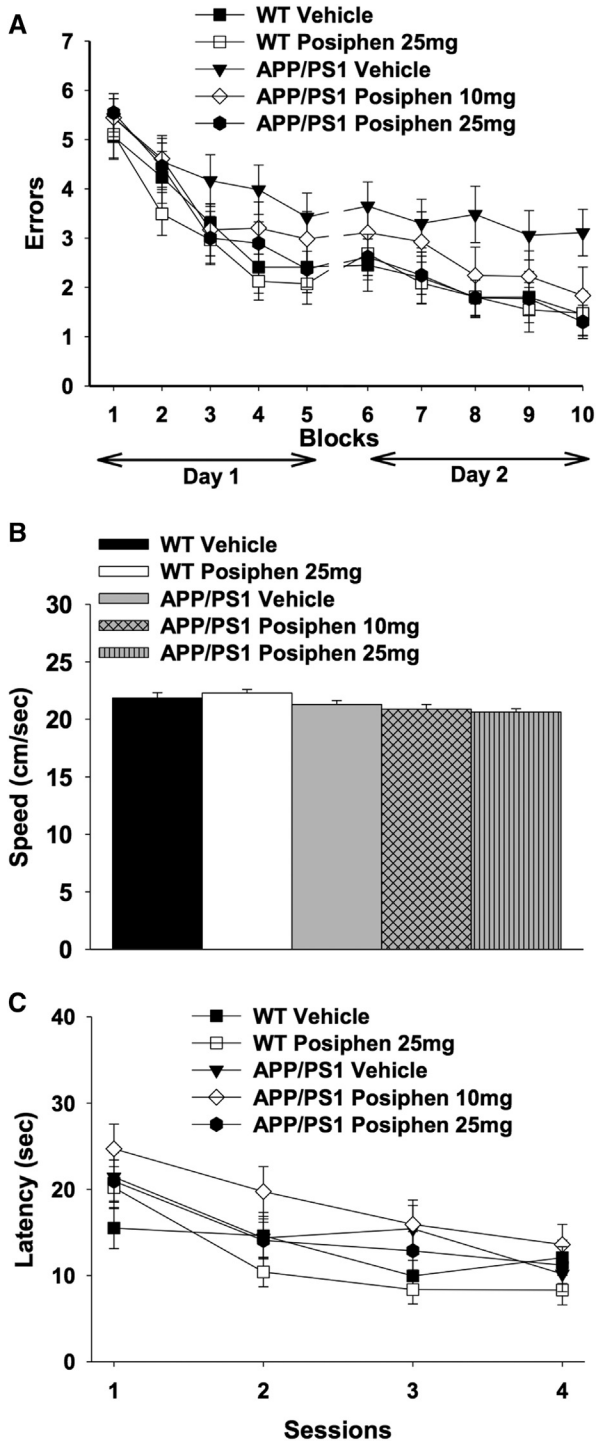


Fig. 1. Posiphen rescues defects in spatial memory of APP/PS1 mice. (A) Daily oral treatment with 25 mg/kg, but not 10 mg/kg, Posiphen starting from 2 months of age for 1 month reduced the number of errors that the APP/PS1 mice made in the 2-day RAWM test, in comparison to saline-treated transgenic mice [WT vehicle vs. APP/PS1 vehicle: F (1, 33) = 4.9, P = .0339; APP/PS1 vehicle vs. APP/PS1 Posiphen 10 mg/kg: F (1, 34) = 1.4, P = .2453; and APP/PS1 vehicle vs. APP/PS1 Posiphen 25 mg/kg: F (1, 35) = 5.13, P = .0298]. WT mice were not affected by 25 mg/kg Posiphen treatment [WT vehicle vs. WT Posiphen 25 mg/kg: F (1, 34) = 0.16, P = .6887]. (B and C) All treatment groups shown in (A) displayed similar speed (B) and latency (C) to a visible platform above the water surface. Mean and SEM are shown. N = 17–19 per group. Abbreviations: APP, amyloid precursor protein; PS1, presenilin-1; RAWM, radial arm water maze; SEM, standard error of the mean; WT, wild-type.

(line 6.2), together with their WT littermates. They were obtained by crossing APP with PS1 animals, and their genotype was confirmed by the use the polymerase chain reaction on samples of the tail. At Medical University of South Carolina, female, 9- to 11-week-old B6.Cg-Tg (APPswe, PSEN1dE9) 85Dbo/Mmjax mice (APP-PS1; Stock No. 34832; the Jackson Laboratory) obtained from the Mutant Mouse Resource and Research Centers were used. Henceforth, both mice will be referred to as APP/PS1.

2.2. Treatments

Posiphen tartrate (>99.5% purity) was diluted in 0.9% sterile saline solution (vehicle) and stored at 4°C.

For the behavioral and LTP studies at Columbia University, Posiphen or saline injection was delivered per os (p.o.—oral gavage; total volume of 250 μL) to WT or transgenic mice daily, starting at 2 months of age until sacrifice, which occurred at the time of assessing LTP. The treatment groups for behavioral studies were as follows:

1. APP/PS1—vehicle (N = 18)
2. APP/PS1—10 mg/kg Posiphen (N = 18)
3. APP/PS1—25 mg/kg Posiphen (N = 19)
4. WT—vehicle (N = 17)
5. WT—25 mg/kg Posiphen (N = 19)

Behavioral assessment started at 3 months of age and lasted for about 2 weeks, during which the treatment continued. After sacrifice, hippocampi were collected and stored at −80°C until used to quantify levels of APP. One of the two fresh hippocampi of some mice was used for LTP (N = 8, 9, 9, 8, and 8, respectively). For such animals, the leftover hippocampus was stored at −80°C.

For the pharmacokinetic studies, 25 mg/kg Posiphen was administered daily (p.o.) to three mice at 3 months of age for 2 weeks. Plasma and cerebellum tissue was used for pharmacokinetic analyses.

For the pharmacodynamics studies at Medical University of South Carolina, 0.9% saline (n = 5) or Posiphen (n = 20; five per time point) was administered daily using intraperitoneal (IP) injection to 9- to 11-week-old APP/PS1 transgenic mice. To ensure stability of the compound, 21 aliquots of 30–50 mg Posiphen was preweighed into glass vials and dissolved in 0.9% saline just before use. The initial dose was 75 mg/kg for 1 week, followed by 50 mg/kg for the following 2 weeks (N = 20). These doses were selected from a previous study that resulted in a substantial decline in APP and analyte levels in WT mice [12]. The reason for lowering the dose was the observation of severe tremors at 75 mg/kg, which was resolved at 50 mg/kg. On the final day, mice were injected at staggered 15-minute intervals and euthanized in 15-minute intervals after 90, 180, 360, and 540 minutes to ensure consistent

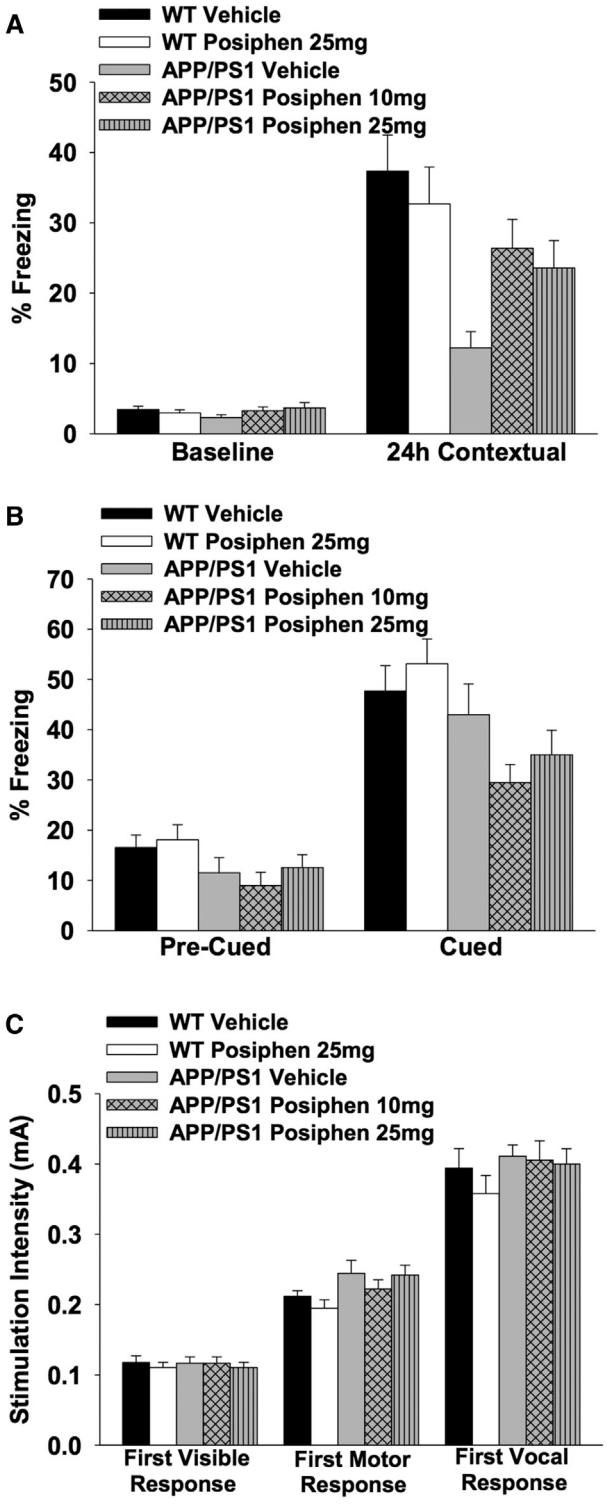


Fig. 2. Posiphen rescues the defect in associative memory of APP/PS1 mice. (A) Daily oral treatment of APP/PS1 mice with either 10 or 25 mg/kg Posiphen starting at 2 months of age for 1 month reestablished normal freezing in a test for contextual fear memory, in comparison to saline-treated APP/PS1 mice (t tests for 24-hour contextual: WT vehicle vs. APP/PS1 vehicle, $P < .0001$; APP/PS1 vehicle vs. APP/PS1 Posiphen 10 mg/kg, $P = .005$; and APP/PS1 vehicle vs. APP/PS1 Posiphen 25 mg/kg, $P = .0185$). WT mice were not affected by Posiphen treatment (t test WT vehicle vs. WT Posiphen 25 mg/kg, $P = .5304$). Freezing (%) was comparable for all groups at baseline. (B and C) Daily oral treatment with Posiphen starting at 2 months of age for 1 month in the animals shown in (A) did not affect cued memory (B) and capability of mice to perceive the electric shock (C) in neither WT nor APP/PS1 mice, as compared to saline-treated controls. Mean and SEM are shown. N = 17–19 per group. Abbreviations: APP, amyloid precursor protein; PS1, presenilin-1; SEM, standard error of the mean; WT, wild-type.

collection times. Brain tissue was collected, coronally sliced into about 16 slices of 1-mm thickness (Zivic slicer), and frozen (−80°C) until extraction for further analysis. The seventh slice from the anterior end, corresponding to the hippocampus and the surrounding cortex and associated midbrain, was used for analysis of APP, CTFα, CTFβ, $A\beta_{42}$, and $A\beta_{40}$. Results were compared to a control group of transgenic mice that received vehicle (saline, N = 5). The last brain slice, corresponding to cerebellum, and plasma from three mice from the 90-minute group was used for pharmacokinetics analyses.

2.3. Electrophysiology

Slice recordings were performed as previously described ([29] and Supplementary Material).

2.4. Behavioral studies

All behavioral studies started at 3 months of age. The 2-day RAWM task and FC training were performed as previously described ([30] and Supplementary Material).

2.5. Posiphen and metabolite pharmacokinetic assays

Concentrations of Posiphen, N^1-norPosiphen, and N^8-nor Posiphen in the murine plasma and brain (cerebellum) were determined by liquid chromatography-mass spectrometry/mass spectrometry at Alliance Pharma (Malvern, PA, see Supplementary Material).

2.6. Biomarker levels

The levels of APP, CTFα, and CTFβ and controls GAPDH and synaptophysin in the brain tissue were determined by semiquantitative Western blotting as described previously ([31] and Supplementary Material), whereas $A\beta_{42}$ and $A\beta_{40}$ levels were analyzed by the sandwich enzyme-linked immunosorbent assay (see Supplementary Material).

2.7. Statistics

Statistical analyses were performed blindly with respect to genotype and treatment. Results of 2-day RAWM and LTP were analyzed with two-way analysis of variance with post hoc correction (multiple comparisons). Protein biomarkers were analyzed by Data Magik Ltd, using a two-tailed one-way analysis of variance test. The rest of the studies were analyzed with t-tests. Results were expressed as mean ± standard error of the mean. The level of significance was set for $P < .05$. The actual P values obtained are presented.

3. Results

3.1. Posiphen effects on cognitive impairments in APP/PS1 mice

Memory loss is the most striking and best-known symptom of AD. In a series of experiments, we tested whether Posiphen is capable of reestablishing normal spatial memory in double transgenic animals. Both WT and APP/PS1 mice were orally treated with Posiphen or vehicle. APP/PS1 animals that had been dosed with vehicle exhibited severe abnormalities in their spatial working memory, as assessed with the 2-day RAWM test. Daily, oral treatment with Posiphen improved the behavioral performance of the double transgenic mice, without affecting the performance of WT mice (Fig. 1A). To test for visual, motor, and motivational deficits, Posiphen and vehicle-treated APP/PS1 mice and WT littermates underwent visible platform task evaluation after performing the RAWM test. We found no difference in speed (Fig. 1B) and latency period (Fig. 1C) to the platform for the various groups of mice. These data indicate that Posiphen is capable of reestablishing normal spatial working memory in APP/PS1 mice.

Another cognitive test that can be used in AD animal models is the FC, associative learning paradigm. We found no difference in the freezing behavior of the vehicle- and Posiphen-treated APP/PS1 mice compared with vehicle- and Posiphen-treated WT littermates during the training phase of the FC testing. Twenty-four hours later, we observed decreased freezing behavior in vehicle-treated APP/PS1 mice compared with vehicle-treated WT littermates, suggesting impaired contextual fear learning. However, treatment with Posiphen reestablished normal freezing in APP/PS1 mice and did not affect the performance of WT mice (Fig. 2A). Neither the APP/PS1 genotype nor Posiphen treatment affected freezing behavior during cued learning (Fig. 2B). Similarly, neither the APP/PS1 genotype nor Posiphen treatment affected the sensory perception of electric foot shock, as assessed by the electric current threshold that caused flinching, jumping, or vocalization (Fig. 2C). Taken together, these results indicate that the impairment in contextual fear learning in APP/PS1 mice can be rescued by treatment with Posiphen.

3.2. Posiphen effects on LTP impairments in APP/PS1 mice

Assessment of the function of the connection between Schaffer collateral and pyramidal neurons from CA1 stratum radiatum was performed using extracellularly recorded field excitatory postsynaptic potentials. As previously shown in double transgenics [29], we found normal basal synaptic transmission in slices from vehicle-treated APP/PS1 mice compared with vehicle-treated WT littermate slices. Next, we examined the effect of Posiphen on the synaptic plasticity at the Schaffer collateral-CA1 connection of APP/PS1 mice [29]. LTP was severely impaired in vehicle-treated double transgenic mice compared with vehicle-treated WT animals. Posiphen treatment dramatically resolved the impairment of LTP in slices of APP/PS1 mice, without modifying LTP in slices from WT littermates (Fig. 3). These findings indicate that Posiphen improves synaptic function in APP/PS1 mice.

3.3. Posiphen pharmacokinetics in APP/PS1 mice

LC-MS/MS analyses of the plasma and brain cerebellum of mice treated with Posiphen, at either dose used, showed that the levels of Posiphen and its metabolites were higher in the brain as compared with plasma: roughly 7–8 fold in the case of the 25 mg/kg, p.o. treatment (Fig. 4A) and 3–6 fold higher in the case of IP treatment with 75–50 mg/kg Posiphen (Fig. 4B). The most abundant metabolite was N^8-norPosiphen. Specifically, in the case of the 25 mg/kg, p.o. treatment (Fig. 4A), the concentration of N^8-norPosiphen is quite higher than Posiphen and N^1-norPosiphen. However, in the case of the IP 75–50 mg/kg treatment (Fig. 4B), the concentrations of the three molecules are comparable. N^1, N^8-bisnorPosiphen was not quantified, as it was found to be a minor metabolite in humans [20].

3.4. Posiphen pharmacodynamics in APP/PS1 mice and duration of observed effects

The levels of APP expression in hippocampi of mice used for the behavioral studies were evaluated by Western blot. Oral treatment with 25 mg/kg Posiphen only caused a

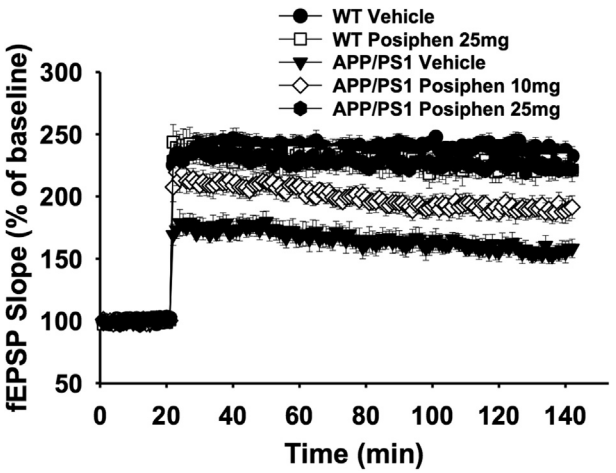


Fig. 3. Daily oral treatment with Posiphen starting at 2 months of age for 1.5 months reestablished normal potentiation in slices derived from APP/PS1 mice, as compared to saline-treated controls [WT vehicle vs. APP/PS1 vehicle: F (1, 14) = 60.54, $P < .0001$; APP/PS1 vehicle vs. APP/PS1 Posiphen 10 mg/kg: F (1, 15) = 10.72, $P = .0051$; and APP/PS1 vehicle vs. APP/PS1 Posiphen 25 mg/kg: F (1, 15) = 54.22, $P < .0001$]. WT mice were not affected by Posiphen treatment [WT vehicle vs. WT Posiphen 25 mg/kg: F (1, 14) = 0.7477, $P = .4018$]. Mean and SEM are shown. N = 8–9 per group. Abbreviations: APP, amyloid precursor protein; fEPSP, field excitatory postsynaptic potential; PS1, presenilin-1; SEM, standard error of the mean; WT, wild-type.

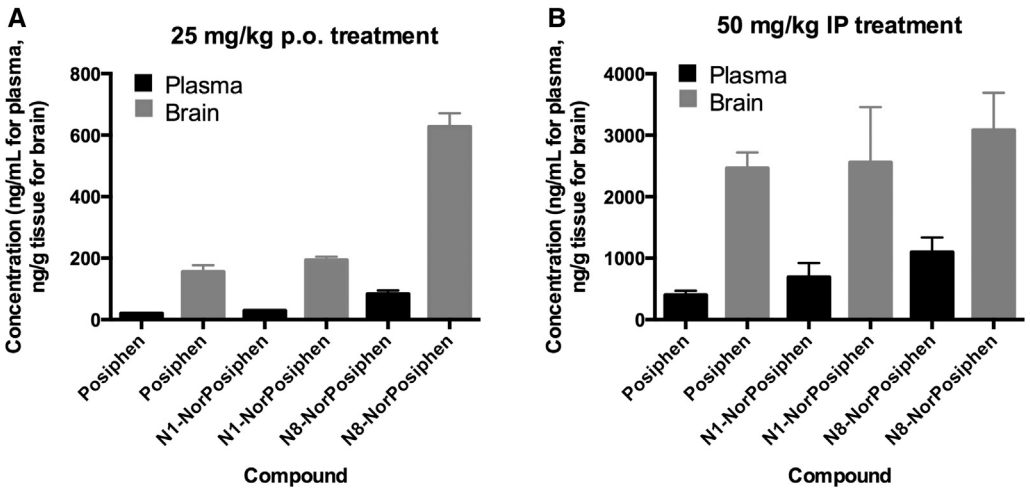


Fig. 4. Distribution of Posiphen and its metabolites in plasma and brain of APP/PS1 mice. Concentration of Posiphen and its main metabolites in plasma and brain of mice treated with 25 mg/kg p.o. for 2 weeks (A) or 75 mg/kg IP for 1 week, followed by 50 mg/kg IP for 2 weeks (B) was determined by LC-MS/MS. N8-norPosiphen is the most abundant form, while all molecules are found mostly in the brain. The brain:plasma ratio of Posiphen, N1-norPosiphen, and N8-norPosiphen is roughly 8, 7, and 7, respectively in case of (A) and 6, 4, and 3, respectively in case of (B). Mean and SEM are shown. N = 3 per group. Abbreviations: APP, amyloid precursor protein; PS1, presenilin-1; p.o., per os; IP, intraperitoneal; SEM, standard error of the mean.

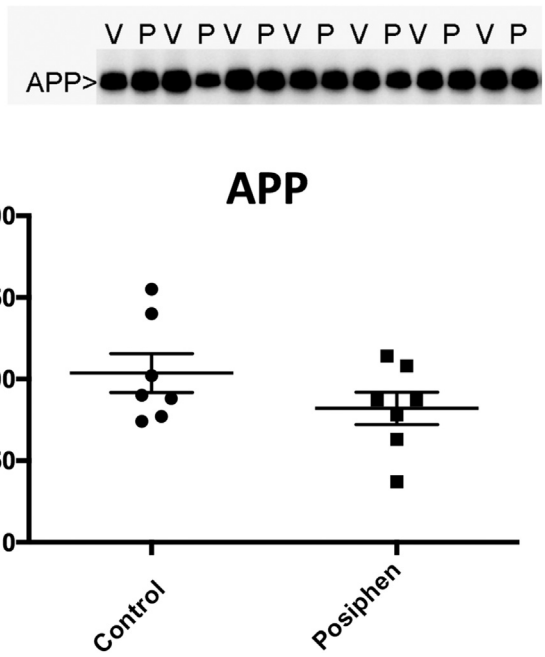
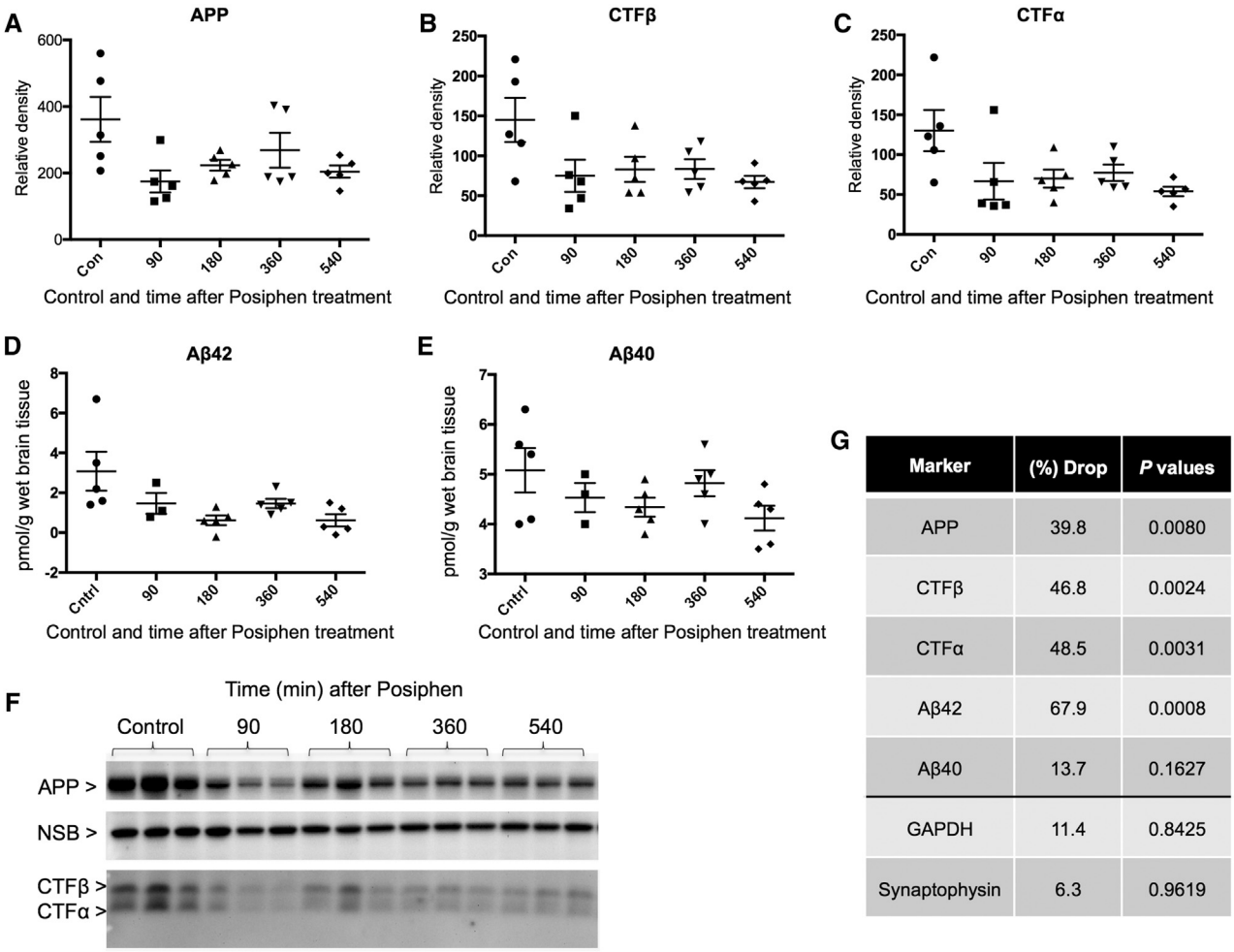


Fig. 5. Effect of daily oral treatment with 25 mg/kg Posiphen (P) (starting at 2 months of age for 1.5 months) on APP levels in the hippocampus of APP/PS1 mice, as compared to vehicle (V, saline). Representative APP Western blot and plotted APP relative densitometric units are presented. A 21% decrease of APP expression was observed in the hippocampus of Posiphen-treated mice in comparison to vehicle-treated controls, although the difference was not statistically significant (t test, $P = .186$). Mean and SEM are shown. N = 7/group. Abbreviations: APP, amyloid precursor protein; PS1, presenilin-1; SEM, standard error of the mean.

roughly 20% trend for reduction of APP expression that did not reach significance (Fig. 5).

To better evaluate the capacity of Posiphen to reduce the levels of APP and its fragments and the duration of such inhibition after the last dose, we treated a parallel series of mice with Posiphen for 21 days. Once daily, administration was performed by the IP route at an initial dose of 75 mg/kg, based on the highest tolerated Posiphen dose in WT mice, as described previously [12]. As our APP/PS1 mice became hyperactive and displayed periodic tremors, the drug dose was reduced to 50 mg/kg from day 7 onward, which had been effective in reducing APP in the hippocampus of Ts65Dn mice [19]. This dose proved to be well tolerated, and animals were euthanized at precise intervals (1.5, 3, 6, and 9 hours) after their final dose on day 21. Key AD biomarkers were quantified in the hippocampus by Western blotting (APP, CTFα, and CTFβ; Fig. 6A–6C and 6F) and enzyme-linked immunosorbent assay ($A\beta_{42}$ and $A\beta_{40}$; Fig. 6D and 6E), versus a vehicle-treated group, to confirm the previously identified reduction in biomarkers and to identify the peak time of inhibition. Posiphen treatment of APP/PS1 mice had a prolonged effect in reducing the levels of each protein. Specifically, Posiphen reduced these levels to a similar extent throughout the time frame tested (starting from 1.5 and up to 9 hours after the last dose; Fig. 6A–6E). Therefore, we compared Posiphen-treated mice from all time points as a single group against the saline-treated control group to determine the percentage of protein expression in comparison to control (Fig. 6G). Posiphen treatment reduced the levels of APP, CTFα, and CTFβ in the hippocampus by approximately 40%–50% and of $A\beta_{42}$ by 68% in comparison to saline, consistent with inhibition of synthesis of APP (Fig 6G). In the case of $A\beta_{40}$, there was only a trend for reduction that did not reach significance. We controlled for equal protein loading with a nonspecific band, recognized by the APP antibody that was not affected by the treatment (Fig. 6F), GAPDH and synaptophysin (Fig. 6G, Western blot data not shown), but we are presenting non-normalized data.



Marker	(%) Drop	*P* values
APP	39.8	0.0080
CTFβ	46.8	0.0024
CTFα	48.5	0.0031
Aβ42	67.9	0.0008
Aβ40	13.7	0.1627
GAPDH	11.4	0.8425
Synaptophysin	6.3	0.9619

Fig. 6. Posiphen treatment of APP/PS1 transgenic mice produces a prolonged decrease in biomarker levels in the hippocampus. Mice were treated IP with saline (control group) or Posiphen at 75 mg/kg for 1 week and 50 mg/kg for 2 weeks. The mice were euthanized in batches of five at 1.5, 3, 6 and 9 hours after the final Posiphen treatment. The seventh in a series of 1-mm thick coronal brain slices was used to compare the levels of APP (A), CTFβ (B), CTFα (C) by Western blots, and $A\beta_{42}$ (D) and $A\beta_{40}$ (E) by ELISA. No trend for recovery of biomarker levels is observed up to 9 hours. Relative densitometric units are presented for (A–C). Mean and SEM are shown. N = 5 per group. (F) Representative example of Western blots for APP, CTFβ, and CTFα. An NSB indicates equal protein loading. (G) The data corresponding to all time points after the last Posiphen dose were averaged and used to calculate the percentage of reduction in expression levels of biomarkers or Western blot loading controls (GAPDH and synaptophysin) after Posiphen treatment, in comparison to the control group (=100%). The expression levels at all time points after Posiphen treatment versus control group were compared by one-way ANOVA, resulting in the listed *P* values. Abbreviations: Aβ, amyloid β; ANOVA, analysis of variance; APP, amyloid precursor protein; CTF, carboxy-terminal fragment; ELISA, enzyme-linked immunosorbent assay; IP, intraperitoneal; NSB, nonspecific band; PS1, presenilin-1; SEM, standard error of the mean.

4. Discussion

Here, we studied the effects of translational inhibition of APP by Posiphen in the APP/PS1 mouse model of AD. First, we examined effects on cognition and LTP, phenomena underlying synaptic plasticity, and then we followed the paradigm of the phase I clinical trial [20] and investigated the pharmacokinetics and pharmacodynamics of Posiphen in our mouse AD model.

The most novel and critical findings of our study are that Posiphen normalizes impairments in spatial working memory, contextual fear learning, and synaptic function in APP/PS1 mice, without causing visual, motor, and motivational deficits. To our knowledge, this is the first study demonstrating the therapeutic efficacy of Posiphen in an AD mouse model. Importantly, these effects were caused by a low-Posiphen p.o. dose (25 mg/kg), which only resulted in a trend for reducing APP levels. In fact, 25 mg/kg Posiphen treatment did not reduce APP expression levels in Ts65Dn mice either [19]. The efficacy of the low dose in improving cognition and LTP suggests that even a small reduction of APP and its toxic fragments is of significant biological consequence. Furthermore, it suggests that behavioral improvements should be the primary read out in future preclinical or clinical studies, instead of levels of APP and its fragments. Finally, it is worth mentioning that the even lower 10 mg/kg p.o. dose of Posiphen, for which the APP brain levels were not tested, normalized contextual fear learning and

significantly ameliorated impairments in synaptic dysfunction, but the improvement in spatial working memory of APP/PS1 mice did not reach significance. Hence, the 25 mg/kg p.o. dose might be a better choice for efficacy in future spatial working memory animal studies. Notably, the Posiphen concentration in plasma associated with this dose in mice was in the order of 6 fold lower that determined in mildly cognitively impaired subjects following a 60-mg dose [17], suggesting that our mouse studies are of translational relevance.

The pharmacodynamics study was carried out to investigate the capacity of a higher Posiphen dose to reduce levels of APP and its fragments in our model, as well as the duration of those effects. As expected [19], the 75–50 mg/kg IP Posiphen dose in our mouse study lowered the levels of both APP and its fragments CTFα and CTFβ by similar factors (∼40%–50%), in agreement with previous reports showing that (1) the reduction of APP expression by Posiphen in cell culture leveled off at ∼50% in comparison to controls [13] and (2) Posiphen treatment reduced the levels of sAPPα and sAPPβ of mildly cognitively impaired patients down to nearly 50% of their pretreatment values, which was about equal to levels in healthy volunteers [20]. $A\beta_{42}$ was reduced by 68%. $A\beta_{40}$ showed a similar pattern to $A\beta_{42}$, but with larger scatter of the data points, causing the level of reduction to be very small. Finally, the prolonged effect of Posiphen treatment on biomarker expression over at least 9 hours following the last dose (the maximum time tested) is in line with the human study data, where the effect of Posiphen on biomarkers remained present for more than 12 hours [20]. This should be considered when designing dosing in future animal and human studies.

When comparing the present mouse pharmacokinetics data to the previous rat and human data [20], we note that, in both cases, Posiphen is preferably distributed to the brain, with a similar brain/plasma concentration ratio. However, although in human plasma and rat plasma and brain, the compound with the highest concentration was Posiphen [20], N^8-norPosiphen proved most abundant in both tissues in mice. Notably, in human cerebrospinal fluid, the most abundant metabolite was N^8-norPosiphen [20]. Finally, N^8-norPosiphen has been shown to be equipotent to Posiphen in reducing APP expression and has no ACHEI activity [14].

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.trci.2017.12.001.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using traditional sources (e.g., PubMed). Despite the existence of mechanistic and proof of concept studies showing that Posiphen lowers amyloid precursor protein and amyloid β levels under experimental conditions and in humans, the therapeutic efficacy of the drug in Alzheimer's disease had not been studied.
2. Interpretation: In line with existing literature, Posiphen's ability to reach the brain tissue and inhibit amyloid precursor protein translation resulted in reduced amyloid levels in APP/PS1 mice. In addition, we showed for the first time that Posiphen normalizes symptoms of Alzheimer's disease in our mouse model, specifically, deficits in spatial working memory, fear associative memory, and long-term potentiation.
3. Future directions: Posiphen is a promising drug for the treatment of Alzheimer's disease, and further preclinical and clinical studies are warranted.

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