Posiphen®: Pharmacokinetics and Mechanism of Action in CSF and Plasma of Mildly Cognitive Impaired Patients

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Abstract

Posiphen® is in clinical development as an oral treatment for Alzheimer's disease (AD). In cell cultures, normal, transgenic and trisomic mice, Posiphen reduces the rate of synthesis of amyloid-β precursor protein (APP) and, thereby, lowers levels of Aβ and all other toxic peptides generated from APP processing.

We decided to conduct a trial in mild cognitive impaired (MCI) patients to confirm this mechanism of action (reduced rate of APP synthesis) in humans and correlate it with the pharmacokinetics of the drug and its metabolites in CSF and plasma. This study evaluates the activity of Posiphen and its metabolites in the CSF of patients with MCI and their relationship to central and peripheral pharmacokinetic parameters.

The patients tolerated the procedure well, and we have data indicating that Posiphen reduces circulating APP levels in CSF and plasma, consistent with inhibition of APP synthesis as a key mechanism of action of Posiphen in humans. We also have extensive pK data of Posiphen and its metabolites in CSF and plasma.

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Introduction

Major hallmarks of Alzheimer's disease (AD) are synaptic loss, brain shrinkage and abnormal protein deposition, particularly amyloid plaques and neurofibrillary tangles. Current AD drugs on the market provide symptomatic relief and improve cognition. Cholinesterases inhibitors (ChE-Is) and memantine are the only FDA approved drugs for AD.

Posiphen® tartrate, an inhibitor of amyloid precursor protein (APP) synthesis[1], is being developed by QR Pharma as a potential disease modifying treatment for AD. Through APP inhibition, Posiphen may halt or slow disease progression by reducing amyloid-β peptide (Aβ) generation, the substrate that forms toxic oligomers. Evidence in the literature suggests that targeting the accumulation of Aβ - a hydrophobic, neurotoxic self-aggregating 40 to 42 amino acid peptide that accumulates preferentially within amyloid plaques in the brain - could change the course of AD[2]. Other data from Genentech suggests that APP in the absence of trophic factors is shed from the surface of neuronal cells and processed into an amino terminal fragment (N-APP) that binds to DR6 receptors and induces nerve cell death[3]. Others have identified a further factor that is cleaved from the C-terminal end of APP (C31) and causes nerve cell degeneration and death in tissue culture cells and in transgenic mice[4] **(Figure 1).** Reducing APP synthesis could be beneficial to the brain, as via the Aβ pathway neurotoxic oligomers and plaque are reduced and via the inhibition of N- and C- terminal fragments nerve cell death is inhibited and brain cells are preserved.

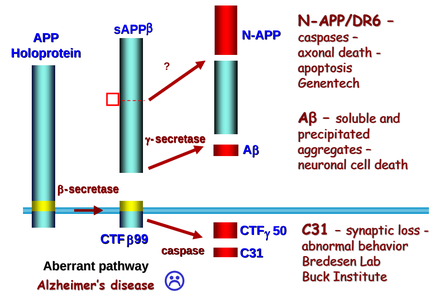


Figure 1. Aberrant Processing Pathway: in the absence of neurotrophic factors β secretase cleaves APP at the N-terminal end of Aβ and leads to the subsequent cleavage of other toxic APP fragments.

Mechanism of Action

Posiphen acts post-transcriptionally[1,5,6] by lowering newly synthesized APP levels, as determined by a brief 10-min incubation in the presence of ^{35}S-labeled amino acids. In contrast, both APP mRNA levels and total protein levels were unaffected (p>0.05, Dunnett) **(Figure 2).**

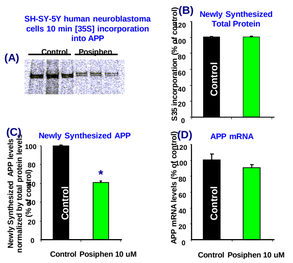


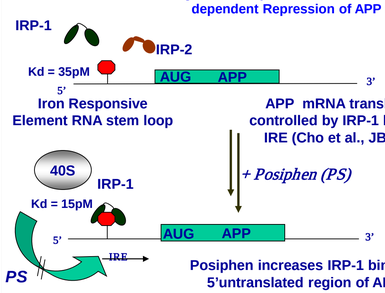
Figure 2: Posiphen lowers the rate of APP synthesis in SH–SY–5Y human neuroblastoma cells by translational regulation. Translation was assessed by addition of [^{35}S]methionine and cysteine for 10 min followed by immunoprecipitation; (1) newly synthesized APP protein was reduced; (2) Newly synthesized total protein was unaffected; (3) Posiphen (10 μM) significantly decreased newly synthesized APP levels (50% reduction, p < 0.05, Dunnett); (4) Treatment with Posiphen did not affect APP mRNA levels (p > 0.05, Dunnett).

The action of Posiphen to lower APP *in vitro* translated to *in vivo* efficacy, as tested in normal, transgenic AD and trisomic mice models[1,7,8].

After Posiphen administration there was a concentration-dependent 50% maximal decline in APP and Aβ . This was achieved with a daily dose of 15 mg/kg i.p. or oral Posiphen, with drug doses greater than 25 mg/kg providing no further action. (Ref 1 and **Figure 4**)

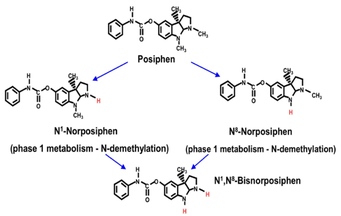
Posiphen's mechanism of action was studied in laboratory of Jack Rogers[6,7,9]. Iron influx increases the translation of APP via an iron-responsive element RNA stem loop in its 5'UTR region. Iron Regulatory Protein 1 (IRP1), but not IRP2, selectively binds the APP IRE with an affinity of 35pM. When IRP1 is bound to the stem loop, it prevents it from binding to the ribosome and prevents translation of the mRNA. Addition of Posiphen to SH-SY-5H human neuroblastoma cells increases the affinity of IRP1 to the stem loop structure to15 pM and lowers the rate of translation of the mRNA by the ribosome (**Figure 3 below**).

Figure 3: Posiphen activates Iron-regulatory protein dependent Repression of APP mRNA Translation



Posiphen and Metabolites

Figure 3. Structure of Posiphen and generation of primary metabolites.



Posiphen in vivo generates 3 primary metabolites, N1-Norposiphen, N8-Norposiphen and N1,N8-Norposiphen.

Posiphen and its metabolites show various activities that are different from one another (**Table 1**). As a good approximation we can say that Posiphen and mebabolites all act on the 5'UTR, and that only N1 has major AChEI activity.

Table 1. Differential actions of Posiphen and primary metabolites

Inhibition of	Posi	N1	N8	N1,N8	Investigator/Lab
APP	+	+	tbd	+	Debomoy Lahiri/ IU
Abeta	+	+	tbd	+	Debomoy Lahiri/ IU
APPneo/C31	+	0	0	+/-	Varghese John/Buck
alpha Synuclein	+	tbd	tbd	+	Jack Rogers/MG
5'UTR Luciferase	+	tbd	tbd	+	Jack Rogers/MG
AChE	0	++	0	+/-	Nigel Greig/NIA
BChE	0	0	0	0	Nigel Greig/NIA

Methods

Recent reports suggest high endogenous variability of Aβ peptides that may be altered by diurnal fluctuations and/or by fed vs. fasted states. Even though these reports have been disputed[10], there is consensus that intra-subject variability is low, whereas inter- subject variability can be quite high. Also intra- and inter-subject variability appear to be greater in CSF than in plasma. To avoid potential inter-subject variability, we decided to use subjects as their own controls and measure CSF and plasma levels for 12 hours both prior to and following Posiphen dosing of 10 days.

5 MCI patients received Posiphen at 240 mg/ day for 10 days (4 x 60 mg was found in a multiple dose safety study to be well tolerated in elderly healthy volunteers). Serial CSF samples were collected via an indwelling lumbar catheter for 12 hours one day before the start of dosing and immediately after the last dose. Plasma samples were taken at the same sampling times. CSF and plasma/serum samples were analyzed for pK and pD.

Clinical Results - Human Safety

Posiphen was tested in 3 phase I human safety trials:
- A single ascending dose (SAD) in healthy, elderly volunteers
- A multiple ascending dose (MAD) safety trial in healthy, elderly volunteers
- A multiple ascending dose (MAD) trial in MCI patients

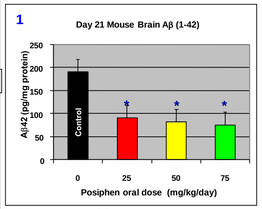
Table 2. Posiphen human safety trials

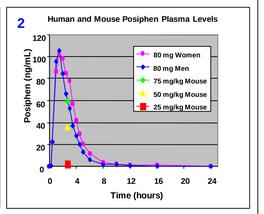
	Dose	Duration
SAD Healthy	160mg	1 Day
MAD Healthy	4x60 mg	10 Days
MAD MCI	4x60 mg	10 Days

Posiphen was found to be well tolerated at doses expected to be 4 to 6 times higher than needed for pharmacological efficacy. The no effect dose was the same for healthy volunteers and MCI patients (**Table 2).**

Efficacious Dose Comparison Mouse/Human Plasma

Figure 4: (1) Mouse brain levels of $A\beta_{1-42}$ are lowered by 21 day oral administration of Posiphen. Relative to vehicle control, Posiphen 25, 50 and 75 mg/kg/day oral dosing reduced mean brain $A\beta_{1-42}$ levels by 52.3%, 56.9%, and 60.2%, respectively (p < 0.01, Dunnetts). **(2)** After the last dose animals were sacrificed at 3 hours and the mean mouse Posiphen plasma concentrations were measured at the time of brain sample collection . These levels were plotted under a plasma concentration curve of a well tolerated Posiphen concentration 80 mg single dose (determined by LC-LC-MS). A comparison of the mouse plasma concentrations that resulted in 50%inhibition of APP/Abeta with the human plasma concentrations at 80 mg shows the expected efficacious drug dose in humans to be about 40 ng/ml, which corresponds to 40 mg per day.





Human pK

Posiphen readily enters the brain in mice and rats with brain levels ranging from 8 to 10 times higher than plasma levels. Comparison of rodent brain CSF, brain and plasma levels with human CSF and plasma levels allowed us to extrapolate to human brain levels for once a day dosing as well as 4 times a day dosing (**Table3).** Posiphen readily achieves level in human brain that allow for maximum inhibition of APP. From Table 3 the effective dose can be calculated to be around 2 x 40 mg/day, which compares well to the Axonyx study shown in Figure 4.

Table 3. APP Inhibitory Activity in Humans Extrapolated

		Extrap 1x/Day		Extrap 4x/Day	
Posiphen	Plasma	Brain	Br Conc	Brain	Br Conc
mg	ng/ml	ng/mg	μm	ng/mg	μm
10	4	35	0.087	77	0.19
20	15	135	0.4	310	0.8
40	50	450	1.1	1350	3.37
60	110	1000	2.5	3500	8.75
80	130	1200	3.1		
160	380	3500	8.7		

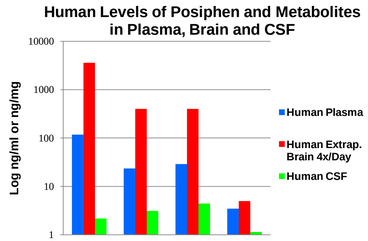
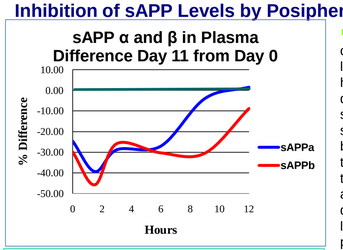
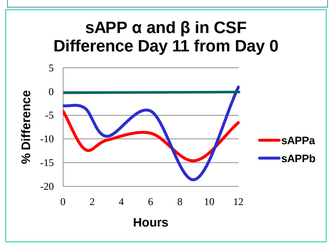


Figure 5: Plasma CSF and Extrapolated Brain Levels of Posiphen and Metabolites following 10 days of 4 x 60mg oral administration of Posiphen to MCI patients.

Inhibition of sAPP Levels by Posiphen





MCI patients were catherized in the lumbar spine for 12 hours at day 0 and at day 11 and CSF samples were taken at specific intervals; blood samples were taken at the same time. Posiphen administration for 10 days lowered the levels of sAPP in plasma by 50% and in CSF by 20% consistent with Posiphen's mechanism of action. **Figure 6: top – plasma & bottom - CSF. T**he two diagrams correspond well with the levels of Posiphen and its metabolites which in plasma are highest at 1.5 and 4 hours and in CSF/brain are highest at 2 to 3 and 8 hours, respectively.

Conclusion

We conducted a human mechanism of action trial in MCI patients and measured the pK of Posiphen and metabolites as well as their pD.

- The patients tolerated the procedure well.
- The human pK of Posiphen and metabolites confirmed that Posiphen enters the brain readily and stays there longer than in plasma. CSF levels are low and measureable.
- Posiphen reduces sAPP levels in CSF and plasma, consistent with the inhibition of APP synthesis as a key mechanism of action of Posiphen in humans.
- Combining the current human pK/pD data with prior preclinical studies in mice and rats allows an estimate of an effective dose in humans of 2x 40mg/day.
- Factors that could be measured:
  - Abeta 38/40/42
  - APP neo/C31
  - Tau/ phospho Tau
  - alpha Synuclein
  - Neurotrophic Factors
  - Inflammatory Factors
  - AChEI/BChEI
  - Suggestions???
- Posiphen appears to be a promising experimental drug for AD as it can effectively lower brain levels of APP by reducing mRNA APP translation in all species tested including humans.

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