Mechanism of Action of Posiphen® in CSF of Mildly Cognitive Impaired Patients

H.W. Holloway[5], N.H. Greig[5], V. John[4], C. Pan[3], M. Murphy[2], MY Chang[1], M.L. Maccecchini[1]

[1]QR Pharma, Radnor, PA 19087; [2]Worldwide Clinical Trials, King of Prussia, PA , 19406; [3]Inarian Neurodiagnostics, Mercer Island, WA 98004;

[4] Buck Institute, Novato, CA 94945, [5]Laboratory of Neurosciences, Intramural Research Program, National Institute on Aging, NIH, Baltimore, MD 21224

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 conducted a clinical trial in mild cognitive impaired (MCI) patients to confirm this mechanism of action (a reduction in the rate of APP synthesis) in humans and correlate it with the pharmacokinetics of the drug and its metabolites in CSF.

Introduction

Major hallmarks of Alzheimer's disease (AD) are synaptic loss, brain shrinkage and abnormal protein deposition, particularly of amyloid plaques and neurofibrillary tangles. Current AD drugs on the market provide symptomatic relief and improve cognition.

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]. 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 further fragments cleaved from the C-terminal end of APP (e.g. C31) that cause 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 plaques would be reduced and, via the inhibition of N- and C- terminal fragments, nerve cell death would be inhibited and brain cells preserved.

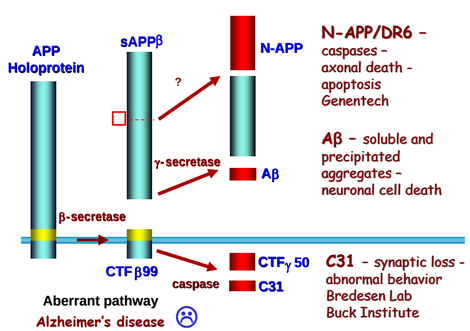


Figure 1. APP Aberrant Processing Pathway

Mechanism of Action

Posiphen acts post-transcriptionally[1,5,6] by lowering newly synthesized APP levels **(Figure 2).**

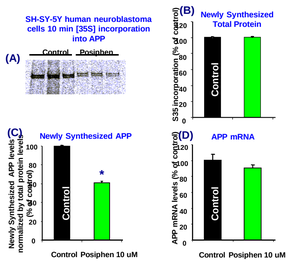
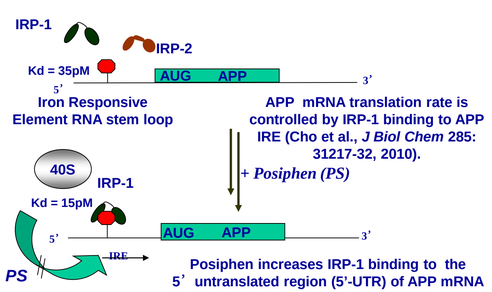


Figure 2: Posiphen lowers the rate of APP synthesis in SH–SY–5Y human neuroblastoma cells by translational regulation. Translation was assessed by [^{35}S]methionine addition for 10 min followed by immunoprecipitation; (1) newly synthesized APP protein was reduced (A&C); (2) newly synthesized total protein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

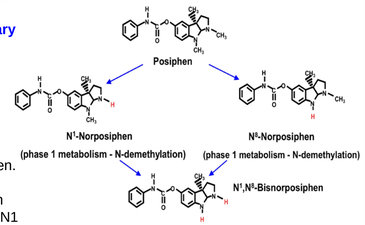
Posiphen's mechanism of action was studied at the molecular level in the laboratory of Jack Rogers[5] (Mass General Hospital, Charlestown, MA). Iron influx increases the translation of APP via an iron-responsive element (IRE) RNA stem loop in its 5'-UTR region. Iron Regulatory Protein 1 (IRP1), but not IRP2, selectively binds the APP IRE with an affinity of 35pM[6]. 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 from 35 pM to15 pM and lowers the rate of translation of the mRNA by the ribosome (**Figure 3**).

Figure 3: Posiphen Increases Iron-Regulatory Protein Dependent Repression of APP mRNA Translation



Posiphen and Metabolites

Figure 4. Structure of Posiphen and generation of primary metabolites.



Posiphen *in vivo* generates 3 primary metabolites, N1-Norposiphen, N8-Norposiphen and N1,N8-Bisnorposiphen. Posiphen and its metabolites all act on the 5'-UTR and only N1 had AChE inhibitory activity **(Table 1.)**

Table 1: Differential Action of Posphen and Metabolites on APP, α-Synuclein and Acetylcholinesterase (AChE)

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
α-Synuclein[7]	+	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[8], 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 used subjects as their own controls and measured CSF and plasma levels for 12 hours both prior to and following Posiphen dosing for 10 days.

Five MCI patients received Posiphen at 240 mg/day for 10 days (4 x 60 mg = 240 mg/day) – a dose that 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

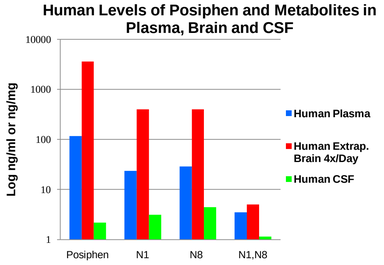
PK – Posiphen and metabolites

PD – sAPPα, sAPPβ, Aβ42, tau, phospho tau

Human PK

- Posiphen readily enters the brain in mice and rats with brain levels ranging from 7 to 9 times higher than concurrent plasma levels. Comparison of rodent 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 **(Figure 5).**
- Posiphen readily achieves level in human brain that allow for maximum inhibition of APP. From our data, the effective dose can be calculated to be around 2 x 40 mg/day.

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



Human PD

- Posiphen administration for 10 days lowered the levels of sAPPα, sAPPβ, tau and phospho-tau (pTau) by about 40% in CSF, consistent with Posiphen's mechanism of action - (**Table 2)** below.

Table 2: Posiphen Affects Biomarkers in CSF

All PD determinations were quantified by at least two independent laboratories using different kits and different antibodies. The data is comparable and consistent.

Human Biomarker	CSF	Assay	Laboratory
sAPP α	-34%	MSD	MY Chang / QR Pharma
	-33%	AlphaLisa	V. John / Buck Institute
sAPP β	-36%	MSD	MY Chang / QR Pharma
	-45%	AlphaLisa	V. John / Buck Institute
Tau	-40%	AlphaLisa	V. John / Buck Institute
	-38%	Innogenetics	C. Pan / Inarian
pTau	-31%	Innogenetics	C. Pan / Inarian
Aβ42	+/-0	Innogenetics	C. Pan / Inarian

Comparison with Healthy Volunteers

- Posiphen given for 10 days to MCI patients lowers their sAPPα, sAPPβ and tau levels back to the levels found in healthy volunteers **(Figure 6).**

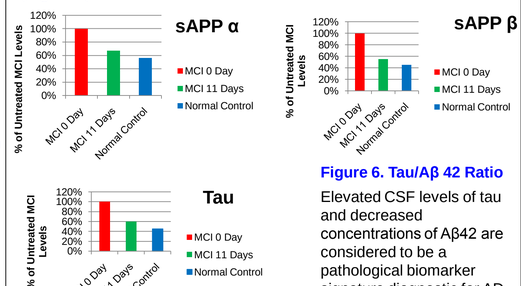


Figure 6. Tau/Aβ 42 Ratio

Elevated CSF levels of tau and decreased concentrations of Aβ42 are considered to be a pathological biomarker signature diagnostic for AD. The ratio tau/Aβ is used to estimate progression to AD. Posiphen shifts this ratio toward non-AD subjects

Ratio	MCI 0 Day	MCI 11 Days	non AD
Tau/Aβ42	0.56	0.35	0.31

Conclusion

- The patients tolerated the procedure well.
- The human PK of Posiphen and metabolites confirmed that Posiphen enters the brain and readily reaches levels 7- to 9-fold higher than in plasma at steady-state. Its half-life in brain is12 hours versus 5 hours in plasma
- Posiphen in humans lowers sAPPα and sAPPβconcentrations, consistent with its mechanism of action.
- Posiphen also reduces tau and phospho-tau by 40%.
- 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 40 mg/day.
- 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[1,9,10] including humans. Additionally it also inhibits tau and phospho-tau.

References

1. Lahiri DK, et al., *J Pharmacol Exp Ther*. 320:386-396, 2007.
2. Selkoe DJ. *Arch Neurol*. 62:192–195, 2005.
3. Nicolaev A, et al., *Nature* 457: 981-9902, 2009.
4. Galvan V, et al. *PNAS* 98: 7605-7610, 2001.
5. Rogers JT, et al., *J Biol Chem*. 277:45518-28, 2002.
6. Cho HH, et al., *J Biol Chem* 285:31217-32, 2010.
7. Rogers JT et al., *J Neural Transm*. 2011 Jan 8. [Epub ahead of print]
8. Soares H, et al., (Pfizer, New London, CT) ICAD 2009 poster P1-259 (Vienna, Austria)
9. Marutle A, et al., *PNAS* 104: 12506-11, 2007.
10. Salehi A, et al., (Dept. Neurology, Stanford Univ., CA) ICAD 2008 poster (Chicago, IL)

Supported by QR Pharma,Inc.

Contact Info: Maria Maccecchini: **maccecchini@qrpharma.com**
or Nigel H. Greig: **greign@mail.nih.gov**