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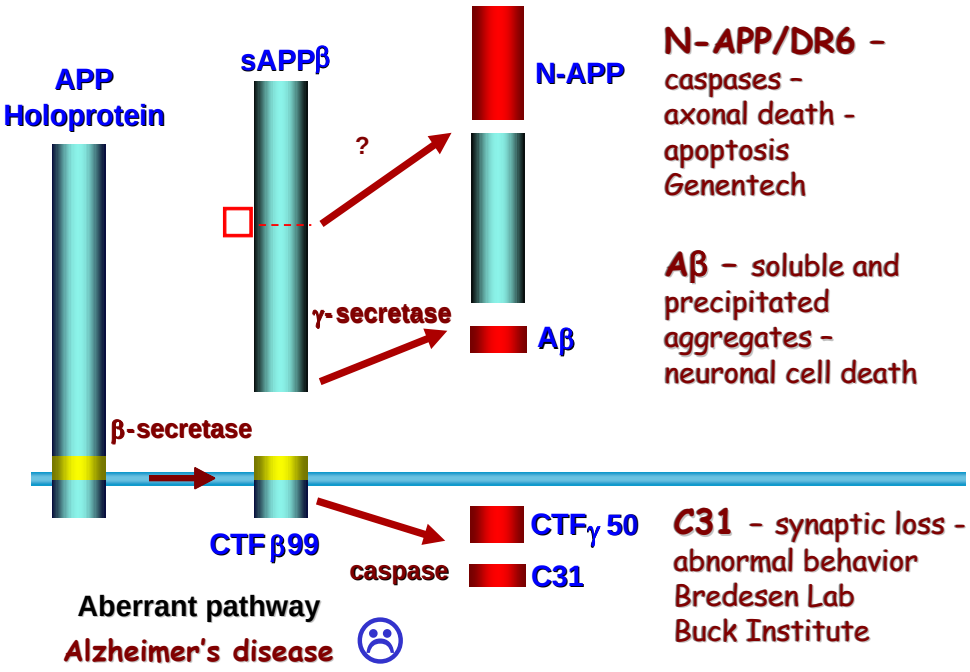
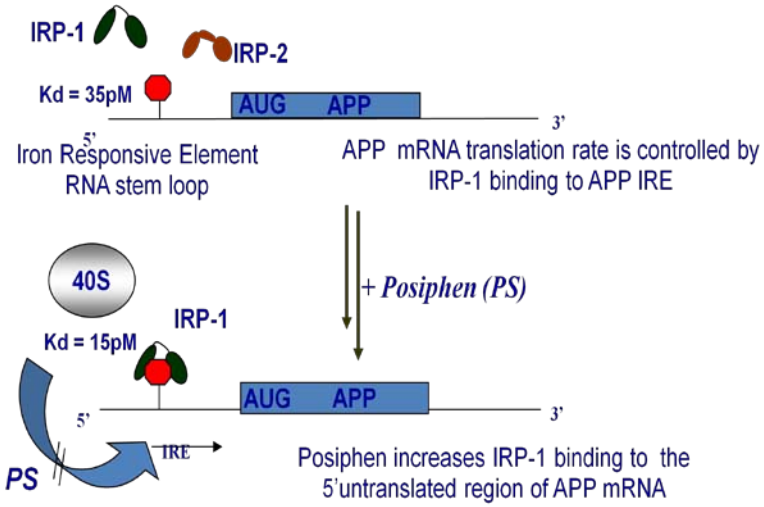
Posiphen® Technical Summary

Introduction:
Posiphen® tartrate, an inhibitor of amyloid precursor protein (APP) synthesis (D.K. Lahiri et al. JPET 320, 12506-125511, 2007[1]), is being developed by QR Pharma as a potential disease modifying treatment for Alzheimer's disease (AD). Through APP inhibition, Posiphen may halt or slow disease progression by reducing APP levels and accordingly β amyloid (Aβ). 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 senile plaques (SP) in the brain, could change the course of AD (D.J.Selkoe, Arch Neurol 62, 2005, 192-195). Other data from Genentech show 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 (A. Nicolaev et al, Nature, Vol 457, 2009, 981-990[2]). The Bredesen Lab identified another 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 (V. Galvan et al. PNAS, Vol 103, No. 18, 2006, 7130–7135[3]).

APP Aberrant Processing Pathway: In the absence of neurotrophic factors β-secretase cleaves APP at the N-terminal end of Aβ and initiates a cascade that leads to the subsequent cleavage of toxic APP fragments. Inhibition of APP, therefore, leads to lower APP levels and lower toxic fragments derived from the precursor. Reducing APP could be beneficial to the brain, because through the Aβ pathway neurotoxic plaque is reduced and through inhibition of toxic N- and C-terminal fragments nerve cell death is inhibited and brain cells are preserved.



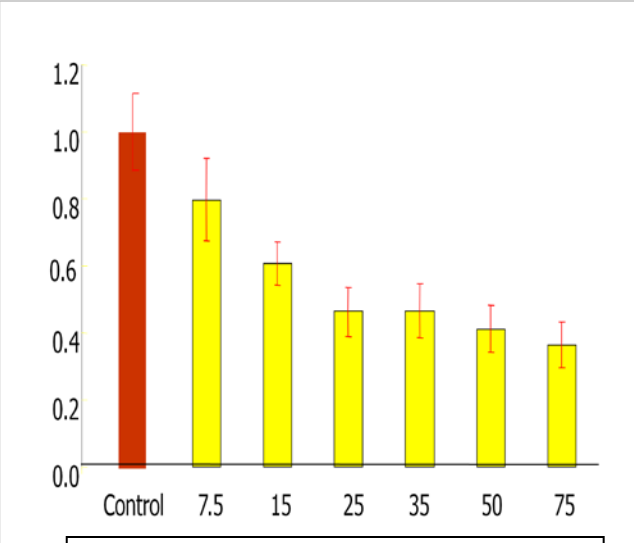
Mechanism of Action:



Posiphen's mechanism of action was studied in the laboratory of Jack Rogers (Cho HH, et al., *J Biol Chem* E-online June). Iron increases the translation of APP by binding to an iron-responsive element (IRE) that forms a stem loop structure in the 5' untranslated region (5'UTR) of APP mRNA. Iron regulatory proteins bind to these stem loop structures and suppress the iron activation of mRNA translation. It turns out that in the case of APP 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 the complex 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

Preclinical:

QR's lead compound, Posiphen tartrate, was invented by Nigel Greig and his group at NIA/NIH (Q-S, Yu, et al., J. Med. Chem. 40, 2895-2901, 1997). The compound acts on the 5' UTR of APP mRNA and inhibits its translation into APP protein, which leads to lower levels of APP, Aβ and β-secretase (D.K. Lahiri et al. JPET 320, 12506-125511,). Posiphen also lowers the levels of N- and C-terminal toxic peptides cleaved from the precursor (Varghese John, Buck Institute, unpublished).



Dose-dependent reduction of APP by Posiphen in brain of mice.
x-axis: Posiphen dose in mg/kg, y-axis: control =1

Posiphen inhibits APP synthesis and was shown to reduce APP and Aβ in both cell lines and animals (K.T.Y. Shaw et al., PNAS 98: 7605-10, 2001). In all primary cultures or cell lines tested APP and Aβ was reduced in a dose-dependent fashion by up to 60% with an IC50 of 600 nM. Mice dosed orally with Posiphen and sacrificed at 7 and 21 days also showed a parallel dose-dependent decrease in brain levels of APP as well as Aβ42. At the lower dose of 25 mg/kg the reduction was 40% and at the two higher doses of 50mg/kg and 75 mg/kg the reduction was 60% for both biomarkers (E. Cullen et al., Poster, Intl. Geneva /Springfield Symposium, 2006). Another study confirmed the reduction in APP and Aβ42 to be dose dependent and level off at a maximum reduction et al. J Pharmacol Exp

In transgenic AD mice Posiphen was shown to reduce APP, improve stem cell survival, recruit stem cells, support differentiation of stem cells into neurons rather than glia and astrocytes and increase BDNF (A. Marutle et al., PNAS Vol 104 # 30, 12506-). In a study of trisomic mice (Ts65Dn, a model for Down Syndrome) Posiphen was shown to reduce APP levels while doubling NGF levels (A. Salehi et al., Poster, ICAD)

Animal Toxicity:
The preclinical safety profile of Posiphen was determined from single oral and intravenous toxicity studies in mice, rats and dogs and from repeated dose oral toxicity studies of up to 30 days in rats and dogs. The results showed that repeated oral administration of Posiphen for up to 30 days to rats and dogs was associated with central nervous system and gastrointestinal effects starting at 30 mg/kg/day (corresponding to about 2.25 grams/human/day). The incidence and severity was dose-dependent and reversible. The NOAEL (no observed adverse effect level) was 20mg/kg/day in dogs and 30mg/kg/day in rats.

Posiphen Metabolites and their Activities:
Metabolism of Posiphen was performed in hepatocytes of 5 species: mice, rats, dogs, monkeys and humans. The same three major metabolites were found in all five species. We synthesized them (two single demethylated N1- and N8-norposiphen and one double demethylated N1,N8-norposiphen) and tested them in a variety of tissue culture systems for their disease modifying activity on the 5'UTR of APP and on their symptomatic ChEI activity.

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

As a good approximation we can deduce from the table that Posiphen and metabolites all act on the 5'UTR and that only N1 has major AChEI activity.

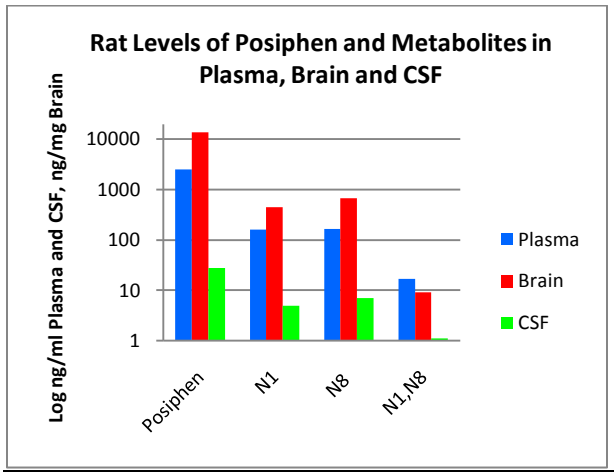
Clinical:
Single and Multiple Ascending Dose Safety: Posiphen was tested in single and multiple ascending dose phase I trials and found to be safe. The Phase 1a study in 60 patients tested single doses ranging from 10 mg to 160 mg per day. In the Phase 1b study, Posiphen was administered orally to 48 patients in doses of 20, 40, and 60 mg QID for 10 days. Single doses were given on the first and last day to determine the pharmacokinetics of the drug. In general the incidence of adverse events, all of mild severity, was somewhat greater at higher Posiphen doses, but most AEs also occurred with similar frequency in the placebo group. Posiphen was absorbed rapidly after oral administration, achieving Cmax within 1.2-1.5 hours and mean T ½'s of 3.8 to 5.2 hours. The Cmax increased disproportionally with dose. There is an active IND and sufficient clinical supplies to continue development

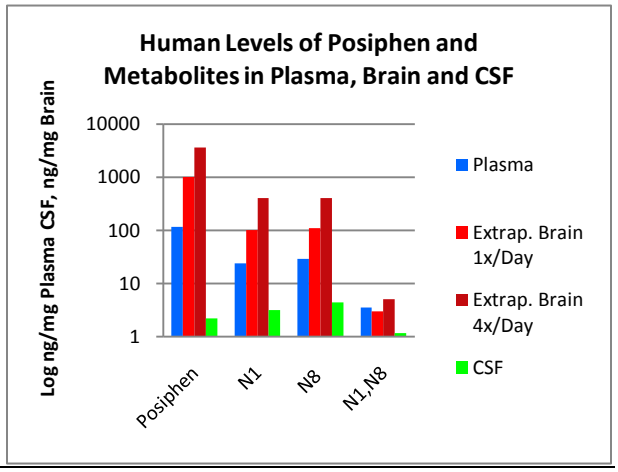
Mechanism of Action (MOA) Study: The company recognized that in order to start a phase II study to measure improvement in cognition as the endpoint and bring Posiphen to market, there were a number of questions that needed to be answered: Does Posiphen get into the brain and once there what does it do? We decided to study mild cognitive impairment (MCI) patients, treat them with Posiphen for 10 days and determine the pK of the compound and its metabolites as well as the levels of APP and other AD associated biomarkers in CSF and plasma. Prior clinical phase I safety data determined 240 mg/day (4 x 60mg) to be the maximum tolerated dose in healthy volunteers,

so we decided to use it for our study. MCI patients were catherized in the lumbar spine for 12 hours prior to administration of the drug at Day 0 and then again after the last dose at day 11; CSF and plasma was drawn over a 12 hour period at both time points.

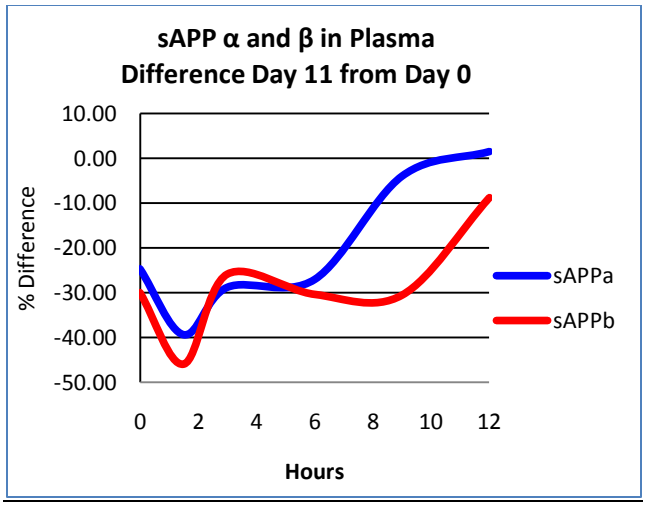
pK of Posiphen and Metabolites: We measured Posiphen and metabolites in plasma of mice, rats dogs and humans; in brain of mice and rats and in CSF of rats and humans after single and multiple dose administration of up to 3 weeks. In all cases we see that in plasma Posiphen achieves the highest levels, with the N1- and N8 metabolites reaching about 20 to 30% of that level and N1, N8-norposiphen being a minor metabolite that barely reaches 1% of Posiphen levels. In brain we again see Posiphen as the major compound at 90 minutes with N1- and N8 reaching higher levels than Posiphen at 180 minutes. Again N1, N8-norposiphen plays a very minor role. We also measured binding to human and mouse brain homogenate and found that Posiphen and metabolites are about 96% bound and 4% free.

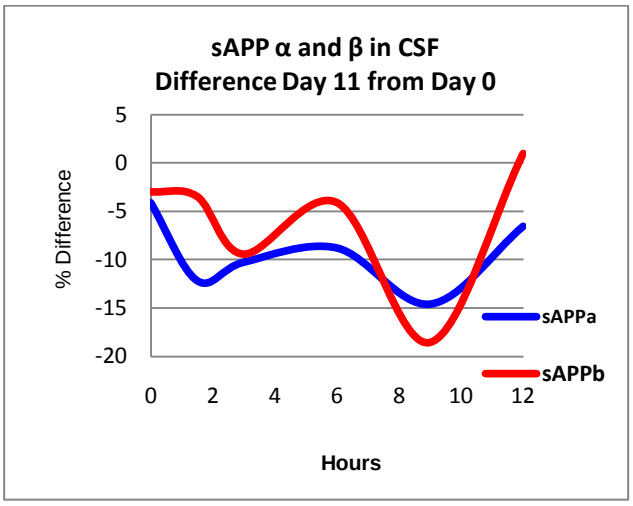
Posiphen readily enters the brain in mice and rats with brain levels ranging from 8 to 10 times higher than plasma levels. Comparison of rodent brain CSF, brain and plasma levels with human CSF and plasma levels allowed us to extrapolate to human brain levels for once a day dosing as well as 4 times a day dosing**.** Posiphen readily achieves levels in human brain that allow for maximum inhibition of APP. We calculated the effective dose to be around 2x40mg/day or 2x60mg/day, which compares well with previous calculations done by Axonyx. Due to the prolonged half life in brain compared to plasma (14 vs. 5 hours) there is a possibility that Posiphen could be dosed just once per day. Below are the graphs of the measured levels of Posiphen and metabolites in plasma, brain and CSF of rats and in plasma and CSF of humans with extrapolated brain levels.





pD and inhibition of APP: 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. The 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 4 to 8 hours, respectively.





Other AD associated markers that we also want to measure are Abeta 38/40/42, APP neo/C31, Tau/phospho Tau, alpha Synuclein, Neurotrophic Factors, Inflammatory Factors and AChEI/BChEI.

Summary:

- The patients tolerated the procedure well.
- Posiphen is as safe in MCI patients as in healthy elderly volunteers.
- 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 2x40mg/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 humans.

Pertinent Abstracts:

The Experimental Alzheimer's Disease Drug Posiphen Lowers Amyloid-β Peptide Levels in Cell Culture and Mice
Debomoy K. Lahiri, DeMao Chen, Bryan Maloney, Harold W. Holloway, Qian-sheng Yu, Tada Utsuki, Tony Giordano, Kumar Sambamurti, and Nigel H. Greig
JPET 320:386–396, 2007
ABSTRACT: Major characteristics of Alzheimer's disease (AD) are synaptic loss, cholinergic dysfunction, and abnormal protein depositions in the brain. The amyloid β-peptide (Aβ), a proteolytic fragment of amyloid β precursor protein (APP), aggregates to form neuritic plaques and has a causative role in AD. A present focus of AD research is to develop safe Aβ-lowering drugs. Posiphen was assessed in cultured human neuroblastoma cells and found to lower sAPP and Aβ levels by reducing the APP synthesis rate. Posiphen administration to mice (7.5–75 mg/kg daily, 21 consecutive days) significantly decreased levels of total APP (tissue mass-adjusted) in a dose-dependent manner. Aβ40 and Aβ42 levels were significantly lowered by Posiphen (≥15 mg/kg) compared with controls. The activities of α, β, and μ-secretase were assessed in the same brain samples; β-secretase activity was significantly reduced. Posiphen can lower Aβ via multiple mechanisms and represents an interesting drug candidate for AD treatment.

APP binds DR6 to trigger axon pruning and neuron death via distinct caspases
Anatoly Nikolaev, Todd McLaughlin, Dennis D. M. O'Leary & Marc Tessier-Lavigne
Nature, Vol 457 | 19 February 2009 | doi:10.1038 | nature07767

ABSTRACT: Naturally occurring axonal pruning and neuronal cell death help to sculpt neuronal connections during development, but their mechanistic basis remains poorly understood. Here we report that β-amyloid precursor protein (APP) and death receptor 6 (DR6, also known as TNFRSF21) activate a widespread caspase-dependent self-destruction program. DR6 is broadly expressed by developing neurons, and is required for normal cell body death and axonal pruning both in vivo and after trophic-factor deprivation in vitro. Trophic-factor deprivation triggers the shedding of surface APP in a β-secretase (BACE)-dependent manner. Loss- and gain-of-function studies support a model in which a cleaved amino-terminal fragment of APP (N-APP) binds DR6 and triggers degeneration. Genetic support is provided by a common neuromuscular junction phenotype in mutant mice. Our results indicate that APP and DR6 are components of a neuronal self-destruction pathway and suggest that an extracellular fragment of APP, acting via DR6 and caspase 6, contributes to Alzheimer's disease.

Modulation of human neural stem cell differentiation in Alzheimer (APP23) transgenic mice by Posiphen the positive enantiomer of phenserine

Amelia Marutle, Masao Ohmitsu, Mats Nilbratt, Nigel H. Greig, Agneta Nordberg, and Kiminobu Sugaya

PNAS ! July 24, 2007 ! vol. 104 ! no. 30, 12506–12511

ABSTRACT: In a previous study, we found that human neural stem cells (HNSCs) exposed to high concentrations of secreted amyloid-precursor protein (sAPP) *in vitro* differentiated into mainly astrocytes, suggesting that pathological alterations in APP processing during neurodegenerative conditions such as Alzheimer's disease (AD) may prevent neuronal differentiation of HNSCs. Thus, successful neuroplacement therapy for AD may require regulating APP expression to favorable levels to enhance neuronal differentiation of HNSCs. We found reductions of APP and glial fibrillary acidic protein (GFAP) levels in the hippocampus of APP23 mice after 14 days treatment with Posiphen (25 mg/kg). No significant change in APP gene expression was detected, suggesting that Posiphen decreases APP levels and reactive astrocytes by posttranscription regulation. HNSCs transplanted into Posiphen-treated APP23 mice followed by an additional 7 days of treatment with Posiphen migrated and differentiated into neurons in the hippocampus and cortex after 6 weeks. Moreover, Posiphen significantly increased neuronal differentiation of implanted HNSCs in hippocampal and cortical regions of APP23 mice and in the CA1 region of control mice. These results indicate that Posiphen reduces APP protein *in vivo* and increases neuronal differentiation of HNSCs.

Posiphen Treatment is Able to Reduce App Levels in Ts65Dn Mouse Model of Down Syndrome

Salehi, M. Faizi, R. Takimoto, J. Valletta, A. Danks, and W.C. Mobley

International Congress on Alzheimer's Disease, Chicago, 2008

ABSTRACT: Down syndrome (DS), the most common genetic cause of mental retardation, is due to the presence of an extra copy of human chromosome 21 (i.e. trisomy 21). The condition is common with an incidence of 1:733 live births. Human chromosome 21 (HSA 21) is estimated to contain over 300 genes. We have taken advantage of the synteny between mouse chromosome 16 and human chromosome 21 and the ability to engineer mice that contain an extra copy of genes homologous to those on HSA 21. The Ts65Dn mouse has an extra copy of ~ 140 mouse gene homologous including an extra copy of APP. Among the most salient findings was a striking decrease in nerve growth factor (NGF) transport from hippocampus to BFCNs that was linked to degeneration of these neurons. In a recent publication (Salehi et al., 2006), we showed that an extra copy of the gene for APP causes both disruption of NGF transport and degeneration of BFCNs. We decided to use Posiphen to target *App* gene expression to restore NGF transport and prevent or rescue BFCN degeneration. In fact Posiphen restored APP levels in TS65Dn mice to normal mouse levels and reverse failed NGF transport and BFCN degeneration.

Selective Translational Control of the Alzheimer Amyloid Precursor Protein Transcript by Iron Regulatory Protein-1

Cho HH, Cahill CM, Vanderburg CR, Scherzer CR, Wang B, Huang XD and Rogers JT

J Biol Chem E-online June 2010

Iron Influx increases the translation of Alzheimer amyloid precursor protein (APP) via an iron-responsive element RNA stem loop in its 5'UTR. Equal modulated interaction of the iron regulatory proteins with canonical IREs controls iron-dependent translation of the ferritin subunits. However, only IRP1 selectively binds to the 5'UTR of APP. A computer predicted optimal stem loop structure was constructed for human, mice and monkey IREs resulted in a 13 base single stranded terminal loop and a conserved GC rich stem. An AGU pseudotriloop is key for IRP1 binding to the canonical ferritin IREs. Intracellular iron chelation increased binding of IRP1 to the APP IRE decreasing intracellular APP expression, whereas IRP1 knockdown caused increased expression of APP.