# Online Measurement of Amyloid Plaque Dissolution in Transgenic Mouse and Human Brain Tissue

Granic, Ivica; Penke, Botond; Kovacs, Gabor G.; Verwer, Ronald W.H.; Selchow, Olaf; Behrle, Eva; Nyakas, Csaba; Luiten, Paul G.M.; Eisel, Ulrich

*Published in:*
Alzheimer & Dementia

**Background:** In Alzheimer's disease, the extracellular deposition of the β-amyloid (Aβ) peptide occurs due to aberrant processing of the full-length Aβ precursor protein (APP) by a group of proteases termed secretase. **Objective(s):** Neurodegeneration is believed to be triggered by the accumulation of potentially toxic Aβ species which are generated following β- and γ- secretase cleavage of APP. We have reported earlier that memantine reduces the levels of the secreted APP (sAPP), and Aβ peptides in the human neuroblastoma (SK-N-SH) cells and in rat primary cortical neurons. **Methods:** To understand the mechanism of Aβ reduction by memantine, the activity of α- and β-secretase enzymes was determined in SK-N-SH cells treated with memantine (4 μg/ ml for four days) using a fluorescent-based enzyme assay utilizing enzyme-specific substrates and reporter molecules. For the α-secretase assay, the APP peptide substrate *YEVHHQKLV* and EDANS/DABCYL as the reporter system were used. This substrate corresponds to the amino acid sequence associated with α-secretase cleavage of APP (681-689). For the β-secretase assay, the APP peptide substrate *REEVNLDAEFKR* corresponding to the amino acid sequence with β-secretase cleavage of APP (668-675) was used. We also determined the effects of memantine on the levels of soluble APP and Aβ peptides in the conditioned media by Western immunoblotting and ELISA assays. **Results:** Our MTT results indicate that there was no cellular loss in memantine-treated cells compared to control, which was corroborated by LDH results showing no cellular toxicity. In our initial experiments, memantine-treated cell extracts showed a reduction in β- secretase activity without any significant change in α-secretase activity. The levels of total sAPP, and Aβ peptides were also reduced in memantine-treated media compared to control. **Conclusions:** Further experiments are in progress to better understand the mechanism of memantine's effect on secretases.

## P4-311 PAN-811 PROTECTS PRIMARY NEURONS FROM Aβ NEUROTOXICITY: A POTENTIAL DRUG FOR ALZHEIMER'S DISEASE

**Hossein A. Ghanbari**, Zhi-Gang Jiang, *Panacea Pharmaceuticals, Inc., Gaithersburg, MD, USA. Contact e-mail: pstup@panaceapharma.com*

**Background:** PAN-811 is a small lipophilic compound with a molecular mass of 195 Dalton, belonging to the (N)-heterocyclic carboxaldehyde thiosemicarbazone class of molecules originally developed for cancer therapy. Our previous result has demonstrated highly effective function in protecting ischemic neurotoxicity through a dual mechanism of divalent metal chelation and free radical scavenging. It has proved to be non-toxic in human clinical trials and crosses brain / blood barrier. **Objective:** We have evidence that oxidative stress is the most likely mediator of Aβ neurotoxicity and our objective is to demonstrate that PAN-811 could be an effective neuroprotector. **Methods:** To prove this hypothesis, first we established that Aβ has neurotoxicity in primary neurons in culture. Using Aβ1-42 or Aβ25-35, the rate and intensity of neurotoxicity was increased by age in culture and oxidative stress. For 2-week old neurons, the neurotoxicity was observed in 12 to 16 days after the insult and required 0% antioxidant level (high oxidative stress) whereas neurotoxicity occurred after 1 day for 3-week old neurons under 90% antioxidant level(mild oxidative stress). There was no neurotoxicity in young neurons low oxidative stress when treated with less than 80μm Aβ. The neuronal cell death was by apoptotic process. Neurotoxicty of Aβ1-42 and Aβ25-35 is specific since a reversed sequence peptide Aβ35-25 was unable to induce any toxicity. **Results:** We have clearly demonstrated that PAN-811 inhibits Aβ-induced neurotoxity. PAN-811 at doses as low as 2μM and as late as 3-hours post insult (Aβ1-42 or Aβ25-35 treatment) effectively protects neurons. **Conclusions:** We conclude that PAN-811 protects primary neurons from Aβ neurotoxicity and should be pursued as a viable candidate for therapeutic drug development for Alzheimer's disease.

## P4-312 A MODEL-BASED COMPARISON OF THE NONCLINICAL AND CLINICAL EFFECTS OF ANTI-Aβ ANTIBODIES ON PLASMA Aβ1-40 LEVELS

**Eric R. Siemers**, Lisa Ferguson-Sells, David G. Waters, Ronald B. DeMattos, Ryan J. Hansen, *Eli Lilly and Company, Indianapolis, IN, USA. Contact e-mail: esiemers@lilly.com*

**Background:** Administration of anti-Aβ antibodies to animals and humans produces increases in the total levels of Aβ in the plasma. This biomarker response provides evidence that the antibodies 'hit the target' and also provides insight for comparison of effects between species. **Objectives:** Although difficult to measure directly, an understanding of antibody effects on 'free' Aβ levels would also be advantageous, as one of the desired effects of antibody administration is to decrease 'free' Aβ levels and shift Aβ equilibria away from deposition into plaque. **Methods:** Mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) models were developed to characterize relationships between plasma anti-Aβ antibody levels and the time-course of the plasma $Aβ_{1-40}$ response in PDAPP mice and Alzheimer's patients. These models allow inference of effects on both 'total' and 'free' Aβ. Simulations were performed to compare the relative biomarker responses in PDAPP mice and humans. **Results:** Following intraperitoneal administration of anti-Aβ antibodies (m266) to PDAPP mice, plasma m266 concentrations showed first-order absorption followed by mono-exponential decay. In humans, intravenous anti-Aβ antibody administration led to bi-exponential pharmacokinetics that were well characterized using a 2-compartment PK model. In both mice and humans, antibody concentrations appeared to increase proportionally with administered dose. Dose-dependant plasma $Aβ_{1-40}$ pharmacodynamic responses were observed in both mice and humans. These PD responses were well characterized using mechanism-based PK/PD models that incorporated antibody-antigen binding principles. Simulation results showed greater effects on 'free' $Aβ_{1-40}$ at the dose levels administered in humans than for the dose levels used for nonclinical efficacy studies in PDAPP mice. **Conclusions:** This analysis illustrates that the dose levels studied in the initial clinical study compare favorably, from a biomarker perspective, with dose levels that demonstrated beneficial effects in PDAPP mice.

## P4-313 ONLINE MEASUREMENT OF AMYLOID PLAQUE DISSOLUTION IN TRANSGENIC MOUSE AND HUMAN BRAIN TISSUE

**Ivica Granic**[1], Botond Penke[2], Gabor G. Kovacs[3], Ronald W.H. Verwer[4], Olaf Selchow[5], Eva Behrle[5], Csaba Nyakas[1], Paul G.M. Luiten[1], Ulrich L.M. Eisel[1], *[1]University of Groningen, Haren, The Netherlands; [2]Albert Szent-Gyorgyi Medical University, Szeged, Hungary; [3]National Institute of Psychiatry and Neurology, Budapest, Hungary; [4]Netherlands Instiute for Brain Research, Amsterdam, The Netherlands; [5]University of Stuttgart, Stuttgart, Germany. Contact e-mail: I.Granic@rug.nl*

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive loss of cognitive functions. The pathology of AD shows a strong degeneration of neuritic processes, the presence of intracellular neurofibrillary tangles and extracellular amyloid plaques.

According to the amyloid hypothesis the amyloid-β peptide (Aβ), which accumulates in the brain of patients suffering from AD, is a major pathogenic force in this disorder. Therefore, the reduction of amyloid deposits in AD brain or inhibition of Aβ fibrillogenesis is a promising target for treating the disease.

As a result, a possible therapeutic approach can be the use of beta-sheet breaker peptides. Beta-sheet breaker peptides are small synthetic peptides (mainly penta-peptides) derived form the Aβ sequences, which are able to bind the amyloid-β peptide and destabilize the beta-sheet-rich structure therefore preventing aggregation and plaque formation.

Here we established a novel method for testing amyloid resolving drugs on brain slice cultures. Amyloid plaques in organotypic brain slice cultures derived from either $APP_{SL}$ × PS1mut mice or tissue from AD patients were stained using thioflavin S. A tissue incubation chamber was designed for chronic treatment of amyloid plaques in living brain tissue with sheet breaker peptides. The plaque dissolving properties of the sheet breakers were measured up to 24 hours using confocal laser scanning microscopy. Changes in plaque density were visualized after application of the beta-sheet breaker peptides RVVIA, LPYFD and RIIGL in living tissue. After incubation of 24 hours plaque reductions of up to 50% were established dependent on sheet breaker composition and concentration. These results indicate the promising potency of amyloid sheet breaker to combat plaque density in 'living' brain tissue of AD patients. The experimental set up using postmortem Alzheimer brain tissue provides a powerful tool for measuring amyloid dissolving properties of newly developed anti-amyloid compounds.

## P4-314 OVEREXPRESSION OF NEPRILYSIN DECREASES NEURONAL AND BEHAVIORAL IMPAIRMENTS IN HUMAN AMYLOID PRECURSOR PROTEIN TRANSGENIC MICE

**William J. Meilandt**, Tiffany Wu, Gui-Qiu Yu, Kimberly Scearce-Levie, Lennart Mucke, *Gladstone Institute of Neurological Disease, San Francisco, CA, USA. Contact e-mail: wmeilandt@gladstone.ucsf.edu*

**Background:** Expression of human amyloid precursor proteins (hAPP) and amyloid-β (Aβ) peptides in neurons of transgenic mice results in the age-dependent development of neuritic plaques and of plaque-independent synaptic and behavioral alterations. Several of the behavioral alterations correlate with the depletion of synaptic activity-related proteins, such as Fos and calbindin-28K, in the dentate gyrus, but not with plaque load. **Objective:** Here we assessed whether neuronal and behavioral alterations in hAPP mice are caused by Aβ or by alternative hAPP metabolites such as C-terminal fragments and secreted hAPP ectodomains. **Methods:** We crossed hAPP mice from our familial Alzheimer's disease-mutant line J20 with transgenic mice that overexpress the Aβ-degrading enzyme neprilysin in neurons [Neuron 40: 1087]. Neprilysin has previously been shown to decrease the plaque burden in hAPP mice, but its effects on neuronal deficits in hAPP mice remain to be defined. **Results:** Neprilysin (NEP) overexpression reduced hippocampal Aβ1-42 and Aβ1-× levels in hAPP/NEP doubly transgenic mice compared with hAPP singly transgenic mice. hAPP/NEP mice also had more Fos-positive granule cells and higher levels of calbindin in the outer molecular layer of the dentate gyrus relative to hAPP mice, although the levels of these proteins did not reach the levels in the nontransgenic and NEP singly transgenic controls. Compared with these controls, hAPP mice, but not hAPP/NEP mice, had increased hippocampal levels of met-enkephalin. NEP overexpression also improved several, but not all, behavioral alterations in hAPP mice. **Conclusions:** These results suggest that neuronal and behavioral deficits in hAPP mice are caused primarily by increased levels of Aβ, underlining the therapeutic potential of Aβ-lowering strategies.

Supported by NIH grants AG022074 and NS43945.

## P4-315 PHARMACODYNAMIC CHANGES IN PLASMA $A\beta_{40}$ FOLLOWING PERIPHERAL ANTIBODY TREATMENT IN TRANSGENIC MICE

**Darrell A. Henze**[1], Stephanie Rosenberg[1], Michelle C. Crouthamel[1], Sethu Sankaranarayanan[1], Toni Williamson[2], Pawel Richards[2], Mark S. Shearman[2], Adam J. Simon[1], Guy R. Seabrook[1], Gene G. Kinney[1], *[1]Merck Research Laboraties, West Point, PA, USA; [2]Merck Sharp and Dohme Research Laboratories, Harlow, United Kingdom. Contact e-mail: darrell_henze@merck.com*

**Background:** Antibodies against the Amyloid Beta (Aβ) peptide oligomers have been proposed as a therapeutic treatment for Alzheimer's disease. One mechanism by which these antibodies work is by binding and sequestering Aβ species in the periphery and thus reducing the amount of Aβ in the CNS. Transgenic PDAPP mice that strongly overexpress human APP have been shown to demonstrate high plasma levels of Aβ in the 24 hr period following a single acute IV dose of Aβ specific antibody (Demattos et al 2001. PNAS 98:8850). **Objective:** To better understand the relationship between Aβ in the CNS and acute plasma elevations of antibody-bound $A\beta_{40}$, we undertook a series of studies examining the effects of different Aβ monoclonal antibodies in two different transgenic mouse lines (APP-YAC (2-5 mos) and Tg2576 (8-10 mos)) that express human APP. Relative to WT controls, at baseline, the 2-4 mos APP-YAC mouse has ~ 3 fold elevations in brain DEA soluble Aβ while the 8-10 mos Tg2576 mouse has ~ 40 fold elevations in brain DEA soluble Aβ. **Methods:** Plasma elevations in Aβ40 were measured using ELISA methodology. **Results:** Using the Aβ selective 4G8, WO2, or Merck mAb1 antibodies , we observed robust increases in plasma $A\beta_{40}$ in the 2-24 hr period following a single IV injection of antibodies in both mouse strains. The maximum elevation was observed between 4 and 12 hrs in the Tg 2576 and APP-YAC mice. The plasma elevations due to WO2 in Tg2576 were dose dependent as were the elevations to Merck mAb1 in APP-YACS . The $A\beta_{40}$ elevations were also observed with subcutaneous administration of 4G8 and Merck mAb1 although the magnitude of the response was slightly smaller than the equivalent dose administered IV. **Conclusions:** Taken together these data show that acute plasma elevations of $A\beta_{40}$ induced by mAb administration can be observed in Tg AD mice and are a useful pharmacodynamic marker of antibody activity.

## P4-316 Aβ DERIVATIVE VACCINE DOES NOT CAUSE BRAIN MICROHEMORRHAGES IN TG2576 MICE AND ITS EFFECTIVENESS IS AGE-DEPENDENT

**Allal Boutajangout**, Ayodeji A. Asuni, Henrieta Scholtzova, Elin Knudsen, Yong-Sheng Li, David Quartermain, Blas Frangione, Thomas Wisniewski, Einar M. Sigurdsson, *NYU School of Medicine, New York, NY, USA. Contact e-mail: boutaa01@med.nyu.edu*

**Background:** Immunotherapy for AD targeting Aβ remains promising despite the setbacks in the initial clinical trial that may have been related to T-cell-mediated encephalitis. Prior to these side effects, we advocated that vaccination with Aβ derivatives that are non-toxic and preferentially elicit a Th2 immune response is likely to be safer for human use. In addition, for enhanced safety, adjuvants that induce a humoral response, such as alum, should be used. Another potential side effect of this type of therapy is cerebral bleeding which has been observed following passive immunization. This phenomenon has not been assessed in active immunization studies. **Objective(s):** To determine: 1) if our Aβ derivatives are effective when combined with alum; 2) whether our approach produces hemorrhages; 3) whether the efficacy of our vaccine is age-dependent. **Methods:** Tg2576 mice and wild-type littermates (n=66) were immunized with soluble, non-toxic Aβ derivative, K6Aβ1-30-$NH_2$ in alum adjuvant, starting at 11- and 19 months. This adjuvant is approved for humans and promotes humoral immunity. Mice went through various sensorimotor and cognitive tests at 22-24 months, and their brains were subsequently analyzed for amyloid burden and associated pathology including microhemorrhages. **Results:** Treatment initiated at 11 months reduced cortical plaque burden by 31% ($p<0.05$), total brain Aβ40 levels by 30% ($p=0.03$), Aβ42 by 37% ($p=0.02$), but soluble Aβ levels remained unaltered. This effect was associated with cognitive improvements in both the radial arm maze and the Hebb-Williams maze. In contrast, treatment initiated at 19 months was not immunogenic, did not reduce plaque burden or improve cognition. In various sensorimotor tasks, Tg mice differed from their wild-type littermates but treatment effect was not observed, indicating that the therapy specifically improves cognition. Cerebral microhemorrhages were observed in the Tg mice but the immunotherapy did not increase the bleeding in contrast to previous Aβ antibody studies. **Conclusions:** These findings