The MODEL-AD consortium preclinical testing pipeline: pharmacokinetics and pharmacodynamics of prophylactic treatment with levetiracetam on the 5XFAD mouse model of Alzheimer's Disease

SJ Sukoff Rizzo[1], SK Quinney[2], KD Onos[1], KJ Keezer[1], DR Jones[2], AR Masters[2], IF Metzger[2], JA Meyer[2], J Peters[2], SC Persohn[2], BR McCarthy[2], AA Bedwell[2], M Sasner[1], G Howell[1], H Williams[1], AJ Oblak[2], BL Lamb[2], and PR Territo[2]

[1]The Jackson Laboratory, Bar Harbor, Maine USA; [2]Indiana University School of Medicine, Indianapolis, Indiana USA

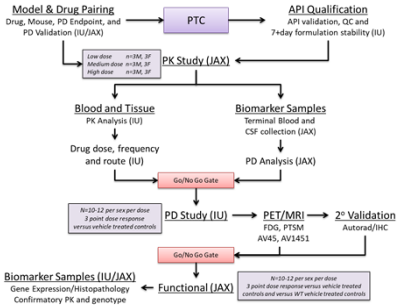
MODEL-AD
Model Organism Development & Evaluation for Late-Onset Alzheimer's Disease

The Jackson Laboratory
Leading the search for tomorrow's cures

Sage BIONETWORKS

INTRODUCTION

The preclinical testing core (PTC) of the Model Organism Development for Late Onset Alzheimer's Disease (MODEL-AD) consortium has established a streamlined preclinical drug testing strategy with go/no-go decision points that allow critical and unbiased assessments of potential therapeutic agents. The goals of the PTC are to develop a testing strategy that maximizes the therapeutic potential of all drug candidates by initiating the dosing strategy prior to the onset of disease relevant biomarker readouts.



The PTC strategy includes a primary screen to determine: 1) drug formulation and qualification of the active pharmaceutical ingredient (API); 2) drug stability in the intended formulation; and 3) *in vivo* pharmacokinetics and target tissue concentrations in models at disease-relevant ages. A secondary screen evaluates target engagement and disease modifying activity utilizing non-invasive PET/MRI as a pharmacodynamic (PD) readout matched to known disease pathology in the model. Compounds demonstrating positive PD effects in the secondary screen are further interrogated via a tertiary screen of functional assays that assess the compounds ability to normalize a disease-related phenotype in cognition and neurophysiological tests. At the conclusion of functional testing, brain tissue (right hemisphere) is sent for gene expression analysis while left hemisphere is sent for confirmatory PK. For the present studies, levetiracetam (LEV), a compound currently in clinical trials for the treatment of cognitive impairment associated with AD, was selected for testing in 5XFAD mice.

METHODS & MATERIALS

For PK studies, LEV was administered acutely p.o. to 6 mo aged female and male 5XFAD mice in which AD-relevant phenotypic alterations in MRI function, memory, and amyloid deposition have been reported. From serial blood samples (0.5, 1, 2, 4, 6 h and terminal) plasma and brain tissue were evaluated for exposure levels. A second independent PK study was conducted to refine the optimal dose range and dosing frequency. All samples were frozen and stored at -80C until analysis. Bioanalytical analysis was performed by LC-MS/MS for the parent and primary metabolite (etiracetam). To determine the initial pharmacokinetics for LEV, non-compartmental analysis (NCA) was performed for both the parent and primary metabolite in female and male mice. Provided the NCA rate constants, a 1-tissue compartment model was fit to the population data using Phoenix WinNonlin. The resultant rate constants for absorption (K_a), elimination (K_e), and volumes of distribution (V_d) were then used to generate simulations for once (QD) and twice (BID) daily dosing.

In line with the PTC's prophylactic dosing strategy for PD studies, BID chronic administration of LEV (10, 30, and 56 mg/kg, p.o.) began at 3 mo of age with all PD endpoints including functional behavioral measures, 18F-FDG (*experiment in progress*) and 18F-AV45 PET/MRI measured at 6 mo of age. The behavioral testing battery included assessments of exploratory and locomotor activity in the open field, hippocampal working memory as measured by spontaneous alternation in a y-maze, and rotarod motor coordination (*see Sukoff Rizzo et al 2018 Current Protocols in Mouse Biology for SOPs*). Behavioral tests were separated by a one day inter-test interval. All PET scanning (15 min/ea.) was performed on the IndyPET3 scanner and post mortem brains were extracted and frozen for autoradiography (Autorad). MRIs were acquired (10 min/ea.) on a Siemens 3T Prisma scanner outfitted with a 4 channel phased array head coil. PET/MRI images were co-registered to Paxinos-Franklin atlas and 27 average brain (56 total for left and right) regions were extracted. LEV was typically administered each day between 7-9am and 3-5pm. On the day of the PD measures, with the exception of 18F-AV45, subjects were administered LEV as a 30 min pretreatment. For Autoradiography, frozen brains were sectioned at 20 um in sextuplet at 3 bregma targets, exposed on phosphor plates, scanned and manually segmented for 16 brain regions across 4 bregma targets which have been mapped to PET/MRI data.

NCA parameters were analyzed by 2-way ANOVA with sex and dose being the primary factors. For open field activity and rotarod, data were analyzed by 2-way RM ANOVA within sex. For PET and Autorad, data were analyzed by 2-way ANOVA, where sex and regions were the primary factors. In all cases, fiduciary level of significance was taken at p<0.05.

FURTHER INFORMATION

- MODEL AD: www.modelad.org
- AMP-AD Knowledge Portal: http://www.synapse.org/ampad
- JAX AD models: https://www.jax.org/alzheimers
- AlzForum research models: http://www.alzforum.org/research-models

NIH National Institute on Aging

ACKNOWLEDGEMENT

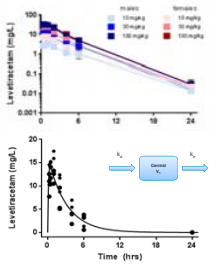
MODEL-AD Centers were established with funding from
The National Institute on Aging (U54 AG054345; U54 AG054349).

IN VIVO PK AND PK/PD MODELING

- Prior to in vivo PK and PD studies, lot-to-lot variability and formulation stability for LEV were assessed. Data indicate <5% variation across LEV lots and LEV was stable in 0.9% NaCl at 4°C for up to 9 days.
- Serial plasma and terminal brain tissue samples following acute oral administration (10, 30, and 100 mg/kg, n=3/sex/dose) revealed brain concentrations were linearly related with plasma concentrations.

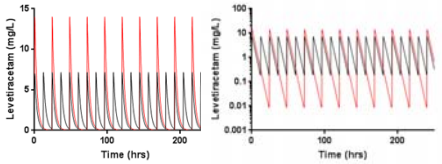
Noncompartmental Pharmacokinetics	10 mg/kg		30 mg/kg		100 mg/kg	
	Female	Male	Female	Male	Female	Male
Levetiracetam						
ke (1/h)	0.30 ± 0.12	0.21 ± 0.03	0.26 ± 0.02	0.25 ± 0.03	0.29 ± 0.07	0.27 ± 0.02
Half-life (h)	2.53 ± 0.84	3.31 ± 0.37	2.72 ± 0.25	2.76 ± 0.30	2.52 ± 0.57	2.54 ± 0.22
Tmax (h)	0.86 ± 0.24	0.85 ± 0.28	0.86 ± 0.29	0.84 ± 0.30	0.51 ± 0.45	0.44 ± 0.14
Cmax (µg/L) [a]	8.10 ± 1.80	3.50 ± 0.73	16.1 ± 1.23	12.4 ± 1.80	28.7 ± 1.60	35.0 ± 2.35
AUCinf (mg/L/h) [a]	28.2 ± 5.50	15.1 ± 5.10	59.7 ± 13.4	44.0 ± 14.5	110 ± 9.31	113 ± 9.09
Vd/F (L/kg) [a]	1.33 ± 0.55	3.45 ± 1.27	2.06 ± 0.61	3.06 ± 1.59	3.37 ± 1.03	3.24 ± 0.02
CL/F (L/h/kg) [a]	0.36 ± 0.07	0.71 ± 0.20	0.52 ± 0.13	0.75 ± 0.31	0.91 ± 0.08	0.89 ± 0.07
Etiracetam						
ke (1/h)	0.23 ± 0.14	0.25 ± 0.24	0.19 ± 0.004	0.19 ± 0.16	0.31 ± 0.15	0.23 ± 0.18
Half-life (h)	3.84 ± 1.91	6.05 ± 6.19	3.68 ± 0.08	5.54 ± 3.89	2.56 ± 1.07	4.43 ± 2.83
Tmax (h)	0.86 ± 0.29	2.36 ± 1.51	1.35 ± 0.56	0.85 ± 0.28	1.03 ± 0.01	0.85 ± 0.28
Cmax (µg/L)[a]	136 ± 43.4	87.7 ± 9.50	367 ± 103	250 ± 25.8	902 ± 167	559 ± 106
AUCinf (µg/L/h)[b]	591 ± 209	1256 ± 568	1483 ± 510	1729 ± 489	2847 ± 639	3620 ± 1236

[a] Significant differences between males and females and dose levels (p<0.05, 2-way ANOVA) after correcting C_{max} and AUC for dose. [b] Significant differences between dose levels (p<0.05, 2-way ANOVA)



Observed (top panel) LEV plasma concentration-time profiles by dose and sex, and 1-compartment model fit (bottom panel) for 30 mg/kg dose.

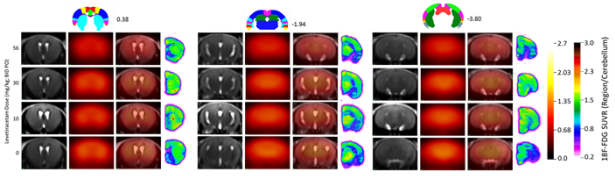
- Simulations of twice daily dosing minimized the C_{max}:C_{min} ratio and would produce the same AUC while providing a more consistent steady state level with C_{min} concentrations within the therapeutic range.

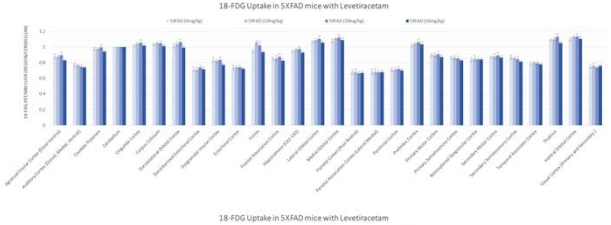


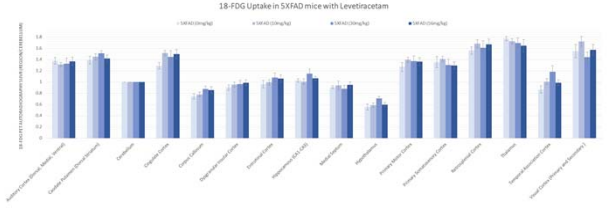
Predicted concentration vs. time curves for chronic LEV 30 mg/kg (red) or 15 mg/kg (black) every 24 hours (left panel) or every 12 hours. Data are presented as linear (left) or log10 (right) transformations vs. time. Given the short half-life, it was determined that BID dosing scheme would be required to yield plasma and brain levels above the minimum therapeutic concentration.

18F-FDG PET / MRI / AUTORADIOGRAPHY

- Prophylactic treatment with LEV (10, 30, or 56 mg/kg; p.o., BID, initiated at 3 months of age) in female and male 5XFAD mice for glucose metabolism at 6 months of age as measured by 18F-FDG PET/MRI and Autoradiography are ongoing and preliminary results are shown below.

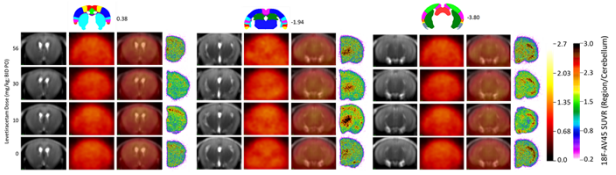


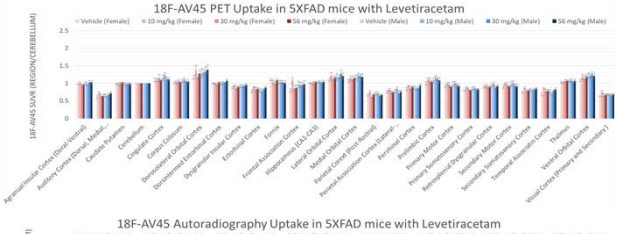


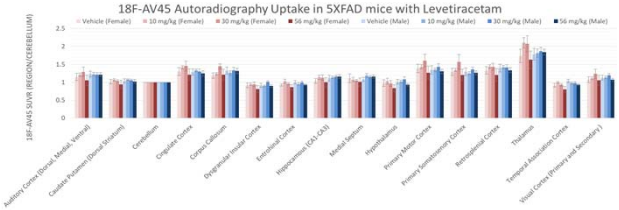


18F-AV45 PET/MRI/AUTORADIOGRAPHY

- Prophylactic treatment with LEV (10, 30, or 56 mg/kg; p.o., BID, initiated at 3 months of age) in female and male 5XFAD mice, failed to alter amyloid deposition at 6 months of age as measured by 18F-AV45 PET/MRI and Autoradiography.
- A total N=73 6mo old 5XFAD mice were imaged (n=32 female; n=41 male; N=7-11 per sex per dose level) with 56 brain regions per subject (N=4088 total regions; 1792 females, 2296 males) extracted from co-registered to Paxinos-Franklin atlas using semi-automated methods.
- Post mortem 18F-AV45 autoradiography in 16 brain regions per subject were hand segmented (N=7008 total; 3936 males, 3072 females) at 3 bregma targets according to Paxinos-Franklin.







BEHAVIORAL EFFECTS OF CHRONIC ADMINISTRATION OF LEV

- Male and female 5XFAD mice were treated with LEV (10, 30, or 56 mg/kg; p.o., BID, initiated at 3 months of age) or vehicle control. Male and female WT littermate controls were treated with vehicle control (10 ml/kg; p.o. BID, initiated at 3 months of age). At 6 months of age, subjects were pretreated with LEV or vehicle 30 min prior to being placed into the open field for a 60 min period or evaluated for motor coordination on an accelerating rotarod (4-40 rpm over 5 min, 3 consecutive trials).
- LEV produced dose-related increase in locomotor activity in 6 month aged 5XFAD mice, relative to sex-matched, vehicle treated 5XFAD controls, as measured by distance traveled in the open field.
- Vehicle treated 6 month aged 5XFAD male and female mice demonstrated increased ability to maintain their balance on the rotarod relative to vehicle treated, sex-matched WT littermate controls. There was no effect of LEV treatment on rotarod performance in 5XFAD mice.
- Spontaneous alternation data (not shown), pending genotype and PK confirmation.