Transcriptomic Analysis of Chronic Levetiracetam Treatment in Aged 5XFAD Mice: Relationship with Pharmacodynamics and Pharmacokinetics

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

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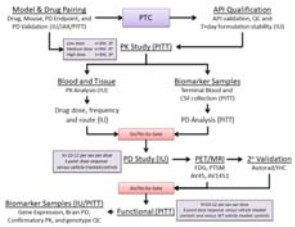
Introduction

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; 2) drug stability; and 3) *in vivo* pharmacokinetics and target tissue concentrations in models at disease-relevant ages. A secondary screen evaluates target disease modifying activity utilizing non-invasive PET/MRI as a pharmacodynamics (PD) readout matched to known disease pathology in the model.



Compounds demonstrating positive PD effects in the secondary screen are further interrogated via a battery screen of functional assays that assess the compounds ability to normalize a disease-related phenotype in cognition and neurophysiological tests. The final component of the PTC screen includes confirmatory exposure levels (PK), and gene expression profiling to evaluate drug related transcriptomic changes.

For the present studies, we selected levetiracetam (LEV), a compound currently in clinical trials for the treatment of cognitive impairment associated with AD, for testing in aging male and female 5XFAD mice.

Methods

- Pharmacodynamic studies: Chronic administration of LEV began at 3 months of age with all PD endpoints measured at 6 mo of age. LEV was administered twice daily (BID), PO. 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. 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. On behavioral testing days, LEV or vehicle was administered as a 30 min pretreatment prior to testing.
- Terminal tissue collection: At the conclusion of behavioral testing, terminal CSF, plasma, and brain tissue were collected. Bioanalytical analysis was performed by LC-MS/MS and Non-compartmental analysis (NCA) for terminal plasma and right brain hemisphere for confirmatory PK. Homogenate from left brain hemisphere was analyzed using a custom Nanostring nCounter® Mouse AD panel that was designed to identify correlations to changes in gene expression specific to clinical late-onset AD. Differential gene expression was determined based on genotype, sex, and treatment.
- All technicians were blinded to dose and genotype during execution of experiments and throughout data collection and analysis. Detailed protocols and raw QCd data are available at www.ampadportal.org.

Observed Pharmacokinetics of Levetiracetam following 3 month chronic PO dosing BID

	Plasma ng/mL (time=0h) (Mean±SD)		Plasma ng/mL (time=0.5h) (Mean±SD)		Cortex ng/g (time=0.5h) (Mean±SD)	
	Female	Male	Female	Male	Female	Male
Levetiracetam						
0 mg/kg	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
10 mg/kg	2.50±3.7	3.6±6.9	6324.4±2631.8	7617.6±1843.7	2503.2±537.9	2741.1±851.6
30 mg/kg	13.8±12.7	8.2±9.3	21049.8±4090.8	26263.8±13758.7	10742.6±4092.9	11821.0±5109.9
56 mg/kg	10.4±7.2	26.9±21.8	33797.6±6081.8	40161.1±14832.5	20281.2±5048.1	21694.3±4038.3
Etiracetam						
0 mg/kg	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
10 mg/kg	< LOQ	< LOQ	39.3±18.1	45.0±8.4	23.0±14.8	24.9±15.1
30 mg/kg	< LOQ	< LOQ	143.9±34.8	138.8±41.6	73.1±35.3	90.6±50.9
56 mg/kg	< LOQ	< LOQ	237.8±44.7	239.8±52.5	150.1±63.7	182.3±65.0

Plasma LOQ=0.3ng/mL; Cortex LOQ=0.8ng/mL.

Acknowledgements

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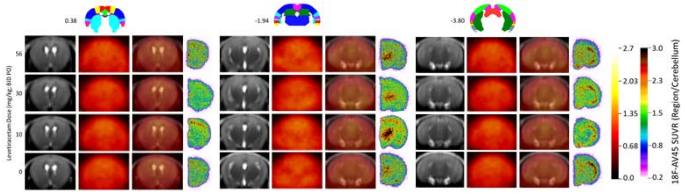
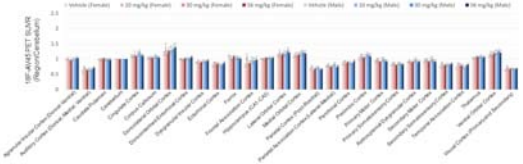
- AMP-AD Knowledge Portal: www.ampadportal.org
- MODEL AD: www.modelad.org
- Jax AD models: https://www.jax.org/alzheimers
- AlzForum research models: http://www.alzforum.org/research-models

Pharmacodynamic Effects of Chronic Levetiracetam Treatment

Chronic prophylactic treatment with LEV failed to alter Aβ levels in 6 month aged male and female 5XFAD mice as measured by 18F-AV45 PET

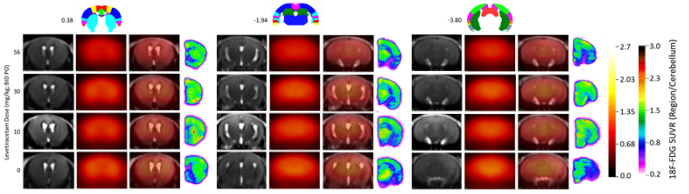


18F-AV45 PET/MRI images represent an average of 5 randomly selected males and females, while autoradiography images are representative males or females. In all cases, images are presented as SUVR to the cerebellum. Each bregma image panel presented as average MRI (left), PET (center-left), Fused (center-right), and Auto-radiography (right) for three bregma targets (0.38, -1.94, -3.80) as a function of chronic LEV dosing (top to bottom).

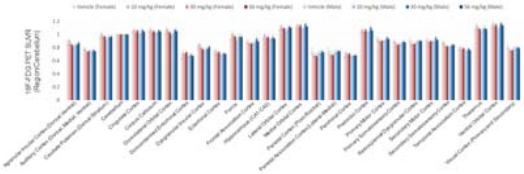


Quantitative analysis of 18F-AV45 PET/MRI uptake in male and female 5XFAD mice as a function of LEV dose. Data are presented as means ± 1 SEM, and analyzed with a 2-way ANOVA, with sex and treatment as factors. As predicted, LEV did not alter 18F-AV45 uptake.

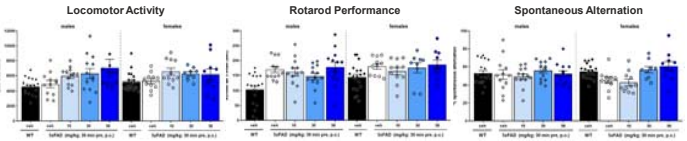
Chronic prophylactic treatment with LEV failed to alter glucose metabolism in 6 month aged male and female 5XFAD mice as measured by 18F-FDG PET



18F-FDG PET/MRI images represent an average of 5 randomly selected male and females, while Autoradiography images are representative males or females. In all cases, images are presented as SUVR to the cerebellum. Each bregma image panel presented as average MRI (left), PET (center-left), Fused (center-right), and Auto-radiography (right) for three bregma targets (0.38, -1.94, -3.80) as a function of chronic LEV dosing (top to bottom).



Quantitative analysis of 18F-FDG PET/MRI uptake in male and female 5XFAD mice as a function of LEV dose. Data are presented as means ± 1 SEM, and analyzed with a 2-way ANOVA, with sex and treatment as factors. LEV did not alter 18F-FDG uptake.

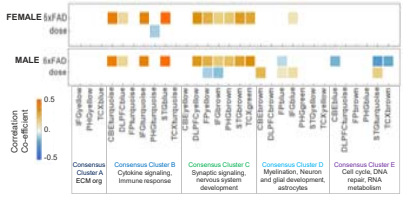


Chronic administration with LEV produced dose related hyperactivity in 6 month aged 5XFAD male and female mice relative to vehicle treated 5XFAD controls in the open field.

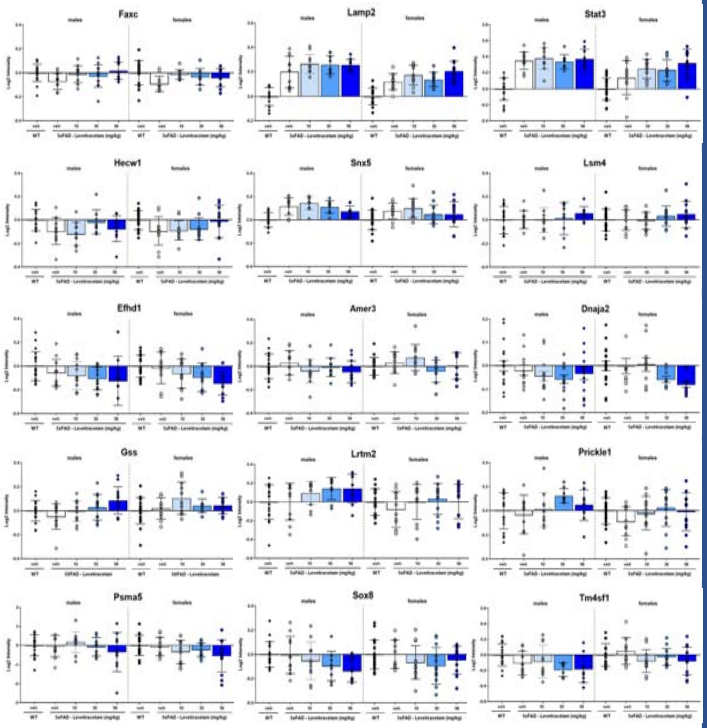
Chronic administration with LEV did not alter motor coordination in 6 month aged male and female 5XFAD mice as measured by latency to maintain balance on a rotarod.

In male mice, there was no effect of LEV treatment on spontaneous alternation. In female mice, vehicle treated 5XFAD demonstrated the expected deficit relative to WT with LEV dose related increases in % alternation.

Gene Expression Profiling of Chronic Levetiracetam Treatment



- Treatment effects were *correlated* with AD effects in Consensus Cluster D: neuron, glial development, myelination
- Treatment effects were *anti-correlated* with AD effects in Consensus Cluster C: Synaptic signaling, CNS development
- Linear regression analysis revealed the following genes with an effect size of p<0.05 for genotype, sex, and/or treatment:
- Faxc, Lamp2, and Stat3 demonstrated dose, sex, and genotype effects
- Hecw1 and Snx5 demonstrated both genotype and dose effects.
- Lsm4 demonstrated both dose and sex effects.
- Efhd1, Amer3, Gss, Dnaja2, Lrtm2, Prickle1, Psma5, Sox8, and Tm4sf1 demonstrated only dose effects



Summary & Conclusions

- Taken together these data suggest that the 5XFAD mouse model may not be an optimal model for studying therapeutic interventions for Alzheimer's disease, independent of its utility to model early onset plague deposition.
- Transcriptomics data provide revealing information on LEV's mechanism of action and may point to specific genetic mouse models where evaluation of the effects of LEV may be more relevant.
- Gene expression profiling may be an important tool for identifying patient populations related to a drug's mechanism of action.