Inhibition of Cholesterol 24-Hydroxylase Improves the Survival of a Mouse Model of Epileptic Phenotype and Modulates Extracellular Levels of Glutamate

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Introduction

- Cholesterol 24-hydroxylase (CH24H, also known as CYP46A1) converts cholesterol into 24*S*-hydroxycholesterol (24HC).
- Human amyloid precursor protein and presenilin-1 (APP/PS1) Tg mice have a high mortality with an epileptic phenotype.[1]
- The mortality of APP/PS1-Tg mice is remarkably reduced by cross-breeding with heterozygous CH24H-knockout (KO) mice.[2]
- This study was designed to characterize the therapeutic potential of CH24H inhibitor in an APP/PS1-Tg model using the investigational compound, TAK-935 (OV935), which is a first-in-class, specific CH24H inhibitor with an anti-epileptic property.[3]

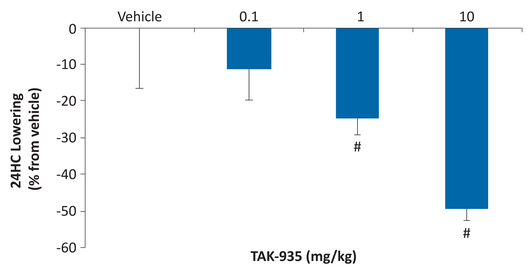
Materials and Methods

- **Animals:** APP/PS1-Tg mice were obtained by cross-breeding APP SW mice (Tg2576) with PS-1M146L knock-in (KI) mice.
- **Drug preparation:** TAK-935 was dissolved in 0.5% methyl cellulose solution at a concentration of 1 mg/mL and diluted to the indicated doses. Drug solution was administered to mice at a volume of 10 mL/kg body weight PO QD.
- **Survival study:** The survival of 7-week-old APP/PS1-Tg mice was monitored for 8 weeks. Mice were orally treated with vehicle or TAK-935 at a dose of 10 mg/kg PO QD.
- **Hippocampal microdialysis:** A guide cannula was implanted in the left hippocampus. Ringer solution containing 100 mM KCl was continuously injected to depolarize the cells. The glutamate (Glu) concentration of collected dialysate was analyzed by reversed-phase high-performance liquid chromatography (HPLC). 10 µM tetrodotoxin (TTX) was used to contain depolarization.

Results

Figure 1. TAK-935 Dose-Dependently Reduced Brain 24HC Levels

- Dose-pharmacodynamics (PD) relationship to brain 24HC was assessed after 3 days of repetitive treatments of TAK-935 in APP/PS1-Tg mice.
- Brain 24HC was lowered at dose levels of 0.1, 1, and 10 mg/kg by 11%, 25%, and 50% compared with the vehicle control group, respectively.
- 10 mg/kg was considered to be a relevant dose to mimic CH24H$^{+/-}$ KO mice.



#*P*<0.025 vs. control, one-tailed Williams' test.

Figure 2. Survival Improvement of APP/PS1-Tg Mice by TAK-935 Treatment

- Premature death of APP/PS1-Tg mice becomes apparent around 6 weeks after birth, and roughly half of the cohort die over 3 months.
- TAK-935 treatment (10 mg/kg PO QD) was initiated from 7 weeks of age and maintained for the subsequent 8 weeks.
- Mortality was 14/30 (46.67%) in the control arm, while it was 2/30 (6.67%) in the treatment arm throughout the period (hazard ratio 5.849).

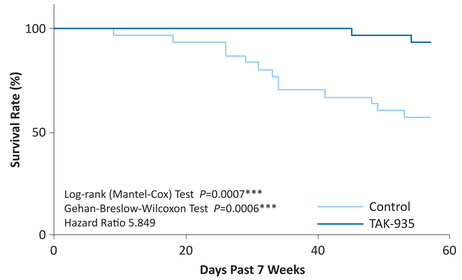
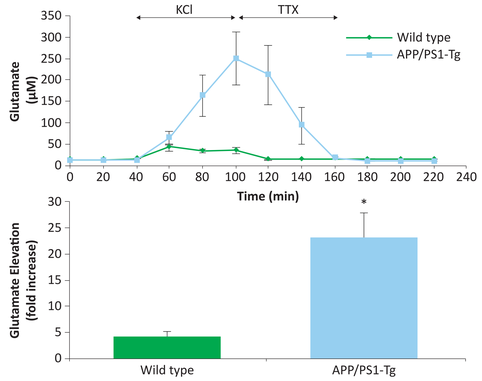


Figure 3. APP/PS1-Tg Mice Are Susceptible to Elevation of Extracellular Glutamate

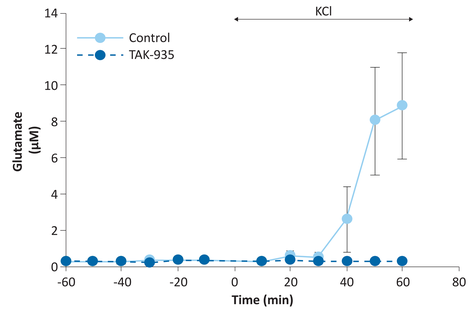
- To unmask the epileptic phenotype of APP/PS1-T APP/PS1-g mice, the hippocampus was perfused with KCl, and extracellular glutamate levels were measured.
- KCl injection caused seizure-like behaviors accompanied by marked elevations in extracellular glutamate only in APP/PS1-Tg mice.
- The fold increase in glutamate levels was 22.8 and 4.0 in Tg and wild-type (WT) mice, respectively.

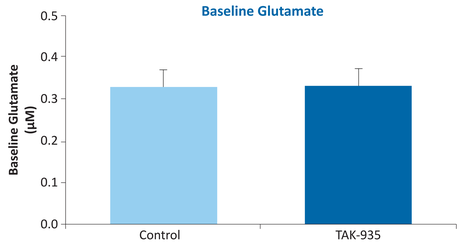


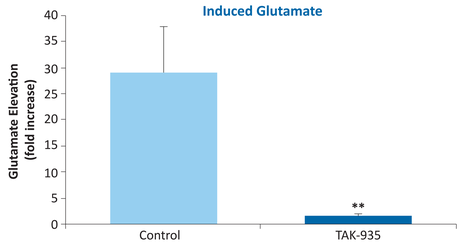
**P*<0.05, Student *t* test.
TTX= tetrodotoxin.

Figure 4. TAK-935 Suppresses Extracellular Glutamate Elevations in APP/PS1-Tg Mice

- 7-week-old APP/PS1-Tg mice were treated with TAK-935 for 2 weeks (10 mg/kg PO QD), followed by microdialysis.
- TAK-935–treated animals showed only marginal elevations in glutamate with no seizure-like behaviors observed in contrast to untreated animals.
- The maximum elevation was 28.9- and 1.6-fold from the baseline for control and TAK-935, respectively.
- TAK-935 did not change the baseline level of extracellular glutamate.



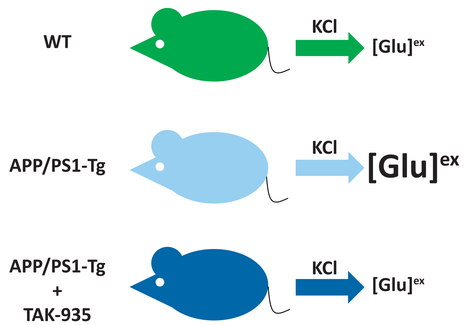




**P*<0.01, Student *t* test.

Summary

- TAK-935 reduced brain 24HC by 50% in APP/PS1-Tg mice, mimicking heterozygous CH24H deficiency.
- TAK-935 reduced the high mortality of APP/PS1-Tg mice.
- TAK-935 normalized the high susceptibility of APP/PS1-Tg mice to extracellular glutamate elevation.
- CH24H may play a role in either or all of the following mechanisms in a hyperexcitatory state:
  1. Synaptic Glu release
  2. Clearance of extrasynaptic K^+
  3. Clearance of extrasynaptic Glu



Conclusion

- CH24H inhibition has a potential mechanism to regulate extracellular K^+/Glu levels, thereby modulating neural excitation.

Acknowledgment

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References

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