Inhibition of Cholesterol 24-Hydroxylase Is a Novel Pharmacological Strategy for Epilepsy Treatment

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Introduction

- There are significant unmet needs in the treatment of epilepsy disorders, particularly in rare pediatric epilepsies.
- A novel pharmacological mechanism could meet the unmet medical needs in the treatment of refractory rare epilepsies.
- Cholesterol 24-hydroxylase (CH24H) also known as CYP46A1 is a brain-specific enzyme responsible for cholesterol catabolism.
- 24-hydrocholesterol (24HC), the end product of CH24H reaction, has recently been characterized as an endogenous positive allosteric modulator of N-methyl-D-aspartate (NMDA) receptors.
- CH24H inhibition could be therapeutic in disorders related to glutamatergic neuronal excitation.

Materials and Methods

Administration of TAK-935

For oral administration, TAK-935 was administered once daily. For subcutaneous infusion, TAK-935 was delivered through sonic pumps.

Mouse Pentylenetetrazol Kindling

Five-week-old male ICR mice were used. Pentylenetetrazol (PTZ; 42.5 mg/kg) was intraperitoneally injected in mice twice a week. Seizure severity was evaluated on a modified Racine scale (0-4). TAK-935 was given to animals 30 minutes before PTZ injection. The number of mice showing score 4 seizures was recorded.

Measurement of 24HC Levels in Brain and Plasma

24HC was extracted from brain homogenate, and its levels were measured by reverse-phase high-performance liquid chromatography. The plasma level of 24HC was determined by an LC/MS/MS system (liquid chromatography/mass spectrometry).

Rotarod Test

The drum was rotated at a speed of 8 rounds per minute. After a 2-minute training session, mice received a 60-second testing session 3 times. The latency to fall off the rotarod was recorded, and motor coordination was evaluated as the mean latency of the 3 trials.

Results

1. Delayed PTZ-Induced Kindling Development by TAK-935

Figure 1 shows the effects of TAK-935 (30 mg/kg po qd) on the acquisition of PTZ-induced kindling over PTZ stimulations in 30 days. The results indicate that the acquisition of kindling was retarded by TAK-935 treatment.

- The mean seizure scores of the TAK-935-treated group remained lower than those of the control group.
- At the end point, the average seizure scores of the control and TAK-935 groups reached similar levels.

2. Correlation Between CH24H Inhibition and Kindling Retardation

The relationship between the effects of TAK-935 on kindling retardation and CH24H inhibition was examined. Subcutaneous remote pumps were used to achieve robust CH24H single-level inhibition (0.03, 0.3, 1, 3, and 10 mg/kg/day). PTZ stimulations were given for 2 weeks to assess seizure latency. After a shorter period on the basis of the previous experiment (1.5 weeks), TAK-935 modified kindling development. A masked suppression of kindling progression was observed in the 2- and 10-mg/kg TAK-935 groups (Figure 2A).

- TAK-935 reduced the total seizure burden, shown as accumulation of seizure scores in Figure 2D. Significant effects of TAK-935 were seen at 1, 10, and 100 mg/kg (Fig. 2D, 5-10 mg/kg) (TAK-935 approximately 97% reduction).

- Brain 24HC values were determined after the final kindling stimulation to evaluate the pharmacodynamic effects of TAK-935 (Fig. 2B). mSN and mN1 rats were used. The brain 24HC level of mice treated with TAK-935 was significantly lower than that of the control group.

- Significant correlation was observed between brain and plasma 24HC (r = 0.78, 0.1%, Pearson), which suggests that the level of brain 24HC could be a monitorable pharmacodynamic marker (Figure 2F).

3. TAK-935 Did Not Affect Motor Coordination

To examine whether TAK-935 has an anti-ictogenic property, it was tested in the acute PTZ seizure model (60 mg/kg IP). TAK-935 was administered to mice for 2 weeks in a regimen that enables a robust CH24H inhibition (Figure 2G).

- The latency to fall off the rotarod was recorded to assess motor coordination. A statistically significant difference related to the vehicle control group was observed in the first treatment group (n = 2 mice). (14 H 0.05, Student's t-test). The total duration of seizures was observed when treated with TAK-935 did not differ from that of the vehicle-treated controls (Figure 2H).

Figure 2. A-C, Effect of TAK-935 on development of PTZ kindling (100 mg/kg IP); D, Correlation Between Brain 24HC Lowering and Cumulative Seizure Score; E, Correlation Between Brain and Plasma 24HC Lowering; F, Correlation Between Plasma 24HC Lowering and Total Seizure Burden; G, Effect of TAK-935 on Rotarod Performance

Table 1. Effect of TAK-935 on Acutely Induced PTZ Seizures

Conclusions

- TAK-935 can modify the development of kindling without suppressing seizures.
- The effects of TAK-935 on kindling development correlated with its 24HC-lowering effects.
- In preclinical models of 2-week-old ICR mice, a 39% reduction in seizure burden was observed. TAK-935 was tested for up to 7 PTZ stimulations in 30 days. The results indicate that the acquisition of kindling was retarded by TAK-935 treatment.

- In these models, TAK-935 did not disturb motor coordination with a strong CH24H inhibition.

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References


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Summary

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- The effects of TAK-935 on kindling development correlated with its 24HC-lowering effects.
- In preclinical models of 2-week-old ICR mice, a 39% reduction in seizure burden was observed. TAK-935 was tested for up to 7 PTZ stimulations in 30 days. The results indicate that the acquisition of kindling was retarded by TAK-935 treatment.

- In these models, TAK-935 did not disturb motor coordination with a strong CH24H inhibition.