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Date: March 25, 2026

Title: **Evaluation of ETSP # 613003 in the Mouse 6 Hz 44 mA Seizure Model and Rotarod Motor Impairment Assays**

Purpose: To determine the effect of **oral (p.o.)** drug administration of an investigational compound on acute seizures in the mouse 6 Hz 44 mA model and motor function in the rotarod assay.

Study ID: 1

Dose (mg/kg): 0 ☑ This is a vehicle test

Strain / Sex / Supplier: CF - 1 / Male / Charles River

Vehicle: 5% DMSO + 45% PEG300 + 5% Tween80 + 45% Saline

Compound Supply Batch: -

Laboratory: Experiments were performed by the Epilepsy Therapy Screening Program Contract Site at the University of Utah under contract number HHSN271201600048C

Experimenter: 14

Notebook Reference: 536; 1-10

INTRODUCTION: The investigational compound was evaluated by the National Institute of Neurological Disorders and Stroke (NINDS) Epilepsy Therapy Screening Program (ETSP) in a rodent preclinical seizure model. The compound was evaluated for its ability to prevent the incidence of evoked seizure in the mouse 6 Hz 44 mA model. In addition, an assessment of motor impairment in the rotarod assay and general behavioral observations for the compound were performed prior to the 6 Hz test. The route of administration for these tests was p.o.

RESULTS:

6 Hz Seizure Model

The 6 Hz test is a model of focal seizures that shows resistance to a number of currently approved antiseizure drugs. Performance of the investigational compound against 6 Hz seizures induced by 44 mA stimulation in mice is shown in Table 1.

Rotarod Assay

The rotarod test is used to identify potential adverse effects of the investigational compound on the motor function of mice. The assessment for impairment using rotarod is performed following drug administration and immediately before seizure tests. Performance of the mice in the rotarod assay is shown in Table 1.

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Table 1: Profile of Antiseizure Activity and Rotarod Performance of ETSP # 613003 in Male Mice (p.o. administration)

Time (hr.):			0.5			2					
Test	**Form.**	**Dose (mg/kg)**	**N/F**	**C[a]**	**# affected**	**N/F**	**C[a]**	**# affected**	**N/F**	**C[a]**	**# affected**
6 Hz	Sol.	-	0/4			0/4					
Rotarod	Sol.	-	0/8			0/8					
Survival at 72 hr. post-drug admin.											

The data for each condition is presented as N/F, where N = number of animals protected (6 Hz 44 mA) or displaying adverse motor effects (rotarod) and F = number of animals tested. Any adverse effects, including deaths, are noted. For 72-hour survival assessment, N = animals that survived and F = number of animals tested. C = comment code. An observer blinded to treatment confirms behavioral observations. Form. = Formulation, Sol. = Solution, Sus = Suspension

[a] Comment code description is provided in Appendix A.

Table 2: Adverse Effects Observed in Animals Dosed with ETSP # 613003 (p.o. administration)

Test	Time (hr.)	Dose (mg/kg)	Comment Code	Description	Count
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	
-				-	

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DEVIATIONS FROM STANDARD PROTOCOL:	ADDITIONAL COMMENTS:
None	These are only the results of the vehicle group

APPENDIX A
METHODS

Methods are attached as Appendix A. An overview of the screening flow and the description of models used by the ETSP can be found at https://panache.ninds.nih.gov/

The *in vivo* data in this report is reported as per the ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines)

Animals

Male albino CF - 1 mice (18 - 30 g; Charles River, Kingston, NY) were used as experimental animals. All animals were housed, fed, and handled in a manner consistent with the recommendations in the National Research Council publication, "Guide for the Care and Use of Laboratory Animals" (1). Mice were group housed in a pathogen-free facility under a 12-h light/12-h dark light cycle and had access to food (Teklad Global 2920X) and water ad libitum, except during experiments. No insecticides capable of altering hepatic drug metabolism enzymes were used in the animal facilities. All animals were euthanized in accordance with the Institute of Laboratory Resources policies on the humane care of laboratory animals. All protocols involving the use of animals have been approved by the Institutional Animal Care and Use Committee at the University of Utah.

Testing Paradigm

Animals were transferred to the experimental room approximately 30 minutes to one hour before testing, for an acclimation period, coded and randomized using GraphPad (http://graphpad.com/quickcalcs/randMenu). N = 4 mice/dose/time point were used in the test. Animals were tested at two time point(s) and - dose(s) given by p.o. administration in a volume of 0.01 ml/g body weight in mice. Additional doses and time points can be used if supported by known pharmacology. The results for each condition are presented as N/F, where N = number of animals protected and F = number of animals tested. For tests of impairment (rotarod), N = number of animals displaying adverse effects and F = number of animals tested. Any adverse effects, including deaths, were noted. The experimenter was blinded to the identity of experimental drug and an observer blinded to treatment confirmed behavioral observations.

6 Hz Mouse Seizure Model

In mice, a current intensity of 22 mA is sufficient to evoke a seizure in 97% of the population tested (CC_{97} (2)); the 44 mA stimulation intensity typically used in this model is 2 times CC_{97}. A drop of 0.5% tetracaine hydrochloride in 0.9% saline (anesthetic/electrolyte) solution was applied to the eyes of each animal prior to placement of the corneal electrodes. The electrical stimulus was delivered, for a 3 second duration, via corneal electrodes by an apparatus similar to that originally described by Woodbury and Davenport (3). Typically, 6 Hz seizures are characterized by an initial momentary stun followed immediately by jaw clonus, forelimb clonus, twitching of the vibrissae, and Straub tail lasting for at least 1 second. Animals not displaying these characteristics within a 1-minute observation period are considered protected (2, 4, 5).

Rotarod Assay

Shortly before 6 Hz test the performance of mice in rotarod test was examined to identify potential effect of the test compound on motor functions (6). When a mouse is placed on a 1-inch knurled rod that rotates at a speed of 6 r.p.m., the animal can maintain its equilibrium for long periods of time. The mouse was considered impaired if it fell off this rotating rod three times during a 1-minute period.

72-hour Survival Assessment

Vehicle group animals were not assessed for 72-hour survival

Exclusion of animals and data points:

No animals were excluded from this study

Biospecimen Collection:

No blood or tissue collection

Vehicle Testing Details:

No associated ESTP# for the vehicle

Compound Preparation for Administration

The test substance was prepared in
5% DMSO + 45% PEG300 + 5% Tween80 + 45% Saline
The compound was administered p.o. in a volume of 0.01 ml/g body weight in mice.

5% DMSO was added then 45% PEG300 was added and vortexed for 1 min followed by 5% Tween80 and vortexed for 1 min and finally 45% saline was added and vortexed for 2 min

Formulation Images

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Comment Codes

Code #	Description
2	Died during test without seizure
3	Death following continuous seizure
4	Death following tonic extension
5	Death following clonic seizure
11	Anesthesia (total loss of feeling or sensation, unresponsive to tail pinch and tapping of the eye)
12	Ataxia (lack of voluntary coordination of muscle movements, can include wobbly gait)
13	Loss of righting reflex (unable to turn over when placed in a dorsal recumbent position)
14	Unable to grasp rotarod (inability to hold on to rotarod in order to begin test)
15	Minimal motor impairment
16	Loss of muscle tone (soft, with low muscle tone)
19	Sedated (very calm or appear to be sleeping, but will respond to external stimuli)
22	Continuous seizure activity
23	Clonic seizures (muscle convulsions of the forelimbs and/or hindlimbs)
24	Intense, repeated jumping straight up
25	Myoclonic jerks (non-rhythmic muscle twitch, jerk, shake or spasm)
26	Wild running (frantic running)
27	Myoclonic jerks (non-rhythmic muscle twitch, jerk, shake or spasm) following investigational compound administration but before the induction of a seizure test
28	Writhing (a stretch, tension to one side, extension of hind legs, contraction of the abdomen, or twisting of the trunk)
29	Hyperactivity (increased velocity of movement, faster motion than typical)
31	Severe tremors (strong rhythmic muscle contraction, shaking movements in the limbs or body leading to complete or near incapacitation)
32	Exophthalmos (eye bulging)
33	Tremors (rhythmic muscle contraction, shaking movements in the limbs or body)
34	Muscle spasms (continuous or recurrent muscle contraction or rigidity)
35	Wet dog shakes (a brief, ~1 second shaking of entire body. not restricted to single body part)
36	Stretching and rolling (extension/elongation of the body; rolling onto one side with or without completely exposing the ventral body surface)
37	Retropulsion (backward locomotion or backward circling)
38	Arching (arching of the back)
41	Diarrhea (loose, watery stool)
42	Salivation (noticeable saliva outside the mouth)
45	Piloerection (hairs become erect and bristle due to hair follicle contraction i.e., goose bumps)
49	Hyperesthesia (increase in sensitivity for all senses, i.e., jumping at noises, running or jumping when touched)
55	Vocalizations (noises audible to humans)
56	Excessive grooming (intense, excessive or disproportionate body cleaning; may be restricted to specific body parts, with or without visible signs of tissue damage)
61	Urinary staining (pigmented urine)
62	Bloody urine (bright red urine)
80	Cold tail (tail feels cold when touched)
81	Cold to the touch (animal’s body feels colder than typical - more severe than cold tail, above)
Z	Other - described in table

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Table A1. Antiseizure Activity and Minimal Toxicity of Prototype Anti-Seizure Medications (ASMs) in the 6 Hz Model in Male Mice

ASM	i.p. Administration			p.o. Administration	
	TD_{50}[a](mg/kg) Rotarod	ED_{50}[a] (mg/kg) and PI[b] 6 Hz 32 mA	ED_{50}[a] (mg/kg) and PI[b] 6 Hz 44 mA	TD_{50}[a](mg/kg) Rotarod	ED_{50}[a] (mg/kg) and PI[b] 6 Hz 44 mA
Carbamazepine	40.6 (33.2 - 43.7)	23.7 (18.3 - 26.3) **PI 1.7**	36.4 (24.7- 51.7) **PI 1.1**	125.5 (74.1 - 168.8)	63.7 (42.5 - 85.9) **PI 1.7**
Clobazam	16.8 (11.5 - 23.7)	4.1 (2.6 - 4.7) **PI 4.1**	7.3 (5.3 -9.6) **PI 2.3**	196.7 (118.6 - 333.0)	7.0 (5.0 - 9.2) **PI 28.1**
Clonazepam	2.4 (1.9 - 3.4)	0.1 (0.09 - 0.14) **PI 24**	0.8 (0.5-1.2) **PI 3**	2.0 (1.4 – 2.9)	NT
Ethosuximide	361.4 (326.9-399.5)	171.6 (157 - 191.5) **PI 2.1**	> 370 **PI < 1**	554.6 (345.2 - 1122.1)	NT
Ezogabine	59.1 (35.5-105.4)	42.5 (32.1 - 53.6) **PI 1.4**	62.2 (42.4-89.6) **PI 0.96**	> 500	54.4 (31.2 - 99.9) **PI > 7.8**
Felbamate	453.0 (363.2 - 563.2)	72.9 (55.3 - 89.6) **PI 6.2**	97.5 (79.3 - 122.1) **PI 4.6**	309.8 (220.1 - 416.6)	NT
Gabapentin	> 500	>350	> 500	NT	NT
Lacosamide	23.8 (21.0-28.4)	5.6 (3.5-8.4) **PI 4.3**	15.2 (10.7-19.5) **PI 1.6**	NT	NT
Lamotrigine	90.1 (63.9 – 112.1)	19.5 (12.9 - 30.8) **PI 4.6**	42.8 (26.1-61.0) **PI 2.1**	135.4 (116.9 - 154.6)	NT
Levetiracetam	> 500	9.9 (6.7 - 14.4) **PI > 50**	> 500	> 500	NT
Phenytoin	41.6 (32.6-48.9)	> 60 **PI < 0.7**	64.0 (42.0-98.9) **PI 0.7**	127.1 (79.8 - 164.5)	NT
Tiagabine	7.6 (4.4-10.3)	ND	0.9 (0.8-1.3) **PI 8.4**	33.2 (25.4 - 46.8)	1.9 (1.2 - 2.7) **PI 17.7**
Topiramate	233.5 (183.2-309.6)	> 400 **PI < 0.6**	> 300 **PI < 0.8**	NT	NT
Valproate	568.9 (539.4-604.9)	121.2 (80.2 -171.5) **PI 4.7**	234.7 (193.6-302.5) **PI 2.4**	> 600	387.4 (276.1-530.6) **PI > 1.5**

[a] 95% confidence interval ( ).
[b] Protective Index; PI = TD_{50}/ED_{50}.
NT = not tested, ND = not determined
A minimum of 4 doses with 8 animals per dose were tested to determine values.

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