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LP352, A 5-HT$_{2C}$ SUPERAGONIST, HAS BROAD ANTIEPILEPTIC ACTIVITY IN PRECLINICAL SEIZURE MODELS

Anne M. Danks, Marco Peters, Randall Kaye

Longboard Pharmaceuticals, La Jolla, CA, USA

LONGBOARD PHARMACEUTICALS

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BACKGROUND

- 5-hydroxytryptamine (5-HT)$_2$ receptor agonists have shown treatment efficacy for a variety of seizure types and disorders
- LP352 is a potent and highly selective 5-HT$_{2C}$ superagonist designed for the treatment of developmental and epileptic encephalopathies
- Proof of concept for the utility of LP352 across a range of seizure etiologies was established in zebrafish and mouse model systems
- Due to the rapid reproductive capacity and ease of genetic manipulation, both zebrafish and mice are highly useful model systems for studying many human diseases
- Both have been validated as experimental models for seizures and epilepsy and show sensitivity to many classes of anti-seizure medications

OBJECTIVES

- **Experiment 1:** To determine the efficacy of LP352 on locomotor activity and brain epileptiform activity in the *scn1lab*$^{-/-}$ zebrafish epilepsy model of Dravet syndrome[1]
- **Experiment 2:** To assess the efficacy of LP352 on locomotor activity and brain epileptiform activity in the zebrafish ethyl ketopentenoate (EKP)[2] seizure model
- **Experiment 3:** To assess the efficacy of LP352 on locomotor activity and brain epileptiform activity in the zebrafish kainic acid (KA)[3] seizure model
- **Experiment 4:** To evaluate the ability of LP352 to increase or decrease the threshold for seizure induction caused by the intravenous (IV) infusion of pentylenetetrazol (PTZ) in mice

METHODS

- For zebrafish studies, locomotor activity of individual larvae in 96 well plates was tracked with an automated tracking device (Daniovision/Ethovision, Noldus)
- Local field potentials were recorded via non-invasive surface recordings from the skin above the optic tectum of zebrafish, and epileptiform activity was quantified
- In mice, LP352 was administered orally prior to IV PTZ administration, and time to the first myoclonic twitch or onset of generalized clonus was recorded

Experiment 1

- Zebrafish larvae containing mutations in the fish ortholog gene (*scn1lab*$^{-/-}$) were treated with LP352 or vehicle, and motor behavior and brain epileptiform activity were measured

Experiment 2

- Wild-type zebrafish larvae were treated with EKP, which reduces synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), to induce generalized seizures
- EKP-treated larvae were exposed to LP352 and locomotor activity and brain epileptiform activity were recorded

Experiment 3

- Wild-type zebrafish larvae were treated with KA, a cyclic analog of L-glutamate that binds to and activates excitatory glutamate receptors, to induce acute and chronic seizures in zebrafish in a model of temporal lobe epilepsy
- KA-treated larvae were exposed to LP352 and brain epileptiform activity was recorded

Experiment 4

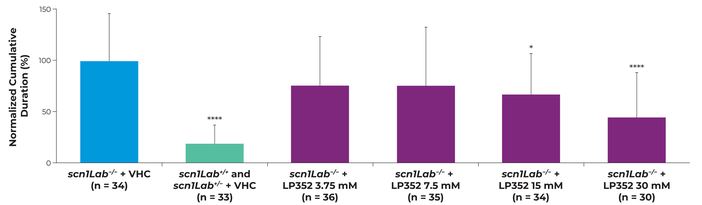
- Mice were given IV PTZ, an antagonist of the GABA-A receptor, to produce myoclonic and tonic-clonic seizures in a model of generalized epilepsy
- Time to the first myoclonic twitch or onset of generalized clonus was recorded in LP352 and vehicle-treated mice

RESULTS

Experiment 1

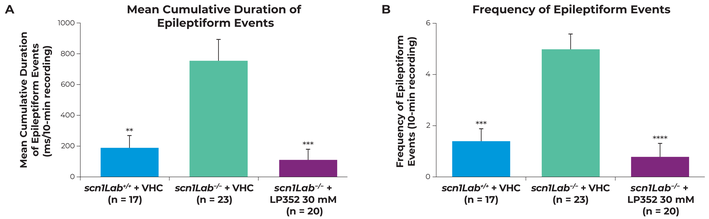
- LP352 treatment reduced locomotor activity (**Figure 1**) and both the frequency and the mean cumulative duration of epileptiform events (84% and 85%, respectively; **Figure 2**)

Figure 1. LP352 Treatment Reduced Locomotor Activity in the Zebrafish Model of Dravet Syndrome



*P<0.05 and ****P<0.0001

Figure 2. LP352 Treatment Reduces Frequency and Duration of Epileptiform Activity in the Zebrafish Model of Dravet Syndrome

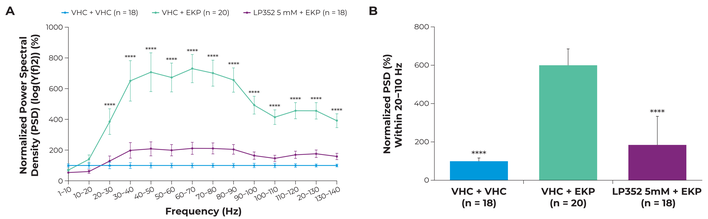


**P<0.01, ***P<0.001, and ****P<0.0001.

Experiment 2

- LP352 treatment reduced brain seizure activity by an average 69.1% in zebrafish models of generalized seizures (**Figure 3**)

Figure 3. LP352 Treatment Reduced Brain Seizure Activity Induced by the Reduction of GABA Activity in Zebrafish

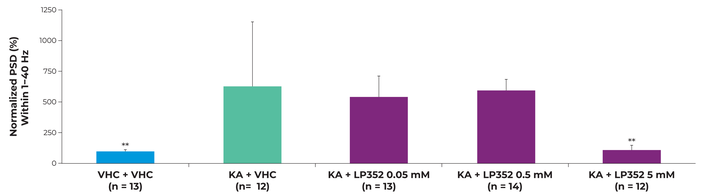


****P<0.0001. Statistically justified outliers were removed.

Experiment 3

- LP352 treatment reduced brain seizure activity induced by excess glutamatergic activity by 82.4% in the zebrafish model of temporal lobe epilepsy (**Figure 4**)

Figure 4. LP352 Treatment Reduces Brain Seizure Activity Induced by Excess Glutamatergic Activity in Zebrafish



**P<0.01. Statistically justified outliers were removed.

Experiment 4

- LP352 administration produced a dose-dependent increase in the time to the first myoclonic twitch and the time of onset to generalized clonus (**Table 1**)

Table 1. LP352 Increases Seizure Threshold Produced by IV PTZ Infusion in Mice

Compound	Dose (mg/kg)	Animal Weight (Grams, Mean ± SEM)	Time of Test	PTZ dose (mg/kg, Mean ± SEM)	
				First Twitch	Clonus
Vehicle	0	32.7 ± 0.7	0.5 h	25.1 ± 1.5	26.4 ± 1.6
LP352	3	31.4 ± 0.4	0.5 h	26.5 ± 0.8	30.3 ± 1.1
LP352	10	31.8 ± 0.4	0.5 h	28.7 ± 0.7	32.3 ± 1.2**

n = 10/group.
**P<0.01.

CONCLUSIONS

- **LP352 broadly reduced a wide variety of seizure activities stemming from numerous underlying causes, including genetic mutations in neuronal sodium channels, reduced GABAergic signaling, and excessive glutamatergic excitation**
- **These data support the potential of LP352 to be useful in treating patients who have developmental and epileptic encephalopathy with heterogeneous underlying pathologies**

Abbreviations **5-HT**, 5-hydroxytryptamine; **EKP**, ethyl ketopentenoate; **GABA**, gamma-aminobutyric acid; **IV**, intravenous; **KA**, kainic acid; **PSD**, Power Spectral Density; **PTZ**, pentylenetetrazol; **SEM**, standard error mean; **VHC**, vehicle.

References **1.** Sourbron J et al. *ACS Chem Neurosci.* 2016;7:588-598. **2.** Zhang Y et al. *Sci Rep.* 2017;7:7195. **3.** Heylen L et al. *Front Mol Neurosci.* 2021;14:753936.