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

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BACKGROUND

- 5-hydroxytryptamine (5-HT)$_2$ receptor agonists have shown treatment efficacy for a variety of seizure types and disorders
- Bexicaserin 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 bexicaserin 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 bexicaserin on locomotor activity and brain epileptiform activity in the *scn1lab*$^{-/-}$ zebrafish epilepsy model of Dravet syndrome[1]
- **Experiment 2:** To assess the efficacy of bexicaserin on locomotor activity and brain epileptiform activity in the zebrafish ethyl ketopentenoate (EKP)[2] seizure model
- **Experiment 3:** To assess the efficacy of bexicaserin on locomotor activity and brain epileptiform activity in the zebrafish kainic acid (KA)[3] seizure model
- **Experiment 4:** To evaluate the ability of bexicaserinto increase or decrease the threshold for seizure induction caused by the intravenous (IV) infusion of pentylenetetrazol (PTZ) in mice
- **Experiment 5:** To evaluate the ability of bexicaserin to reduce the incidence of spontaneous hippocampal paroxysmal discharges (HPDs) caused by intrahippocampal kainic acid (IHK) infusion 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, bexicaserin was administered orally prior to IV PTZ administration, and time to the first myoclonic twitch or onset of generalized clonus was recorded (Experiment 4). Spontaneous HPDs were induced by unilateral IHK infusion; after 4 weeks recovery, digital electroencephalograms (EEGs) were recorded in freely moving mice from 20 minutes prior to 90 minutes post bexicaserin administration (Experiment 5)

Experiment 1

- Zebrafish larvae containing mutations in the fish ortholog gene (*scn1lab*$^{-/-}$) were treated with bexicaserin 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 bexicaserin 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 bexicaserin 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 bexicaserin and vehicle-treated mice

Experiment 5

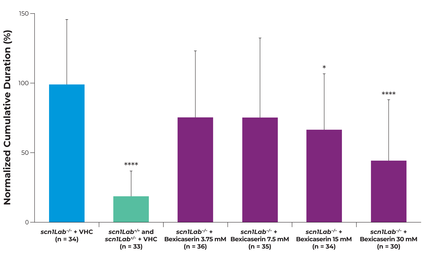
- Mice were administered unilateral intrahippocampal infusions of KA to induce spontaneous HPDs in a chronic IHK model of mesial temporal lobe epilepsy
- Digital EEG recordings of high amplitude hippocampal discharges were made in freely moving mice treated with bexicaserin or vehicle

RESULTS

Experiment 1

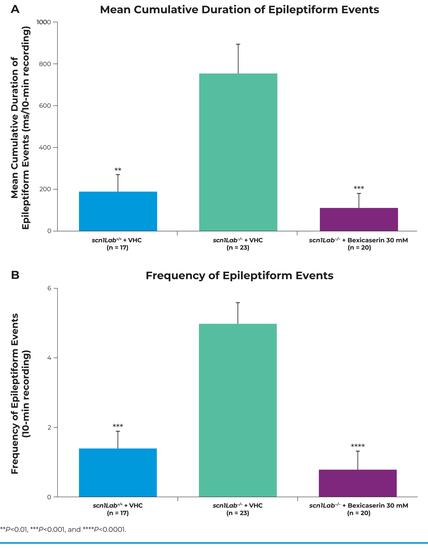
- Bexicaserin treatment reduced locomotor activity (**Figure 1**) and both the frequency and the mean cumulative duration of epileptiform events (84% and 85%, respectively; **Figure 2**)

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



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

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

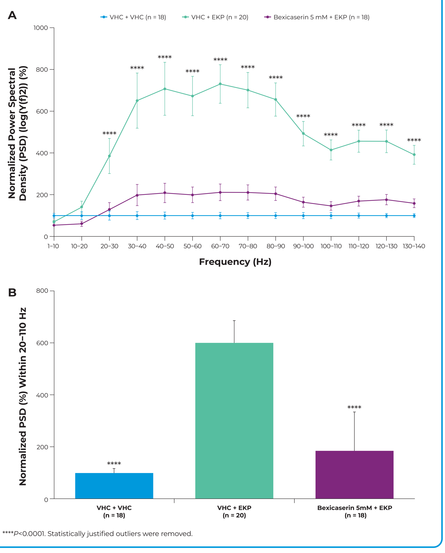


**P<0.01, ***P<0.001, and ****P<0.0001.

Experiment 2

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

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

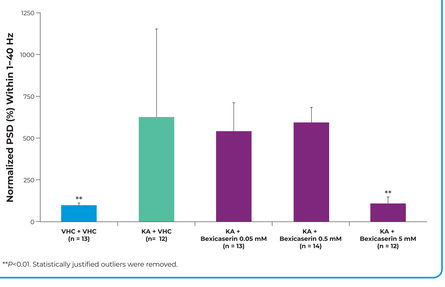


****P<0.0001. Statistically justified outliers were removed.

Experiment 3

- Bexicaserin 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. Bexicaserin Treatment Reduces Brain Seizure Activity Induced by Excess Glutamatergic Activity in Zebrafish



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

Experiment 4 and 5

- Experiment 4: bexicaserin administration produced a dose-dependent increase in the time to the first myoclonic twitch and the time of onset to generalized clonus (**Table 1**)
- Experiment 5: bexicaserin administration reduced HPDs caused by IHK infusion (**Table 1**)

Table 1. Bexicaserin Increases the Seizure Threshold Produced by IV PTZ Infusion and Decreases HPDs Produced by IHK Infusion in Mice

Compound (p.o.)	Dose (mg/kg)	PTZ dose (mg/kg, Mean ± SEM)		IHK
		First Twitch	Clonus	Reduction in HPDs (%)
Vehicle	0	25.1 ± 1.5	26.4 ± 1.6	17.17 ± 3.31
Bexicaserin	3	26.5 ± 0.8	30.3 ± 1.1	–
Bexicaserin	10	28.7 ± 0.7	32.3** ± 1.2	53.92* ± 22.24

n = 10/group.
*P<0.05, **P<0.01.

CONCLUSIONS

- **Bexicaserin 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 bexicaserin to be useful in treating patients who have developmental and epileptic encephalopathy with heterogeneous underlying pathologies**

Abbreviations **5-HT**, 5-hydroxytryptamine; **EEG**, electroencephalogram; **EKP**, ethyl ketopentenoate; **GABA**, gamma-aminobutyricacid; **HPD**, hippocampal paroxysmal discharges; **IHK**, intrahippocampal kainic 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.

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LONGBOARD PHARMACEUTICALS

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