Exploring 5-HT₂ Receptors as Targets for Treating Epilepsy in Fragile X Syndrome: A Preclinical Study of *Fmr1* Knock-out Mice

Tanishka S. Saraf, Yiming Chen, Jessica L. Armstrong, Clinton E. Canal

Mercer University College of Pharmacy, Atlanta, Georgia

INTRODUCTION

Epilepsy prevalence is ~12% in children with autism spectrum disorder (ASD) and ~25% in children with fragile X syndrome (FXS), a monogenetic, neurodevelopmental disorder characterized by intellectual disabilities, severe anxiety, attention deficit hyperactivity disorder, and sensory hypersensitivity—FXS is also the most common, known cause of ASD. Seizure risk increases by three times in individuals with comorbid FXS and autism.

Juvenile (P23–P25) *Fmr1* knock-out mice—a genetic model of FXS/ASD—have a high prevalence of sound-induced (audiogenic) seizures, recapitulating the increased incidence of seizures and sensory hypersensitivity in FXS and ASD.

Several early studies linked alterations in serotonin (5-hydroxytryptamine, 5-HT) to ASD, and selective serotonin reuptake inhibitors (SSRIs) are among the most commonly prescribed medicines to treat psychiatric symptoms in ASD and FXS. Critically, there are at least 14 genetically-encoded 5-HT receptors (5-HTRs)—all indirectly activated by SSRIs—yet detailed information about the impact of specific 5-HTRs on seizures in FXS and ASD is conspicuously lacking.

A recent, small clinical trial reported that **lorcaserin (Belviq®)**, a moderately selective 5-HT_{2C} receptor agonist for obesity, reduced seizure frequency in children with Dravet syndrome (Griffin et al., 2017, PMID: 28073790) and Lennox-Gaustaut syndrome (Tolete et al., 2018, PMID: 30258026). This project explores the anti-epileptic potential of 5-HT₂ receptor agonists in FXS and ASD, beginning with tests of lorcaserin to prevent audiogenic seizures in *Fmr1* knock-out mice.

METHODS

***Fmr1* knock-out** (FVB.129P2-*Pde6b*⁺ *Tyr*^{c-ch} *Fmr1*^{tm1Cgr/J}) and wild-type control (FVB.129P2-*Pde6b*⁺ *Tyr*^{c-ch}/AntJ) mice were purchased from the Jackson Laboratory to develop a colony. Genotyping of litters by PCR was performed based on established protocols.

Audiogenic seizures were assessed by exposing male and female juvenile *Fmr1* knock-out mice to a 120 dB alarm (Radioshack) for 5 min, 30 min after intraperitoneal administration of vehicle (MilliQ water), lorcaserin (AdooQ Bioscience) 1 (L1), 3 (L3), 5.6 (L5.6), or 10 mg/kg (L10), M100907 0.03 mg/kg (M0.03), M0.03+L5.6, or MPEP. Juvenile wild-type mice were treated with vehicle before exposing to the alarm. Responses, including wild running and jumping (WRJ), tonic-clonic seizures (TCS) with and without respiratory arrest, were scored and recorded.

Radioligand saturation binding was performed using cell membranes of brain tissue collected from juvenile male and female wild-type and *Fmr1* knock-out mice, as previously described (Canal et al., 2011; PMID: 20165943). [³H]Mesulergine and [³H]Ketanserin (PerkinElmer) with cold ligands to block off-targets were used to label 5-HT_{2C}Rs and 5-HT_{2A}Rs, respectively.

Adult, male C57BL/6J were used to test in vivo interactions between lorcaserin and racemic (±)-2,5-dimethoxy-4-iodoamphetamine (DOI); specifically effects on the head-twitch response (HTR) and locomotion.

5-HT₂ receptor phosphoinositide hydrolysis assays were performed as previously described (Chen et al., 2019; PMID: 30845376) using human 5-HT_{2A} and 5-HT_{2C} receptors transfected in HEK293 cells.

LORCASERIN PHARMACOLOGY

lorcaserin (Belviq®)

Lorcaserin exhibits full agonist activity at human 5-HT_{2A}Rs and 5-HT_{2C}Rs, with ~17-fold selectivity for 5-HT_{2C}Rs (Felsing, Canal, Booth, 2019; PMID: 30689993 and unpublished data).

(±)-DOI exhibited potent full agonist activity at human 5-HT_{2A}Rs and 5-HT_{2C}Rs, with ~6-fold selectivity for 5-HT_{2A}Rs (Canal, unpublished data).

Lorcaserin, 3 and 5.6 mg/kg, did NOT attenuate the HTR elicited by (±)-DOI, 1 mg/kg, in adult, male C57BL/6J mice. Rather, we observed small, but significant *increases* in HTR frequency (Felsing, Liu, and Canal, unpublished data). Data shown as mean (SD).

Despite NOT attenuating the HTR elicited by DOI, lorcaserin significantly decreased locomotor activity in adult, male C57BL/6J mice. Lorcaserin's hypolocomotor effects on its own are not shown (Felsing, Liu, and Canal, unpublished data). Data shown as mean (SD).

EFFECTS OF LORCASERIN ON AUDIOGENIC SEIZURES IN JUVENILE *FMR1* KNOCK-OUT MICE

Significantly less wild type (WT) mice that received vehicle and *Fmr1* knock-out (KO) mice that received MPEP (positive control) displayed WRJ and TCS/seizures (Fisher's exact test, *****P*<0.0001 and **P*<0.05, respectively) and died due to seizure induced lethality (Fisher's exact test, ###*P*=0.0002 and **P*<0.05, respectively), relative to vehicle. Lorcaserin, 3 mg/kg, decreased audiogenic seizure prevalence and lethality by 19% and 32%, respectively, however, effects did not reach the predetermined significance level (Fisher's exact test, *P*=0.3071 and *P*=0.0585, respectively).

No significant sex differences were observed, however, qualitatively, males were more susceptible to AGS in all treatment groups as they displayed more severe phenotypic manifestations such as a higher percentage of lethality and decreased onset latency to responses ultimately leading to early death, than females.

Mean (SD) of (A) response onset latency and (B) response duration in *Fmr1* knock-out mice. Responses progress from WRJ to TCS to D (death). No significant difference was observed in vehicle and lorcaserin treated groups. WRJ onset latency with MPEP was significantly different (Kruskal-Wallis non-parametric test, **P*<0.05)

ASSESSMENT OF 5-HT_{2A} AND 5-HT_{2C} RECEPTOR EXPRESSION IN BRAINS OF *FMR1* KNOCK-OUT MICE

Preliminary data suggest decreased expression of cortical 5-HT_{2A}Rs in juvenile male mice (A) and decreased expression of 5-HT_{2C}Rs in coronal sections containing striatum and choroid plexus in juvenile female *Fmr1* knock-out (KO) mice (B) relative to wild-type (WT) FVB mice.

DISCUSSION

Lorcaserin (3 mg/kg) reduced seizure prevalence and lethality in juvenile *Fmr1* knock-out mice, though, effects were not statistically significant. Overall, lorcaserin did not significantly prevent or prolong latency to or shorten duration of audiogenic seizures; instead, at doses other than 3 mg/kg, lorcaserin potentiated seizures, displaying mild (but not statistically significant) proconvulsant effects. At 5.6 mg/kg, onset latency to all responses, number and duration of WRJ before TCS increased, thus, prolonging the time spent in seizure avoidance behavior. Coadministration of M0.03 and L5.6, increased seizure prevalence but decreased severity of seizures with a similar pattern of anticonvulsant activity (increasing onset latency) observed at 5.6 mg/kg. Thus, lorcaserin's 5-HT_{2C}R-specific effects at higher doses, may involve potential anticonvulsant action. These complex mixed effects of lorcaserin in FXS may involve molecular mechanisms that are distinct from seizure attenuation effects in Dravet and Lennox-Gastaut syndromes. Preliminary results suggest decreases in 5-HT_{2A}Rs and 5-HT_{2C}Rs in brains of juvenile *Fmr1* knock-out mice. These data suggest 5-HTRs are altered in *Fmr1* knock-out mice, and might be therapeutic targets for FXS or ASD.

Lorcaserin is marketed as a selective 5-HT_{2C} agonist, but its selectivity over 5-HT_{2A} is only ~17-fold. Lorcaserin also has agonist activity at 5-HT_{1A} receptors with an affinity as low as 50 nM (FDA briefing document, NDA22529). In drug discrimination tests, the full efficacy, 5-HT_{1A} agonist, 8-OH-DPAT occasioned greater than 90% lorcaserin (0.56 mg/kg)-lever responding in 57% of rats tested (Serafine et al., 2016, PMID: 27640338). According to the FDA briefing document, brain levels of lorcaserin could reach 1.7 μM from the clinical dose. At this concentration, lorcaserin could activate 5-HT_{2A}Rs and 5-HT_{1A}Rs in addition to 5-HT_{2C}Rs. Pharmacodynamic effects of lorcaserin for individuals with ASD or FXS, therefore, remain uncertain.

Future experiments will assess potential contributions of 5-HT_{1A}, and will evaluate other 5-HT₂ ligands, including DOI.