Impact of the KCNQ2/3 channel opener ezogabine on reward circuit activity and clinical symptoms in depression: results from a randomized controlled trial

Supplemental Material

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1. Supplemental Methods

Screening

As part of the screening procedures, all participants underwent a medical screening that included medical history and physical examination, EKG, clinical hematological and biochemical blood analyses, urine toxicology, and urine pregnancy test for female participants. At screening and at the end of the study, participants also underwent an ophthalmic exam according to the FDA recommendation, as ezogabine carries a black box warning for retinal abnormalities with funduscopic features similar to retinal pigmental dystrophies. In addition, a second electrocardiogram was completed prior to escalating to the maximum tolerated dose in order to monitor for QT interval prolongation, which is reported to occur with the study drug according to the FDA package inset.

Study Exclusion Criteria

Exclusionary diagnoses included any primary psychiatric diagnosis other than a depressive disorder as defined by DSM-5 [co-morbid anxiety disorders and posttraumatic stress disorder (PTSD) were allowed], lifetime diagnosis of a major cognitive disorder, or substance use disorder in the past six months. Other exclusion criteria included pregnancy or breastfeeding, urine toxicology positive for illicit drugs at screening or prior to magnetic resonance imaging (MRI), history of retinal abnormalities (i.e., pigment changes, retinal dystrophy) or findings of retinal pathology on ophthalmic exam at screening; and any unstable medical illness. Clinically significant abnormalities of laboratory tests, physical examination, or electrocardiogram (EKG), including a prolonged QT interval (operationalized as a QTc of > 480 msec) at screening, and any contraindication to MRI were also exclusionary. History of non-response to electroconvulsive therapy in the current depressive episode and use of antidepressants and other medication with CNS activity for a duration equivalent to five half-lives of the medication at the time of randomization were exclusionary. Participants who expressed interest in the study and were on a current antidepressant were referred back to their treating physician to consider a taper of the medication, if clinically indicated. Use of non-benzodiazepine hypnotic agents as needed, or benzodiazepines not in excess of the equivalent of 2 mg of lorazepam daily was allowed.

Randomization and Masking

The treatment assignment scheme was computer-generated, using randomly permuted blocks and stratified by center. A computer-generated randomization scheme developed by the coordinating site (Icahn School of Medicine at Mount Sinai) assigned a unique treatment code, which dictated the

treatment assignment and matching study drug kit for each subject. The identity of test and control treatments were not known to investigators, research staff, or patients.

Incentive Flanker Task Description

The IFT (Figure 2.A) is a variation of the MID that incorporates a longer mean cue time, jittered cue time, and catch trials, all features that facilitate differentiation between reward expectancy and reward responsiveness. During the flanker conflict portion of the task, participants are instructed to press the left response button for target letters S/K and the right response button for letters H/C (letter assignment is counterbalanced across subjects). Target letters are flanked by letters representing same or different button presses. Flanker stimulus duration will be manipulated using a dynamic algorithm which adjusts duration to maintain accuracy for each trial type at ~60%. This feature allows for individual differences in RT, while simultaneously ensuring sufficient numbers of both correct and incorrect trial types for analysis. Cues presented prior to letter stimuli (2-6 s) designate the monetary value for each trial: (1) "gain" cues indicate that subjects earn 50 cents with a correct response (and fail to gain with an error); (2) "loss" cues indicate that subjects can avoid a loss of 50 cents with a correct response (and lose money with an error); (3) "neutral" cues indicate that no money is at stake. Two-thirds of all cues will be followed by the letter stimuli. Immediately after the response, outcome feedback is presented for 2 s, followed by a blank inter-trial interval (ITI) for 2–6s before the next trial begins with a new cue. One-third of cues will be followed by a blank screen for 2s ("catch" trials), which will break co-linearity between the cue and feedback, allowing differentiation of brain activity related to expectancy and feedback response despite the slow hemodynamic response of the Blood-Oxygen-Level-Dependent (BOLD) signal. Even though BOLD signals to the button press response and outcome are not distinguishable in the current paradigm, comparisons between gain, loss, and null outcomes will subtract out brain activity related to motor responses occurring in each condition.

Probabilistic Reward Task (PRT)

The PRT is a signal detection test that provides an objective assessment of reward learning (1) and was completed by all study participants at the baseline (Study visit 0) and primary outcome visit (Study visit 5). The task consisted of three blocks (100 trials per block) and was completed on a 17" PC monitor using E-Prime (version 1.1; Psychology Software Tools, Inc, Pittsburgh, PA). Participants were presented with schematic faces on which a straight horizontal line mouth of 11.5 mm ("short") or 13 mm ("long") in length was presented for 100 msec. Participants were to indicate, via keypress, whether the long or the short mouth had been presented in order to receive a monetary reward of 20¢. Unknown to subjects, correct identification of one of the mouth lengths (the "rich stimulus") was rewarded three times more

frequently ("Correct! You won 20 cents") than the other (the "lean" stimulus). Stimulus type (e.g., short vs. long mouth) assigned as the "rich" stimulus was counterbalanced across subjects. An equivalent alternate version wherein the length of a vertical line nose changed was used to avoid practice effects. Within a given participant, the nose or mouth version was counterbalanced for the baseline vs. post-treatment visit. The degree of response bias toward the rich stimulus was used for operationalizing reward responsiveness. The change in response bias from Block 1 to Block 3 was used as a measure of reward learning.

Prior to data analysis, PRT data underwent a quality-control check wherein trials with below chance accuracy and/or >10% reaction time outliers were excluded from analysis. The measure of interest was change in response bias (operationalized as the difference between the last and the first block of the task), as a function of time (pre- and post-treatment). Change in discriminability was also examined in order to ensure that increases in response bias were not associated with general improvements in task performance.

2. Supplemental Results

Ezogabine Plasmatic Levels

Blood samples were collected on the primary outcome visit (Study visit 5) and analyzed for 40 subjects (19 in the ezogabine group and 21 in the placebo group) using High Performance Liquid Chromatography/ Tandem Mass Spectrometry (LC-MS/MS) by NMS labs. One subject in the ezogabine group did not provide the sample due to inability to complete the blood draw despite several attempts. Average levels of ezogabine and N-acetyl-ezogabine in the ezogabine group were 777.5 ng/mL (± 538.4 ng/mL) and 559.5 ng/mL (± 371.9 ng/mL), respectively. Three subjects randomized to ezogabine did not show detectable levels of ezogabine or N-acetyl-ezogabine. None of the subjects randomized to placebo had detectable levels of ezogabine or N-acetyl-ezogabine.

Reward Learning

Forty-five subjects completed the PRT at both pre-treatment and post-treatment. Of these, twenty-four had valid PRT data at both baseline and at post-treatment based on established quality-control procedures (see above for a description of the quality-control procedure). Due to the administration of a 2-block version of the task at one of the study sites for the majority of the trial, the data presented reflects a 2-block rather than a 3-block version. Reward learning increased nominally in the ezogabine group

compared to placebo, but this did not reach significance (estimate: 0.13, SEM: 1.71, DF: 22, p=0.1). No significant differences emerged on discriminability (a measure of subject's ability to discriminate between the stimuli that is a proxy of task difficulty), indicating that increases in response bias from pre-treatment to post-treatment were not simply due to general improvements in task performance (all ps>0.05). Change in reward learning did not correlate with change in VS response to reward anticipation within the ezogabine group or across both treatment groups. Finally, change in reward learning correlated with change in clinical symptoms of anhedonia as measured by the SHAPS (r=-0.46, p=0.02) and TEPS anticipatory (r=0.42, p=0.04) across both groups, however the correlation was not significant within the ezogabine group alone.

3. Supplemental Tables and Figures

Figure S1. Study Flow Diagram

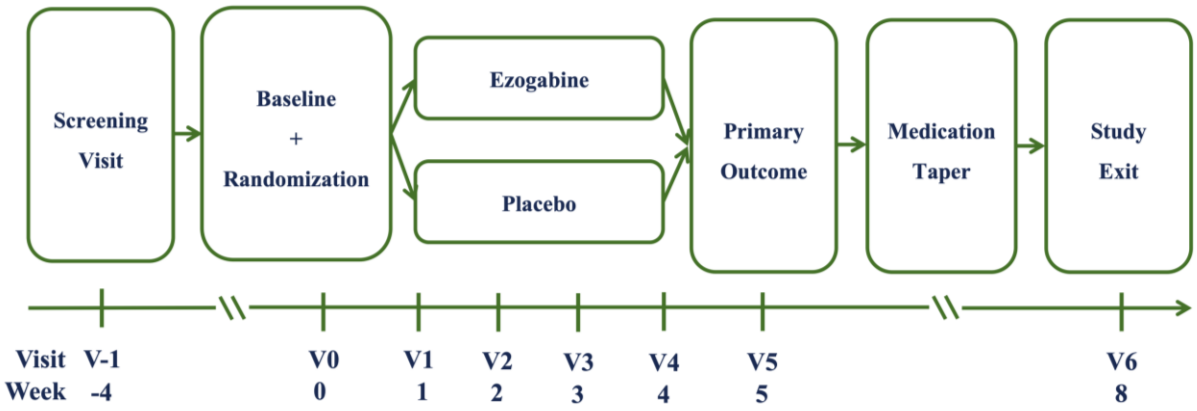
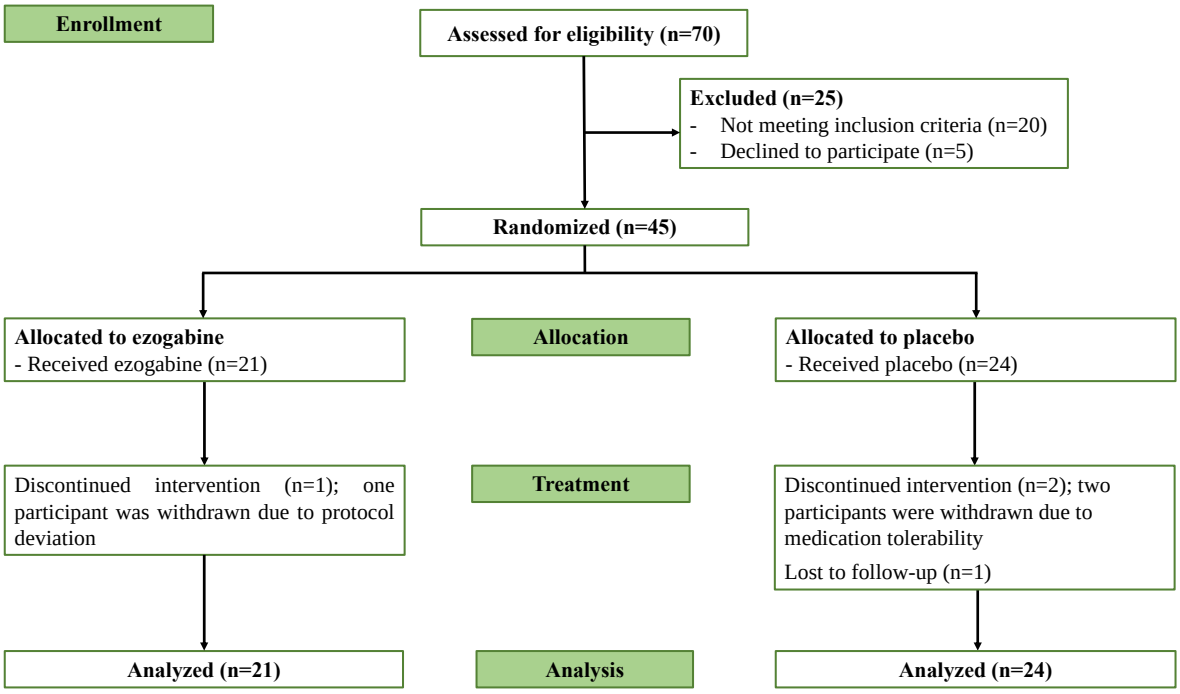


Figure S2. Consort Chart Representing Patient Flow by Study Arm, ezogabine versus placebo[a]



[a]Analyzed data represents the Intent-To-Treat (ITT) sample. Of this, 42 subjects completed the study primary outcome visit, and 40 participants had valid pre- and post-treatment fMRI data during the IFT.

Table S1. Summary of Adverse Events by Treatment Group[a]

	Ezogabine (N = 21)		Placebo (N = 24)		p value
Abdominal pain	2	3.8	3	5.1	0.73
Anxiety	1	1.9	1	1.7	0.95
Back pain	0	0	1	1.7	0.32
Confusional state	4	7.6	0	0	0.05
Constipation	1	1.9	2	3.4	0.62
Cough	1	1.9	1	1.7	0.95
Diarrhea	5	9.4	1	1.7	0.09
Disturbance in attention	3	5.7	0	0	0.08
Dizziness	21	39.7	5	8.6	0.00
Dry mouth	1	1.9	0	0	0.32
Headache	5	9.4	7	12	0.68
Increased appetite	1	1.9	0	0	0.32
Malaise	1	1.9	0	0	0.32
Memory impairment	1	1.9	2	3.4	0.62
Nasal congestion	1	1.9	0	0	0.32
Nausea	3	5.7	5	8.6	0.56
Oropharyngeal pain	1	1.9	0	0	0.32
Palpitations	1	1.9	1	1.7	0.95
Panic attack	2	3.8	0	0	0.16
Polyuria	0	0	1	1.7	0.32
Rash	2	3.8	0	0	0.16
Restlessness	0	0	1	1.7	0.32
Sedation	0	0	1	1.7	0.32
Somnolence	2	3.8	3	5.1	0.73
Upper respiratory tract infection	0	0	1	1.7	0.32
Vision blurred	3	5.7	1	1.7	0.28
Vomiting	2	3.8	1	1.7	0.52
Other	9	17	8	13.7	0.66

[a]Table 3 summarizes occurrence of adverse events (AE) in the trial that occurred subsequent to at least one dose of study medication between randomization and study exit (V8) eight weeks after randomization. AEs are reported as number of events (number of events/100 patient-months).

4. Supplemental References

1. Pizzagalli DA, Jahn AL, O'Shea JP. Toward an objective characterization of an anhedonic phenotype: a signal-detection approach. Biol Psychiatry. 2005 Feb 15;57(4):319–27.