Preclinical Psychopharmacological Properties of JZP-4, a Novel and Potent Mood Stabilizer

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Introduction

- Anticonvulsants are among the most widely used pharmacological agents for CNS disorders. Appplications range from epilepsy, essential tremor, neuropathic pain, bipolar disorders and drug withdrawal (Foreman et al., 2008; French et al., 2004; Goldsmith et al., 2003, Moreley-Forster, 2006 and Weisler et al., 2006). However, due to many adverse effects of these compounds, there remains a need for drugs with broad spectrum efficacy and tolerability.
- Lamotrigine, a better tolerated, anticonvulsant has been shown to be used in the treatment of epilepsy, Lennox-Gastaut Syndrome and bipolar II (Bowden, 1998; Goldsmith et al., 2003, Leach et al., 2002; Shelton, 2002). Although lamotrigine is well tolerated, it has to be titrated slowly to therapeutic doses as it can cause an immunologic hypersensitivity reaction (Ketter et al., 2005).
- JZP-4 (3-(2,3,5-trichloro-phenyl)-pyrazine-2,6-diamine) is structurally related to lamotrigine but has a trichlorophenyl ring rather than a dichlorophenyl ring and a pyrazine rather than a triazine ring (Figure 1). JZP-4 is a potent calcium and sodium channel blocker that is currently in clinical trials as an anticonvulsant and moodstabilizer.
- Compared to Lamotrigine, JZP-4 has lower half life making it pharmacokinetically possible to achieve therapeutic levels with shorter titration periods (Eller et al., in prep).
- The current study focuses on evaluating the efficacy of JZP-4 in mouse and rats models of mania, depression and anxiety.

Methods

Animals

Male CFW (4 weeks old) mice from Charles River Laboratories were used in the Four Plate Test. MaleC57 bl6J mice (8 weeks old) from Jackson Labs were used in the elevated plus maze and amphetamine/chlordiazepoxide hyperactivity tests. Male Sprague Dawley rats (~250g) from Harlan were used in the rat forced swim and locomotor tests. Upon receipt, animals were assigned unique identification numbers (tail marked) and were group housed in OPTI mice ventilated cages with4 mice/ cage or standard rat cages with 3 rats/cage. All animals remained housed in groups of four during the remainder of the study. Animals were maintained on a 12 hr /12 hr light/dark cycle with the light on at 7:00 a.m EST. The room temperature was maintained between 20 and 23℃ with a relative humidity maintained between 30% and 70%. Chow and water were provided *ad libitum* for the duration of the study. Prior to testing, all animals were examined on a regular basis, handled, and weighed to assure adequate health and suitability. In each test, animals were randomly assigned across treatment groups. Animals were not disturbed between test days.

Elevated Plus Maze

The elevated plus maze test assessed anxiety. The maze (Hamilton Kinder) consists of two closed arms (14.5 h x 5 w x 35 cm length) and two open arms (6 w x 35 l cm) forming a cross, with a square center platform (6 x 6 cm). All visible surfaces are made of black acrylic. Each arm of the maze was placed on a support column 56 cm above the floor. Antistatic black vinyl curtains (7' tall) surround the EPM to make a 5' x 5" enclosure. Animals were brought to acclimate to the experimental room at least 1 hr before the test Mice were placed in the center of the elevated plus maze facing the closed arm for a 5-min run. All animals were tested once. The time spent, distance traveled and entries in each arm were automatically recorded by the computer. Mice were injected with lamotrigine, JZP-4, diazepam, 45% cyclodextrin or Jazz vehicle 30 min prior to testing. The EPM was thoroughly cleaned after each mouse.

Four Plate Test

The four plate test is an animal model of anxiety in which simple ongoing behavior (exploration of novel surroundings) is suppressed by the delivery of mild electric foot-shock contingent to quadrant crossing (Aron, et al., 1971). The four plate apparatus (Bioseb, Chaville France) consists of a cage (18×25×16 cm) floored by four identical rectangular metal plates (8×11 cm) separated from one another by a gap of 4 mm. The plates are connected to a shocker unit that can generate electric foot shocks. Drug naive CFW mice were injected with vehicle, JZP-4 or lamotrigine and placed in holding cages for 45 min. A separate group of mice were injected with either alprazolam or water and paced in holding cages for 30 min. Following pretreatment, mice were gently placed in the four plate chamber and allowed to explore the enclosure for 18 seconds. After the exploration period, every time the mouse crossed from one plate to another, the experimenter, blind to the dosing conditions, administered a mild electric foot-shock, and referred to as a punished crossing. The intensity and duration of the foot-shock were 0.5mA for 0.5 seconds. The number of punished crossings was recorded during the 2 min test.

Rat Forced SwimTest

All experiments were done during the rats' light cycle. The Force Swim chamber was constructed of clear acrylic (height = 40 cm; diameter = 20.3 cm) and filled with water (23 ± 1 ° C) at 16 cm deep during habituation and 30 cm deep during test. The rats were exposed on the first day to a 15-min pre-swim session. After completion of the preswim session, rats were injected with water, desipramine, JZP-4 or Jazz Vehicle. Twenty-four hours following the pre-swim, animals were placed back in the same chamber for a 5-minute test session. Pretreatment time for all compounds was 30 min. The frequency of swimming, climbing and immobility was analyzed during the 5-min test session.

Rat Locomotor Activity

Locomotor activity was assessed using photocell monitored cages (Hamilton Kinder, San Diego). Each cage consists of a standard plastic rodent cage surrounded by a stainless steel frame containing photocell beams. Two sets of infrared photobeam cells are located along the long axis of the frame to detect ambulatory distance traveled and rearing activity. Rats were injected with vehicle, LTG or JZP-4 at 3 10 or 30 mg/kg i.p. and placed in holding cages for 30 min following which they were placed individually in the activity chambers. Ten rats were used per treatment group. Locomotor and rearing activities during the 60-min test were measured by consecutive photobeam breaks as the animal moved.

Open Field

The open field test (OF) is used to assess both anxiety-like behavior and motor activity. The open field chambers are plexiglas square chambers (27.3 x 27.3 x 20.3 cm; Med Associates Incs., St Albans, VT) surrounded by infrared photobeam sources (16 x 16 x 16). Distance traveled is measured from consecutive beam breaks. Measures of total distance covered during locomotion were used as an index of activity. Following pretreatment with either vehicle, valproate, JZP-4 or Lamotrigine, mice were injected with water, d-amphetamine or d-amphetamine/CDP mixture and placed in the OF chambers for a 60 min test session. At the end of each open field test session the OF chambers were thoroughly cleaned.

Statistical Analysis

Data were analyzed either by analysis of variance or student's t-test followed by Dunnett's post hoc analysis when appropriate. An effect was considered significant when the p value was less than 0.05. Statistical outliers that fell above or below two standard deviations from the mean were excluded from the final analysis

Figure 1: Chemical Structures of Lamotrigine and JZP-4

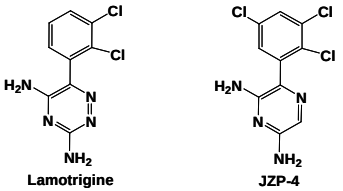
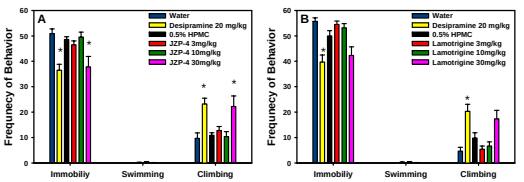
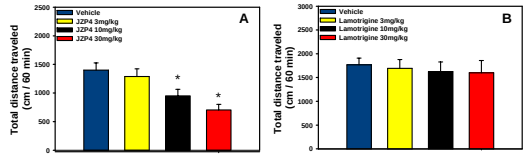


Figure 2: JZP-4 exhibits antidepressant-like properties in rats



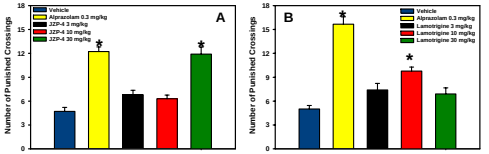
Rats were injected with water, desipramine, 0.5% hydroxyporpylmethyl-cellulose (HPMC), JZP-4 or lamotrigine (i.p) once after training and again 30 min prior to test. Data represent mean ± SEM.

Figure 3: Neither JZP-4 nor lamotrigine showed locomotor-stimulant effects in rats



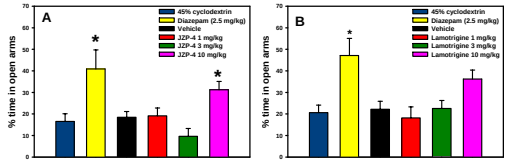
Rats were injected with 0.5% hydroxyporpylmethylcellulose (Vehicle), JZP-4 or lamotrigine (i.p) 30 min prior to a placing in the locomotor activity chambers for 60-min test. Data represent mean ± SEM.

Figure 4: JZP-4 and lamotrigine increased the number of punished crossings in mice



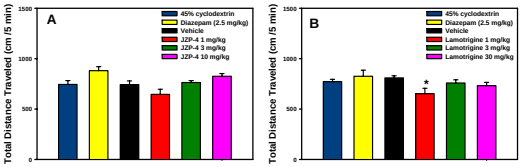
Mice were injected with 0.5% hydroxyporpylmethylcellulose (HPMC), JZP-4 or lamotrigine (i.p) 45 min prior to testing. Data represent mean ± SEM.

Figure 5: JZP-4 increased the percentage of the time mice spent in the open arms of the Elevated Plus Maze



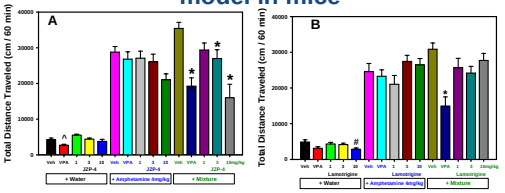
Mice were injected with 45% hydroxyporpy-β-cyclodextrin (po), diazepam (po), 0.5% hydroxyporpylmethylcellulose (vehicle), JZP-4 or lamotrigine (i.p) 30 min prior to testing. Data represent mean ± SEM.

Figure 6: JZP-4 did not cause sedation in the Elevated Plus Maze



Mice were injected with 45% hydroxyporpy-β-cyclodextrin (po), diazepam (po), 0.5% hydroxyporpylmethylcellulose (vehicle), JZP-4 or lamotrigine (i.p) 30 min prior to testing. Data represent mean ± SEM.

Figure 7: JZP-4 showed anti-manic effects in the amphetamine/CDP-induced hyperactivity model in mice



Mice were injected with 0.5% hydroxyporpylmethylcellulose (vehicle), valproate (VPA; 400 mg/kg), JZP-4 or lamotrigine (i.p) 30 min prior to injection with either amphetamine or mixture. Mice were placed in the open field for a 60 min test. Data represent mean ± SEM.

Summary

- JZP-4 showed higher potency and efficacy than lamotrigine in the mouse amphetamine/CDP-induced hyperactivity model of mania. In addition, JZP-4 (30 mg/kg) decreased immobility and increased climbing behaviors in the rat forced swim test similar to the positive reference desipramine suggesting anti-depressant-like properties of the compound. Although lamotrigine showed a trend to decreasing immobility, the effect did not reach significance. The anti-drepressant-like activity of JZP-4 in the rat forced swim test was not a result of a stimulant property of the compound as seen in the locomotor activity studies. The greater potency of JZP-4 in the mania and depression models could well be related to its higher potency at the sodium channels or the additional action of JZP-4 on calcium channels.
- Both JZP-4 and lamotrigine showed anxiolytic-like activities as seen in the increased number of punished crossings in the four late test in mice. In addition JZP-4 was active in the elevated plus maze. At 10 mg/kg, mice treated with JZP-4 spent more time in the open arms compared to vehicle. Lamotrigine showed a non-significant trend to also increasing the % time mice spent in the open arms.
- These data suggest that JZP-4 may have the pharmacological attributes of an effective mood stabilizer both for suppressing the switching from mania to depression in bipolar disorder and

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