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Brilaroxazine (RP5063), a novel serotonin-dopamine stabilizer, displays antipsychotic efficacy in rodents

Laxminarayan Bhat, Kouacou Adiey, Seema R Bhat, and Prabhu Mohapatra

Reviva Pharmaceuticals Holdings, Inc., Cupertino, California, USA (19925 Stevens Creek Blvd., Ste 100, Cupertino, CA 95014)

INTRODUCTION

Schizophrenia, a complex, chronic, and debilitating psychiatric syndrome, affects ~1% of the world's population[1]. It consists of complex mix of positive, negative, and mood symptoms, cognitive impairment, and immune system abnormalities[2,3].This condition's pathobiology involves an imbalance of dopamine (D) and serotonin (5-HT) levels in the brain due to a dysfunctional D-S signaling system, along with neuroinflammation[2-6].

Current antipsychotics, predominantly D or D and 5-HT receptor selective compounds, are far from optimal. Issues include refractory response, suboptimal and lack of broad-spectrum effectiveness vs. major symptoms, adherence, and neurological and cardiometabolic side effects. Substantial unmet medical needs remain[7].

Brilaroxazine (RP5063)- with high affinity for 5-HT$_{1A/2A/2B/7}$ and dopamine D$_{2/3/4}$ and moderate affinity for D$_1$, serotonin transporter, and nicotinic acetylcholine receptor, $\alpha_4\beta$- possesses a broad *in vitro* profile against key D and 5-HT receptors involved with schizophrenia[8,9]. Pre-clinical work in pulmonary artery hypertension (PAH), idiopathic pulmonary fibrosis (IPF), and psoriasis provides initial evidence of brilaroxazine's effect on pro-inflammatory cytokines and chemokines, also found in psychiatric disorders[10-13].

OBJECTIVE

This work addressed the research question- does brilaroxazine exert effective pharmacologic activity in three standard translational rodent models for schizophrenia?

METHODS

Apomorphine Climbing Test[14] involved 5 groups of 10 Naval Medical Research Institute mice (body weight 24-29 g): brilaroxazine (1, 3, and 10 mg/kg i.p.), haloperidol (0.5 mg/kg i.p.), and vehicle (0.2% hydroxypropylmethylcellulose [HPMC] in physiologic saline).

Animals received treatments (i.p.) 30 minutes before apomorphine injection (1 mg/kg subcutaneously [s.c.]). After placement of each animal adjacent to a wire grid wall, evaluation of its behavior occurred every ten minutes using a five-point scale at each time point (10, 20, and 30 mins) over 30 minutes for the intensity of climbing. Climbing intensity scoring involved a five-point scale (0 = normal behavior; 1 = excitation/sniffing; 2 = occasional climbing [2 paws]; 3 = occasional climbing [4 paws]; and 4 = permanent climbing [4 paws]). The total score comprised three measurement points of 10, 20, and 30 minutes. Analysis involved Kruskal-Wallis and Mann-Whitney U tests, with P<0.05 as significant.

Apomorphine-induced Deficit in Prepulse Inhibition (PPI)[15,16] involved 5 groups of 15 Wistar rats (body weight, 260-304 g): brilaroxazine (3, 10, and 30 mg/kg i.p.), haloperidol (1 mg/kg i.p.), and vehicle (0.2% HPMC in physiologic saline).

Rats were placed in chamber 15 minutes after apomorphine induction and habituated for 10 minutes (70 dB intensity background noise). Four phases then proceeded over 23 minutes: (1) no stimulus for basal movement levels; (2) prepulse, involving the presentation of a 20-millisecond (ms) white noise burst at 87, 90, or 93 dB (not to produce a clear startle response); (3) 40 ms burst of white noise at 115 dB (producing a startle response); and (4) 87, 90, or 93 dB prepulse stimulus followed by an 80 ms 115-dB stimulus.

Observations involved: (1) average response over the entire recording period; (2) peak response; and (3) time to peak response. Calculating individual PPI consisted of averaging the eight trials of each type and assessing the percentage reduction in startle amplitude (average and peak values) caused by the 87-, 90-, or 93-dB prepulse. The time to peak response represented a measure of reaction time. Analysis involved ANOVA, followed by planned comparisons against apomorphine alone. Comparison of haloperidol with apomorphine used an unpaired student's t-test. P<0.05 was deemed significant.

Dizocilpine effect on locomotion, stereotypy, and rearing[17], involved 6 groups of 10 Wistar rats: brilaroxazine (3, 10, and 30 mg/kg i.p.), olanzapine (6 mg/kg i.p.) and vehicle (5% Pharmasolve + 45% polyethylene glycol 400 + 50% water for injection) with (and without) induction. The setting involved an open field, a black-colored area 51 × 51 × 36 cm enclosed by black plastic walls of the same dimensions. The field contained an imaginary center 24 cm from the periphery of each side. Testing involved five phases over 80 minutes: (1) T=0 minute – animals received controls and treatments, then transferred to their home cages; (2) T=30 minutes – animals placed in the open field and tracked for their horizontal Locomotion; (3) T=45 minutes – animals removed from the open field; (4) T=45 minutes – animals received dizocilpine or vehicle (i.p.), then transferred to the home cage; (5) T=60 minutes – animals placed in an open field; and (6) T=75 – animals removed from the open area, transferred to a transparent cage for observation.

Assessments involved (1) spontaneous locomotor activity between 30 and 45 minutes in the distance measured (cm); (2) dizocilpine-induced locomotor activity between 60-75 minutes in the distance measured (cm); and (3) dizocilpine-induced stereotypy (sniffing, circling behavior, gnawing, and grooming [0 = absent, 1 = equivocal, 2 = present, 3 = intense, and 4 = intense and continuous]) and number of rears between 70-75 minutes. Data analysis involved ANOVA, then Newman-Keuls multiple comparison test. P<0.05 was deemed significant.

RESULTS

FIGURE 1

Brilaroxazine decreased apomorphine-induced climbing across the 1, 3, and 10 mg/kg doses versus controls (p<0.001).

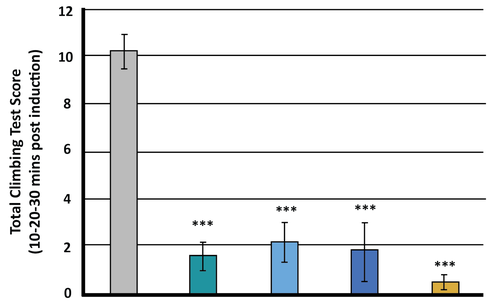

*Figure 1: Effects of brilaroxazine at 1 mg/kg (B1), 3 mg/kg (B3), and 10 mg/kg (B10), haloperidol 0.5 mg/kg (H), and vehicle (V) in the apomorphine-induced climbing test in the mouse (n=10/group). ***p<0.001 (vs. apomorphine plus vehicle; Mann-Whitney U test).*

FIGURE 2

Figure 2 illustrates the results from the apomorphine PPI evaluation. Brilaroxazine at 10 and 30 mg/kg i.p. 30 minutes before the test (i.e., 15 minutes before apomorphine induction) attenuated the apomorphine-induced PPI deficit in a dose-dependent fashion. The 10 mg/kg dose increased PPI at an intensity of 87 dB compared with apomorphine controls (p<0.05). The 30 mg/kg increased PPI at all 3 prepulse intensities (p<0.01 in all cases). Brilaroxazine did not affect spontaneous movements without stimulus or the reaction to the pulse alone, but it slightly decreased the reaction to the prepulse (30 mg/kg at the intensity of 87 dB, p<0.01).

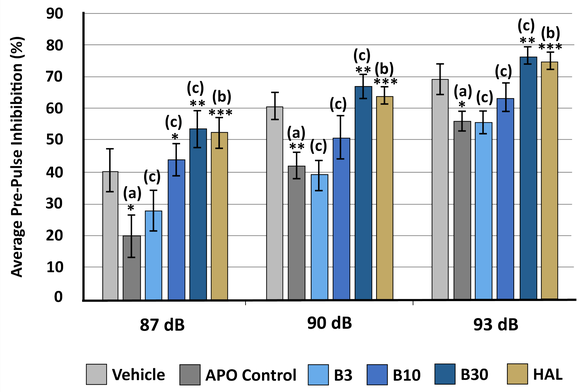

Figure 2: Mean response effects of brilaroxazine at 3 mg/kg (B3), 10 mg/kg (B10), and 30 mg/kg (B30), haloperidol at 1 mg/kg (H), and vehicle (V) in the apomorphine prepulse inhibition test at 87, 90, and 93 dB (n=14 or 15/group).

Notations: *a: Compared vs. vehicle control: * = p < 0.05; ** = p < 0.01. [Student's t-test]. b: compared with apomorphine control: *** = p < 0.001. [Student's t-test]. c: Compared with apomorphine control: no indication = not significant;* = p < 0.05; ** = p < 0.01. [One-way ANOVA followed by Dunnett's t-test in case of significant effect].*

FIGURE 3

In the dizocilpine-induced model, brilaroxazine decreased spontaneous locomotor (30-45 minutes) activity by 15% (p<0.05, 3 mg/kg), 30% (p<0.001, 10 mg/kg), and 40% (p<0.01, 30 mg/kg) and dizocilpine-induced locomotion (60 – 75 minutes) by 25% (p<0.05%, 3mg/kg), 49% (p<0.01, 10 mg/kg), and 47% (p<0.01%, 30 mg/kg).

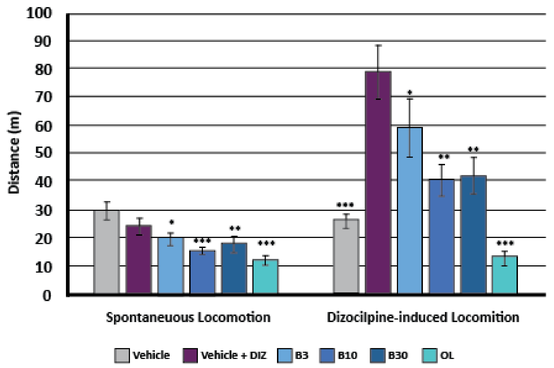

*Figure 3: Effect of brilaroxazine at 3 mg (B3), 10 mg (B10), and 30 mg (B30), olanzapine (6 mg/kg, i.p.) (O) on (A) spontaneous locomotion (T=30–45 minutes) and (B) dizocilpine-induced Locomotion (T=60–75 minutes). (n=9-10/group). *p<0.05; **p<0.015; ***p<0.001 versus dizocilpine-induced vehicle (group 1; 1-way ANOVA followed by the Newman- Keuls multiple comparison test).*

FIGURE 4

Brilaroxazine reduced stereotypy (T = 75–80 minutes) by 51% and 58% (p<0.001, 10- and 30-mg/kg, respectively), and rearing (only 10-mg/kg, NS).

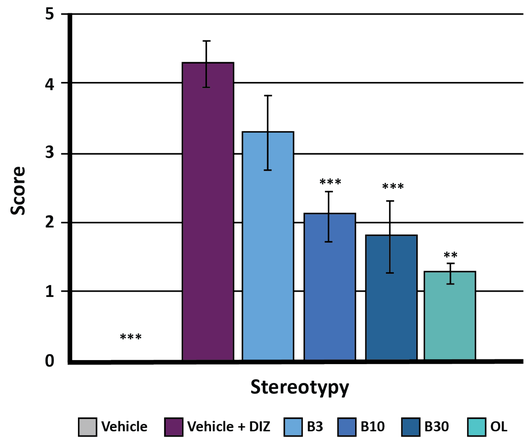

*Figure 4: Effect of brilaroxazine at 3 mg (B3), 10 mg (B10), and 30 mg (B30), olanzapine (6 mg/kg, i.p.) (O) on dizocilpine -induced stereotypy (n=9-10/group). **p<0.01; ***p<0.001 versus dizocilpine (1-way ANOVA followed by Newman-Keuls multiple comparison test).*

DISCUSSION

This research represents the first findings of brilaroxazine's treatment effect for schizophrenia in animal models of induced behaviors. It provides pre-clinical proof-of-concept support that brilaroxazine mitigates behaviors modeled to reflect those schizophrenia patients' experience.

These studies use the most relevant translational rodent models[14-17]. Considering relevant signaling pathways and symptom presentation, they evaluate brilaroxazine's spectrum of antipsychotic activity. The brilaroxazine studies reflect the triad of target receptor pharmacology, significant behavioral symptoms, and predictive data for translation to the clinic. The two apomorphine studies model acute D receptor stimulation. The dizocilpine experiment examines brilaroxazine's effects on the D and 5-HT through its interaction with the NMDA receptor.

Brilaroxazine's activity effect might be explained by its effects on 5-HT$_{1A/2A/2B/6/7}$ and D$_{2/3/4}$ receptors[8, 18-20]. Furthermore, pre-clinical data in the PAH, IPF, and psoriasis rodent models indicate that brilaroxazine impacts the release of pro-inflammatory cytokines and chemokines (e.g., TNFα, IL-1β, IL-6, and MCP-1)[10–13]. Observations from these studies and other wor,[18] suggest that brilaroxazine possesses a multifaceted basis for impacting these symptoms and distinguishes it from other antipsychotics.

In the clinic, phase 1 multiple dose study (10-100 mg) showed improvements with brilaroxazine (p<0.05) over placebo in a Positive and Negative Symptom Scale (PANSS) secondary analysis of patients with a baseline PANSS score >50. The 50 mg dose improved Trails A and B Test results for cognitive and executive functions assessment for patients treated for Days 5, 10, and 16[9,21].

The phase 2 REFRESH study was completed in patients with acute schizophrenia and schizoaffective disorder[8]. Compared with the placebo, all dose groups (15-, 30-, and 50-mg) were numerically superior, and the 15- and 50-mg doses of brilaroxazine were significant (p<0.05) in improving the primary endpoint, the PANSS total score[8]. The median rates of improvement in PANSS total score over baseline in the 15- and 50-mg groups were 23% and 22%, respectively. Both doses were superior to placebo concerning PANSS subscales for positive symptoms, negative symptoms and social functioning, and Clinical Global Impressions-Improvement scores.

CONCLUSION

Brilaroxazine demonstrated significant antipsychotic effects on pharmacologic-induced behaviors associated with psychosis and schizophrenia in three standard translational surrogate rodent models. Currently, this compound is well into phase 3 clinical trials. Such data should reinforce the efficacy profile seen with modulating the D and 5-HT pathways and neuroinflammation in these animal models and in phase 1 and 2 clinical trials.

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