Brexpiprazole has a low risk of dopamine D₂ receptor sensitization and inhibits rebound phenomena related to D₂ and serotonin 5-HT₂A receptors in rats

Naoki Amada¹ | Hitomi Akazawa¹ | Yuta Ohgi¹ | Kenji Maeda² | Haruhiko Sugino³ | Nobuyuki Kurahashi⁴ | Tetsuro Kikuchi⁵ | Takashi Futamura¹

¹Department of CNS Research, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan
²Department of Lead Discovery Research, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan
³Global Business Development, Otsuka Pharmaceutical Development and Commercialization, Ltd., Princeton, New Jersey
⁴Global CNS Business, Otsuka Pharmaceutical Development and Commercialization, Ltd., Princeton, New Jersey
⁵Pharmaceutical Division, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

Correspondence
Naoki Amada, Department of CNS Research, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima, 771-0192, Japan.
Email: amada.naoki@otsuka.jp

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Abstract

Background: Long-term antipsychotic treatment in patients with schizophrenia can induce supersensitivity psychosis and tardive dyskinesia which is thought to be caused by dopamine D₂ receptor sensitization. We evaluated the effects of brexpiprazole on D₂ receptor sensitivity after subchronic treatment in rats. We also evaluated whether brexpiprazole could suppress enhanced response to D₂ receptors in rats subchronically dosed with another atypical antipsychotic.

Methods: The maximum D₂ receptor density (Bₘₐₓ) and apomorphine (a D₂ receptor agonist)-induced stereotypy were measured in rats orally dosed with vehicle, haloperidol (1 mg/kg), or brexpiprazole (4 or 30 mg/kg for Bₘₐₓ, 6 or 30 mg/kg for stereotypy) for 21 days. Then, effects of oral administrations of brexpiprazole (3 mg/kg), aripiprazole (10 mg/kg), and olanzapine (3 mg/kg) against increases in apomorphine-induced hyperlocomotion and (+)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI: a 5-HT₂A receptor agonist)-induced head twitches were evaluated in rats subcutaneously treated with risperidone (1.5 mg/kg/d) via minipumps for 21 days.

Results: Haloperidol and brexpiprazole (30 mg/kg; approximately tenfold ED₅₀ of apomorphine-induced stereotypy) but not brexpiprazole (4 or 6 mg/kg) significantly increased the Bₘₐₓ and apomorphine-induced stereotypy. Brexpiprazole (3 mg/kg) and olanzapine (3 mg/kg) significantly suppressed both increases in apomorphine-induced hyperlocomotion and also DOI-induced head twitches in rats subchronically treated with risperidone, but aripiprazole (10 mg/kg) significantly suppressed only apomorphine-induced hyperlocomotion.

Conclusion: Brexpiprazole has a low risk of D₂ receptor sensitization after a repeated administration and suppresses the rebound phenomena related to D₂ and 5-HT₂A receptors after a repeated administration of risperidone.
1 | INTRODUCTION

Schizophrenia is a chronic disorder that affects 1% of the world population. Initially, the main strategy to treat schizophrenia was based on dopamine D₂ receptor antagonism. In addition, these dopamine D₂ receptor antagonists or second-generation antipsychotics also have antagonistic properties against serotonin 5-HT₂A receptors, as well as a variety of effects on other monoamine receptors, such as 5-HT₁A receptors and α₁-adrenoceptors. These broad target effects have the objective of either improving antipsychotic efficacy with additional effects on affective symptoms or cognitive deficits) or reducing side effects (eg, extrapyramidal symptoms [EPS]). The increased availability of second-generation antipsychotics over the years with different pharmacological profiles has permitted drug-switching strategies in patients showing insufficient therapeutic responses or severe adverse events.

However, due to tolerability issues, treatment with antipsychotic drugs having D₂ receptor antagonist activity may not be the optimal strategy to modulate dopaminergic activity. The discovery and development of D₂ receptor partial agonists have provided an improved treatment option for patients with stabilizing effects on dopamine function. Presently, several D₂ receptor partial agonists such as aripiprazole and brexpiprazole are clinically available.

Brexpiprazole, discovered by Otsuka Pharmaceutical Co., Ltd., and co-developed with H. Lundbeck A/S, is a serotonin-dopamine activity modulator (SDAM) being developed as a novel treatment for psychiatric disorders. Brexpiprazole has subnanomolar binding affinities (Ki < 1 nmol/L) for human serotonin 1A (h5-HT₁A) and 2A (h5-HT₂A), long form of human dopamine D₂ (hD₂L), and human alpha-1B (hα₁b) and alpha-2C (hα₂C)-adrenoceptors. Brexpiprazole acts as a partial agonist at h5-HT₁A and hD₂ receptors, and as an antagonist at h5-HT₂A and noradrenaline hα₁b/C receptors. Brexpiprazole has a lower intrinsic activity at the D₂ receptor and higher affinities to the 5-HT₂A and 5-HT₁A receptors than aripiprazole.

Brexpiprazole displayed antipsychotic-like effects in antipsychotic drug screening models in rats and monkeys in which brexpiprazole seemed to have functional D₂ receptor antagonist activity. In addition, D₂ receptor agonistic activity of brexpiprazole was confirmed in animal models, indicating that brexpiprazole acts as a D₂ receptor partial agonist not only in vitro but also in vivo. Furthermore, its risk for producing catalepsy (EPS) and hyperprolactinemia in animals is lower than the risk observed for risperidone.

The antipsychotic effect of brexpiprazole has been verified in numerous clinical studies in which brexpiprazole improved psychotic symptoms. Overall, it is well-tolerated with low side effects in patients with schizophrenia.

A key issue for D₂ receptor partial agonists is to ascertain how much intrinsic activity (or relative efficacy) is ideal in leading to optimal stabilization of dopaminergic transmission. If D₂ receptor agonistic activity is too high, this can lead to lack of robust antipsychotic efficacy as well as pronounced side effects related to increased D₂ receptor tonus, for example, nausea, vomiting, and motor side effects such as hyperkinesias and restlessness. Conversely, the level of D₂ receptor antagonist activity can also be challenging and lead to an increased risk of EPS, hyperprolactinemia, tardive dyskinesia, and dopamine supersensitivity psychosis.

In fact, long-term D₂ receptor blockade can evoke dopamine D₂ receptor supersensitivity both in animals and humans, which is considered to be a possible cause of tardive dyskinesia and supersensitivity psychosis. In this regard, a number of studies have demonstrated that the repeated administration of antipsychotic drugs increased D₂ receptor density in the striatum and enhanced stereotyped behavior induced by apomorphine in rodents. In addition, other researchers reported that haloperidol significantly increased methamphetamine-induced hyperactivity and the maximum number of binding sites (Bₘₐₓ). Favorably, however, aripiprazole did not increase these parameters.

It has been suggested that the long-term administration of antipsychotics would increase the sensitivity of other receptors as well which may, together with D₂ receptor supersensitivity, contribute to rebound symptoms after their discontinuation. Such rebound symptoms can manifest as psychosis, EPS, and insomnia. Atypical antipsychotics such as risperidone, olanzapine, and quetiapine have higher affinity for the 5-HT₂A receptor than the D₂ receptor. Therefore, long-term treatment of these drugs may influence not only D₂ receptor sensitivity but also 5-HT₂A receptor sensitivity, which may contribute to some symptoms seen with antipsychotic drug switching in patients.

It has not been investigated whether the repeated administration of brexpiprazole evokes D₂ receptor supersensitivity or whether brexpiprazole can suppress behavioral responses to receptor supersensitivities caused by the repeated administration of other antipsychotic drugs. Therefore, firstly we evaluated the effects of 21-day repeated administration of brexpiprazole on Bₘₐₓ of striatal D₂ receptors and apomorphine-induced stereotyped behavior in rats. Secondly, we evaluated whether an acute exposure of brexpiprazole would suppress D₂ receptor supersensitivity using apomorphine-induced hyperlocomotion in rats treated with risperidone for 21 days, and 5-HT₂A receptor supersensitivity using DOI-induced head twitches because risperidone may influence 5-HT₂A receptor sensitivity after its repeated administration as it is a 5-HT₂A receptor antagonist.
2 | MATERIALS AND METHODS

2.1 | Animals

For evaluation of D₂ receptor sensitivity after a repeated administration of brexiprazole and haloperidol, 20 male Wistar rats (Japan SLC, Inc., 7 weeks old at the start of treatment) and 24 male Wistar rats (7 weeks old at the start of treatment) were used for measurement of $B_{max}$ of striatal D₂ receptors and apomorphine-induced stereotyped behavior, respectively. For evaluation of acute oral treatment with brexiprazole, aripiprazole, and olanzapine on behavioral responses associated with D₂ and 5-HT₂A receptor sensitization caused by a subchronic risperidone treatment, 57 male Wistar rats (9 weeks old at the start of risperidone treatment) were used. They were group-housed (5 or 6 per cage) in individual cages with water and food (Oriental Yeast Co, Ltd) supplied ad libitum and maintained under artificial lighting between 7:00 AM and 7:00 PM. The room temperature and humidity were maintained at 23 ± 2°C and 60 ± 10%, respectively.

2.2 | Treatment

Brexiprazole, aripiprazole, and olanzapine (synthesized at Otsuka Pharmaceutical Co., Ltd.) were suspended in 5% (w/v) gum arabic-distilled water solution (vehicle). Haloperidol (Serenace®, Sumitomo Dainippon Pharma Co., Ltd.) was diluted with the vehicle solution. Doses of these compounds were expressed as free bases and administered at a volume of 5 mL/kg. Risperidone (Sigma-Aldrich) was dissolved in a small amount of glacial acetic acid and diluted with saline, adjusted to pH 4-5 with NaOH. The 5-HT₂A receptor agonist, DOI, and the D₂ receptor agonist, apomorphine, were dissolved in saline.

For the evaluation of $B_{max}$ of striatal D₂ receptors, brexiprazole (4 or 30 mg/kg), haloperidol (1 mg/kg), or vehicle was administered orally (p.o.) to rats once daily for 21 days (Figure 1A). Similarly, for the evaluation of apomorphine-induced stereotypy, brexiprazole (6 or 30 mg/kg), haloperidol (1 mg/kg), or vehicle was administered p.o. to rats once daily for 21 days (Figure 1A). In the evaluation of apomorphine-induced stereotypy, the doses of brexiprazole (6 mg/kg) and haloperidol (1 mg/kg) were used as approximately twofold doses of ED₅₀ values against apomorphine [0.7 mg/kg, subcutaneously (s.c.)]-induced stereotypy in rats.²⁸,⁴¹ The dose of brexiprazole at 30 mg/kg was used as a supratherapeutic dose (approximately tenfold of the ED₅₀ value) of this compound against the animal model.

For evaluation of acute oral treatment of brexiprazole, aripiprazole, and olanzapine on D₂ and 5-HT₂A receptor sensitization caused by a chronic risperidone treatment, rats were anesthetized with isoflurane (Mylan) and an ALZET osmotic minipump (model 2ML4) containing risperidone or vehicle was inserted into the subcutaneous space through a small incision on the back. Risperidone (1.5 mg/kg/d) or vehicle was administered continuously (2.5 μL/h) for 21 days through the implanted pumps (Figure 1B). Previous rat studies showed that 30%-60% of D₂ receptors⁴² and 70%-90% of 5-HT₂A receptors⁴³ were occupied by 1 mg/kg/d risperidone when delivered by an osmotic minipump. The osmotic pumps were removed from the rats on the day 22. Vehicle, brexiprazole (3 mg/kg), aripiprazole (10 mg/kg), or olanzapine (3 mg/kg) were administered p.o. to these rats in order to evaluate effects of the treatment conditions on DOI-induced head twitches and apomorphine-induced stereotypy 7 and 11 days after the osmotic pump removal, respectively. Brexiprazole at 3 mg/kg, aripiprazole at 10 mg/kg, and olanzapine at 3 mg/kg were used as doses slightly

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**FIGURE 1** Schematic diagram of experimental procedures. A, Evaluation of D₂ sensitivity after repeated administrations of brexiprazole and haloperidol. B, Evaluation of acute oral administrations of brexiprazole, aripiprazole, and olanzapine on 5-HT₂A and D₂ receptor sensitization induced by a chronic risperidone treatment.
over their ED$_{50}$ values against apomorphine (0.7 mg/kg, s.c.)-induced stereotypy.$^{18,44}$

### 2.3 | D$_2$ receptor binding assay

Rats were sacrificed by decapitation 3 days after the last administration of the compounds, and their striata were quickly dissected from the brains on ice (Figure 1A). Rat striatal membrane was prepared as follows.$^{41}$ The striata were homogenized in ice-cold buffer A [50 mmol/L Tris-HCl buffer (pH 7.4)] using an ultrasonic homogenizer (Microson, Misonix, Inc.) for 15 to 30 seconds. The homogenates were centrifuged at 46,300 g for 10 minutes at 4°C (Centrifuge: himac CP100WX, Hitachi, Ltd., Rotor: PR55T-335, Hitachi, Ltd.). The pellets were suspended with ice-cold buffer A. After incubation for 10 minutes at 37°C, the suspensions were centrifuged under the same condition. The final pellets were suspended in ice-cold buffer B (50 mmol/L Tris-HCl buffer containing 120 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L CaCl$_2$, and 1 mmol/L MgCl$_2$, pH 7.4). All membrane preparations were stored at −80°C until use.

To estimate the $B_{\text{max}}$ and $K_d$ values of D$_2$ receptors in the striatal membrane preparations, the binding assays were performed as follows. The membrane preparations were incubated for 60 minutes at 25°C with buffer B containing $[^3]H$-raclopride. The incubated samples were run through glass fiber filter-bottom 96-well microplates (Unifilter® 96GF/B, PerkinElmer, Inc.) pretreated with 0.1% polyethyleneimine, using a cell harvester (FilterMate™, PerkinElmer, Inc.). The filter plates were rinsed with ice-cold buffer A and dried up in an oven at 50°C. The radioactivity on the filter plates was measured by TopCount (TopCount NXT™, PerkinElmer, Inc.) with the liquid scintillation cocktail (MicroScint™ 0, PerkinElmer, Inc.). The radioactivity of radioligands was measured by both TopCount and liquid scintillation counter (LSC-5101, Aloka Co., Ltd.) with Aquasol™-2 (PerkinElmer, Inc.) in order to calculate the counting efficacy of the radioactivity. Nonspecific binding to D$_2$ receptors was defined in the presence of 10 μmol/L (+)-butaclamol hydrochloride. Specific binding was calculated by subtracting nonspecific binding from total radioligand binding. All binding assays were carried out in triplicate, and protein concentrations of the membrane preparations were measured using Pierce™ BCA Protein Assay Kit [Pierce (at present, Thermo Fisher Scientific)]. The $B_{\text{max}}$ and $K_d$ values were calculated by nonlinear regression analysis (one site binding, Michaelis-Menten equation: $Y = B_{\text{max}} \times X / (K_d + X)$, $X =$ concentration of radioligand (nmol/L), $Y =$ specific binding (fmol/mg protein]) using GraphPad Prism® software version 3.0 (GraphPad Software, Inc.).

### 2.4 | Apomorphine-induced stereotyped behavior

Apomorphine-induced stereotyped behavior was measured 3 and 5 days after the last administration of the compounds (Figure 1A). Each rat was placed into an acrylic cylinder (diameter 23 cm × height 30 cm) and acclimated to this new environment for 30 minutes. After the acclimation, the rats were s.c. injected with apomorphine (0.15 mg/kg; Sigma-Aldrich).$^{45}$ Apomorphine was dissolved in saline immediately before injection. Stereotyped behavior was recorded by an observer blind to the treatment groups (compounds and doses), for a 1-minute interval every 10 minutes over the 20- to 40-minute period after the apomorphine injection. The total score for the three observations was calculated using the scoring scale described in Table 1.$^{18,41,46,47}$

### 2.5 | DOI-induced head twitch

Rats were orally administered with vehicle, brexpiprazole (3 mg/kg), aripiprazole (10 mg/kg), or olanzapine (3 mg/kg) 7 days after the osmotic pomp removal (day 29; Figure 1B). DOI dose-dependently increases the number of head twitches in rodents.$^{48-50}$ Therefore, we used a low dose of DOI at 0.5 mg/kg (s.c.), one-tenth of a dose that we normally use in antipsychotic evaluation,$^{17}$ to see enhancement of response to 5-HT$_{2A}$ receptors after a repeated administration of risperidone. One hour after the test drug administration, the rats were injected with DOI (0.5 mg/kg, s.c.). Each rat was placed in an acrylic cylinder (diameter 23 cm × height 25 cm), and the number of head twitches, head-waving behavior, was counted for 20 minutes by an observer blind to the treatment groups immediately after the DOI injection.

### 2.6 | Apomorphine-induced hyperlocomotion

Rats were orally administered with vehicle, brexpiprazole (3 mg/kg), aripiprazole (10 mg/kg), or olanzapine (3 mg/kg) following a 4-day washout period after evaluation of DOI-induced head twitch (11 days after the osmotic pomp removal; day 33; Figure 1B). One hour after the test drug administration, the rats were injected with apomorphine (0.1 mg/kg, s.c.), a dose that does not increase locomotor activity in naive rats. Apomorphine was dissolved in

| **Score** | **Behavior** |
|----------|-------------|
| 0        | The appearance of the animals is the same as drug-naive rats |
| 1        | Continuous sniffing, constant exploratory activity |
| 2        | Continuous sniffing, periodic exploratory activity |
| 3        | Continuous sniffing, discontinuous biting, gnawing or licking Very brief periods of locomotor activity |
| 4        | Continuous biting, gnawing or licking, no exploratory activity |

**TABLE 1** The scoring scale of stereotyped behavior
saline containing 0.1% (w/v) ascorbic acid. Each rat was placed into a plastic cage (30 x 40 x height 28 cm), and the locomotor activity was measured using a passive infrared ray sensor in the Supermex system (Muromachi Kikai) for 1 hour immediately after apomorphine injection. The experiment was conducted in a blinded manner.

2.7 Statistical analysis

The values were expressed as mean ± SEM for \( B_{\text{max}} \) value, \( K_d \) value, the number of head twitches, and locomotor activity. The stereotypy score was expressed as mean ± SEM and median (minimum and maximum). All statistical analyses were performed using SAS Software for Windows (SAS Institute). The differences were considered statistically significant, when \( P \) value was < .05.

The differences in \( B_{\text{max}} \) and \( K_d \) values between vehicle-treated group and brexipiprazole-treated groups, and between haloperidol-treated group and brexipiprazole-treated groups were analyzed using a two-tailed 1-way analysis of variance (ANOVA) followed by Dunnett’s test. The differences in \( B_{\text{max}} \) and \( K_d \) values between vehicle-treated group and haloperidol-treated group were analyzed by a two-tailed unpaired t test.

The differences in the total stereotypy score between vehicle-treated group and brexipiprazole-treated groups or haloperidol-treated group, and between haloperidol-treated group and brexipiprazole-treated groups were analyzed using a two-tailed Steel test.

3 | RESULTS

3.1 Effects of the 21-day repeated administration of brexipiprazole and haloperidol on striatal dopamine \( D_2 \) receptor density (\( B_{\text{max}} \)) and affinity (\( K_d \))

The repeated administration of haloperidol (1 mg/kg) significantly increased the \( B_{\text{max}} \) (maximal number of \([H]\)-raclopride-specific binding) in the striatum compared to vehicle treatment (Table 2). Although the repeated administration of brexipiprazole (30 mg/kg) significantly increased the \( B_{\text{max}} \) similar to haloperidol treatment, brexipiprazole (4 mg/kg) had no effect on the \( B_{\text{max}} \) compared to vehicle treatment.

Neither of the repeated administrations of brexipiprazole (4 and 30 mg/kg) and haloperidol (1 mg/kg) significantly changed the \( K_d \) values of \( D_2 \) receptors in rat striatum compared to vehicle treatment (Table 2).

3.2 Effects of the 21-day repeated administration of brexipiprazole and haloperidol on apomorphine-induced stereotypy

Significantly enhanced apomorphine (0.15 mg/kg, s.c.)-induced stereotypy compared to vehicle-treated group was observed in

| TABLE 2 | Effects of the 21-d repeated administration of brexipiprazole and haloperidol on striatal dopamine \( D_2 \) receptor density (\( B_{\text{max}} \)) and affinity (\( K_d \)) in rats |

| Treatment  | Dose (mg/kg) | \( B_{\text{max}} \) (fmol/mg protein) (Mean ± SEM) | \( K_d \) (nmol/L) (Mean ± SEM) |
|------------|--------------|-------------------------------------------------|---------------------------------|
| Vehicle    | 0            | 1183 ± 104                                     | 4.6 ± 1.2                       |
| Brexiprazole | 4            | 1358 ± 78                                      | 5.2 ± 1.3                       |
|            | 30           | 1647 ± 77**                                    | 6.2 ± 1.3                       |
| Haloperidol | 1            | 1766 ± 174†                                    | 6.4 ± 1.1                       |

Note: Data are expressed as mean ± SEM. The differences between vehicle-treated group and brexipiprazole-treated groups were analyzed by a two-tailed one-way ANOVA followed by Dunnett’s test (\( F_{2,12} = 7.26, P < .01 \) for \( B_{\text{max}} \), \( F_{2,12} = 0.41, P = .671 \) for \( K_d \)). **\( P < .01 \) vs vehicle-treated group by a two-tailed one-way ANOVA followed by Dunnett’s test.

| TABLE 3 | Effects of the 21-d repeated administration of brexipiprazole and haloperidol on sensitivity of \( D_2 \) receptors in rats |

| Treatment  | Dose (mg/kg) | n | Median score (min-max) | Median score (min-max) |
|------------|--------------|---|------------------------|------------------------|
| Vehicle    | 0            | 6 | 4.0 (3–5)              | 4.0 (2–5)              |
| Brexiprazole | 6            | 6 | 5.0 (4–6)**            | 5.5 (3–6)              |
|           | 30           | 6 | 7.0 (6–9)**            | 4.5 (4–8)              |
| Haloperidol | 1            | 6 | 7.5 (5–10)**           | 7.0 (5–10)**           |

Note: Data are expressed as median (minimum and maximum) score. **\( P < .01 \) vs vehicle by two-tailed Steel test.
haloperidol (1 mg/kg)-treated group 3 and 5 days after the last administration (Table 3 and Figure 2). On the other hand, animals treated with brexpiprazole (6 mg/kg) did not show significantly enhanced apomorphine-induced stereotypy compared to vehicletreated animals. Moreover, the stereotypy score of brexpiprazole (6 mg/kg) treated group 3 days after the last administration was significantly less than that of haloperidol-treated group. Although animals treated with brexpiprazole (30 mg/kg) also showed significantly enhanced apomorphine-induced stereotypy 3 days after the last administration, these animals did not show enhanced stereotypy 5 days after the last administration.

3.3 | Effects of brexpiprazole on DOI-induced head twitches in risperidone-sensitized rats

The chronic treatment of risperidone (1.5 mg/kg/d, s.c.) significantly increased the number of head twitches induced by DOI compared to the chronic vehicle treatment (P < .01; Figure 3), suggesting the chronic risperidone treatment sensitized 5-HT2A receptors in the rats. Brexpiprazole (3 mg/kg) and olanzapine (3 mg/kg) significantly suppressed the increase in the number of DOI-induced head twitches in risperidone-sensitized rats (both P < .01), while aripiprazole (10 mg/kg) did not suppress the behavior.

3.4 | Effects of brexpiprazole on apomorphine-induced hyperlocomotion in risperidone-sensitized rats

The chronic treatment of risperidone (1.5 mg/kg/d, s.c.) significantly increased locomotor activity induced by apomorphine (0.1 mg/kg, s.c.) compared to the chronic vehicle treatment (P < .01; Figure 4), suggesting the chronic risperidone treatment sensitized D2 receptors in the rats. Brexpiprazole (3 mg/kg), aripiprazole (10 mg/kg), and olanzapine (3 mg/kg) significantly suppressed the increase in apomorphine-induced hyperlocomotion in risperidone-sensitized rats (all P < .01).

4 | DISCUSSION

Brexiprazole is a SDAM acting as a partial agonist at h5-HT1A and hD2 receptors, and as an antagonist at h5-HT2A receptors, which was developed as a novel treatment for psychiatric disorders and optimized to achieve clinical efficacy with minimal EPS potential.17,18 Brexpiprazole has already demonstrated its antipsychotic effects in clinical trials as well as antipsychotic profile in animal models.18–20

In this study, we demonstrated that brexpiprazole has lower risk to evoke D2 receptor supersensitivity by repeated administration compared to haloperidol. The 21-day repeated administration of haloperidol at 1 mg/kg significantly increased the Bmax of striatal D2 receptors, whereas brexpiprazole at 4 mg/kg did not significantly increase it. This 4 mg/kg dose of brexpiprazole is nearly 2- or 1.5-fold higher than the ED50 values in apomorphine (0.25 mg/kg, s.c.)-induced hyperactivity and apomorphine (0.7 mg/kg, s.c.)-induced stereotypy, respectively.18 Brexpiprazole increased the Bmax only at the higher dose, 30 mg/kg, which is approximately 15- or 10-fold higher dose of the above-mentioned ED50 values, respectively. The repeated administration of haloperidol (1 mg/kg), the twofold dose of its ED50 value against apomorphine (0.7 mg/kg, s.c.)-induced stereotypy in rats,41 also significantly enhanced apomorphine (0.15 mg/kg, s.c.)-induced stereotypy. On the other hand, brexpiprazole did
FIGURE 2 Effects of repeated administration of brexpiprazole and haloperidol on sensitivity of D_2 receptors in rats. A, Graphic explanation of the data expression using the box plot. B, The score of apomorphine (0.15 mg/kg, s.c.)-induced stereotyped behavior measured 3 d after the last administration of vehicle, brexpiprazole (6 or 30 mg/kg, p.o.), or haloperidol (1 mg/kg, p.o.). The stereotypy score was significantly higher in haloperidol (1 mg/kg)-treated group and brexpiprazole (30 mg/kg)-treated group than vehicle-treated group. The stereotypy score in brexpiprazole (6 mg/kg)-treated group was significantly lower than that in haloperidol (1 mg/kg)-treated group. C, The score of apomorphine (0.15 mg/kg)-induced stereotyped behavior measured 5 d after the last administration of vehicle, brexpiprazole (6 or 30 mg/kg), or haloperidol (1 mg/kg). The stereotypy score was significantly higher in haloperidol (1 mg/kg)-treated group than vehicle-treated group. The stereotypy score of apomorphine (0.15 mg/kg, s.c.)-induced stereotyped behavior measured 5 d after the last administration of vehicle, brexpiprazole (6 mg/kg/p.o.), or haloperidol (1 mg/kg/p.o.) was significantly lower than that in haloperidol (1 mg/kg)-treated group. n = 6/group. ^P < .05 vs haloperidol by two-tailed Steel test. *P < .05, **P < .01 vs vehicle by two-tailed Steel test.

FIGURE 3 Effects of brexpiprazole, aripiprazole, and olanzapine on (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI)-induced head twitches in risperidone-sensitized rats. The number of head twitches was counted for 20 min immediately after DOI (0.5 mg/kg, s.c.) injection. Data are expressed as mean ± SEM (n = 11-12/group). The 21-d chronic treatment of risperidone (1.5 mg/kg/d, s.c.) significantly increased the number of head twitches induced by DOI injection compared to the 21-d chronic vehicle treatment. Brexpiprazole (BREX, 3 mg/kg, p.o.), aripiprazole (ARI, 10 mg/kg, p.o.), and olanzapine (OLZ, 3 mg/kg, p.o.) significantly suppressed the increase of DOI-induced head twitches in risperidone-sensitized rats. Aripiprazole (ARI, 10 mg/kg, p.o.) did not suppress the increase of DOI-induced head twitches in risperidone-sensitized rats. The difference between chronic vehicle + acute vehicle-treated group and chronic risperidone + acute vehicle-treated group was analyzed by a two-tailed Student’s t test. **P < .01 vs chronic vehicle + acute vehicle-treated group by a two-tailed Student’s t test. The differences between chronic risperidone + acute vehicle-treated group and chronic risperidone + acute BREX, ARI, or OLZ were analyzed by a two-tailed one-way ANOVA followed by Dunnett’s test (F_{3,34} = 36.3, P < .01). ##P < .01 vs chronic risperidone + acute vehicle-treated group by a two-tailed one-way ANOVA followed by Dunnett’s test.

FIGURE 4 Effects of brexpiprazole, aripiprazole, and olanzapine on apomorphine-induced hyperlocomotion in risperidone-sensitized rats. Locomotor activity was measured for 1 h immediately after apomorphine (0.1 mg/kg, s.c.) injection. Data are expressed as mean ± SEM (n = 9-11/group). The 21-d chronic treatment of risperidone (1.5 mg/kg/d, s.c.) significantly increased hyperlocomotion induced by apomorphine injection compared to the 21-d chronic vehicle treatment. Brexpiprazole (BREX, 3 mg/kg, p.o.), aripiprazole (ARI, 10 mg/kg, p.o.), and olanzapine (OLZ, 3 mg/kg, p.o.) significantly suppressed the increase of apomorphine-induced hyperlocomotion in risperidone-sensitized rats. The difference between chronic vehicle + acute vehicle-treated group and chronic risperidone + acute vehicle-treated group was analyzed by a two-tailed Student’s t test. **P < .01 vs chronic vehicle + acute vehicle-treated group by a two-tailed Student’s t test. The differences between chronic risperidone + acute vehicle-treated group and chronic risperidone + acute BREX, ARI, or OLZ were analyzed by a two-tailed one-way ANOVA followed by Dunnett’s test (F_{3,34} = 36.3, P < .01). ##P < .01 vs chronic risperidone + acute vehicle-treated group by a two-tailed one-way ANOVA followed by Dunnett’s test.

not significantly enhance the apomorphine-induced stereotypy at 6 mg/kg, the twofold dose of its ED_{50} value against apomorphine (0.7 mg/kg, s.c.)-induced stereotypy in rats, which is equivalent to haloperidol 1 mg/kg regarding anti-apomorphine-induced stereotypy effect, and had a less potential to enhance this behavior even at the overdose, 30 mg/kg (the tenfold dose of the ED_{50} value), compared to haloperidol (1 mg/kg). It has been reported that an oral repeated administration of haloperidol-induced D2 receptor supersensitivity in rats was confirmed as significant increases of methamphetamine-induced hyperactivity and B_{max} of striatal D_2 receptors, whereas aripiprazole treatment did not increase these parameters compared to vehicle treatment.30,31 This indicates that long-term D_2 receptor blockade may lead to D_2 receptor supersensitivity. Similar to aripiprazole, brexpiprazole is a D_2 receptor partial agonist, although brexpiprazole’s intrinsic activity is lower than that of aripiprazole.17 Therefore, this feature of brexpiprazole may be the reason that brexpiprazole had less potential to induce D_2 receptor supersensitivity compared to haloperidol in the studies performed.

The D_2 receptor supersensitivity induced by a long-term treatment of antipsychotic drugs is known to be a possible cause of dopamine supersensitivity psychosis and tardive dyskinesia in patients with schizophrenia.27-29 Compared to long-term treatment of typical antipsychotics, the occurrence of tardive dyskinesia by treatment of atypical antipsychotics is lower.37 It has been clinically demonstrated that aripiprazole had a significantly lower risk of tardive dyskinesia.
than haloperidol in patients with schizophrenia.\textsuperscript{51} In a phase 3 fixed-dose (1-4 mg) 52-week placebo-controlled maintenance study of brexpiprazole, the proportion of patients with schizophrenia causing dyskinetic events was 1% in both placebo group and brexpiprazole group,\textsuperscript{52} indicating that the risk of dyskinesia by long-term brexpiprazole treatment is low. This is consistent with the findings of the preclinical investigation in rats. However, it has been known that atypical antipsychotics still cause supersensitivity psychosis in schizophrenic patients.\textsuperscript{29} The prevalence rate of supersensitivity psychosis following treatment with atypical antipsychotics is 30% in patients with schizophrenia and 70% in patients with treatment-resistant schizophrenia. Therefore, it is important to choose a drug with lower risk of D\textsubscript{2} receptor sensitization to avoid the occurrence of supersensitivity psychosis.

Moreover, we also confirmed in this study that the 21-day subchronic treatment of risperidone (1.5 mg/kg/d) significantly increased responses to apomorphine and DOI in rats, indicating that a chronic treatment of risperidone can sensitize D\textsubscript{2} and 5-HT\textsubscript{2A} receptors. It has been suggested that D\textsubscript{2} receptor sensitization is involved in rebound symptoms such as rebound psychosis.\textsuperscript{35,53} Notably, rebound psychosis occurs when patients switch antipsychotic drugs.\textsuperscript{39,54} Brexpiprazole (3 mg/kg) and olanzapine (3 mg/kg), but not aripiprazole (10 mg/kg), significantly suppressed both behaviors (increased responses to apomorphine and DOI) induced in risperidone-sensitized rats, suggesting that brexpiprazole, olanzapine, and aripiprazole may have a lower potential to induce rebound symptoms/psychosis related to D\textsubscript{2} receptor sensitization, whereas brexpiprazole and olanzapine but not aripiprazole may suppress symptoms occurred by 5-HT\textsubscript{2A} receptor sensitization when switched from atypical antipsychotics such as risperidone. However, it is important to consider that olanzapine has metabolic side effects at a higher rate than other atypical antipsychotics including aripiprazole.\textsuperscript{40,55} It has been reported that 2-week treatment of olanzapine at a lower dose than 3 mg/kg induced weight gain in rats.\textsuperscript{56} Therefore, olanzapine at 3 mg/kg is a side effect dose. Accumulating evidence shows that switching to aripiprazole may provide favorable outcomes by potentially improving psychosis and by decreasing metabolic problems such as weight gain.\textsuperscript{57-60} However, aripiprazole may transiently worsen psychosis in some cases after an abrupt discontinuation of a prior atypical antipsychotic drug.\textsuperscript{61,62} Given that 5-HT\textsubscript{2A} receptor antagonism could suppress the expression of antipsychotic-induced dopamine supersensitivity,\textsuperscript{63} stronger affinity of brexpiprazole for 5-HT\textsubscript{2A} receptor than that of aripiprazole might contribute to the inhibition of antipsychotic-induced supersensitivity response. Taken together, brexpiprazole may have a lower risk of rebound symptoms than aripiprazole, when switched from long-term treatment of serotonin-dopamine antagonists such as risperidone.

5 | CONCLUSIONS

Our findings show that brexpiprazole has less potential to evoke D\textsubscript{2} receptor supersensitivity in rats after repeated administration compared to the typical antipsychotic haloperidol, which may suggest a lower propensity for brexpiprazole to cause dopamine supersensitivity psychosis and tardive dyskinesia. In addition, brexpiprazole may have a lower risk for producing rebound symptoms associated with D\textsubscript{2} receptor and 5-HT\textsubscript{2A} receptor sensitization when switching from other antipsychotics such as risperidone.

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CONFLICT OF INTEREST

All the authors are employees and own stock of Otsuka Pharmaceutical Co., Ltd.

AUTHOR CONTRIBUTIONS

NA, HA, and YO conceived and designed the study, performed experiments, analyzed data, wrote the paper. TF conceived and designed the study, performed experiments, analyzed data, reviewed the paper. KM conceived and designed the study and reviewed the paper. NK and TK conceived the study and reviewed the paper. HS reviewed the paper. All authors contributed to finalizing of the paper and had final responsibility for the decision to submit for publication, took part in either drafting and/or revising the paper, and approved the final version of the paper.

DATA REPOSITORY

All data are available in the Supporting Information data files.

ANIMAL STUDIES

The care and handling of the animals was in accordance with “Guidelines for Animal Care and Use in Otsuka Pharmaceutical Co, Ltd.”

ORCID

Naoki Amada \(\url{https://orcid.org/0000-0002-9625-6240}\)

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