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Full Paper

Acetaminophen improves tardive akathisia induced by dopamine D_2 receptor antagonists

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ABSTRACT

Tardive akathisia is a movement disorder characterized by internal restlessness with an uncontrollable urge to move, leading to repetitive movements. It is a common side effect of long-term treatment with dopamine D_2 receptor antagonists. In the present study, we analyzed the FDA Adverse Event Reporting System and IBM MarketScan Research Database to find a drug that can be used concomitantly with dopamine D_2 receptor antagonists and still reduce the risk of akathisia. Acetaminophen was determined to be the most effective akathisia-suppressing drug. In an experimental validation of the hypothesis, chronic treatment of rats with haloperidol caused akathisia symptoms, including increased stereotyped behavior and locomotor activity, and decreased immobility time. Acute treatment with acetaminophen significantly attenuated haloperidol-induced akathisia. In the ventral striata of these rats, acetaminophen prevented haloperidol-induced decrease in the number of c-Fos$^+$ preproenkephalin$^+$ neurons. These results suggest that acetaminophen is effective in suppressing tardive akathisia by activating indirect-pathway medium spiny neurons.

1. Introduction

Akathisia is a movement disorder characterized by feelings of internal restlessness with an urge to move, leading to repetitive movements.[1] The exacerbation of akathisia symptoms may increase the risk of suicidal ideation, violent or aggressive behavior.[2] Akathisia is the most common movement disorder associated with the use of dopamine D_2 receptor (D2R) antagonists, such as antipsychotics, and presents in approximately 20–30% of patients treated with antipsychotics.[3]

Tardive akathisia onset may occur late in the course of treatment with D2R antagonists (usually after several months or more); however, it may also occur after drug withdrawal or reduction.[4] Moreover, symptoms may persist from months to years, even after the withdrawal. Tardive akathisia is often observed alongside tardive dyskinesia.[5] Treatments with anticholinergics, propranolol, clonazepam, and opioids have been explored as therapeutic strategies to alleviate akathisia; however, tardive akathisia is resistant to these pharmacologic therapies.[6] Thus, new strategies to inhibit tardive akathisia are required.

Drug repurposing involving hypothesis generation from clinical big data and hypothesis testing in animal experiments may help identify treatments for drug-induced adverse events such as tardive akathisia. Previously, we analyzed self-reports of adverse events extracted from the Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) and insurance claims obtained from JMDC Inc., showing that antinociceptive and antipyretic acetaminophen could inhibit orofacial dyskinesia.[7] The hypothesis was then validated in animal experiments. In this study, we focused on IBM MarketScan Research Database (IBM., New York, USA) as another source of insurance claims data, since the number of akathisia cases reported in JMDC's database was insufficient for analyzing akathisia. The numerous cases of akathisia reported in IBM MarketScan Research Database enabled us to find a drug for treating akathisia that can be used concomitantly with D2R antagonists. We then validated its efficacy in D2R antagonist-induced hyperactivity models of rats.

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2. Materials and methods

2.1. Analysis of FAERS database

Adverse event reports from 2004 to 2019 were obtained from the FDA website (https://www.fda.gov/drugs/drug-approvals-and-databases/fda-adverse-event-reporting-system-faers). Duplicate reports were eliminated as previously reported,[8] and the remaining 11,438,031 reports were analyzed. Arbitrary drug names, including trade names and abbreviations, were manually annotated to unified generic names using the Medical Subject Headings descriptor ID. Reports of akathisia were defined by the preferred terms "akathisia" in MedDRA (version 23.0). Statistical analyses of FAERS data were performed using R v4.1.0 and R studio v1.4.1717 software (R Foundation for Statistical Computing, Vienna, Austria) as previously described.[9] In volcano plots, *Z* scores were used instead of *P*-values to save space.

2.2. Analysis of IBM database

Insurance claims data covering the period from January 1, 2017 to December 31, 2019 were purchased from IBM Corp. (New York, USA). The dataset contains medical diagnoses and prescription claims of inpatients (n = 3,284,576) and outpatients (n = 36,440,716) covered by a private insurance plan and the Medicare program. Of these, only outpatient data were analyzed. All data were linked by a consistent, deidentified patient ID with daily time stamps.

Individual diagnoses were assigned according to the International Classification of Diseases 10 (ICD-10) codes. Because accurate diagnosis of akathisia is difficult, cases of akathisia were broadly defined using the following ICD-10 codes: G2570, drug induced movement disorder, unspecified; G2571, drug induced akathisia; and G2579, other drug induced movement disorders. In propensity score matching, the following categorization was used for risk factors: mood disorder, F30–F39; alcohol, substance abuse/dependence, F10–F19; diabetes mellitus, E10–E14; hepatic disease, K70–K77. To identify patients who were prescribed D2R antagonists, we defined the aripiprazole cohort as that treated with "aripiprazole" and "aripiprazole lauroxil." In the haloperidol cohort, patients who received only haloperidol injections or haloperidol lactate injections were excluded, and those who routinely received haloperidol, haloperidol decanoate, or haloperidol lactate were included. The acetaminophen cohort comprised patients who received drugs containing acetaminophen (Supplementary Table 1), excluding drug combinations with opioids (hydrocodone, oxycodone, codeine, tramadol). The following categorization was used for propensity score matching: antiparkinsonian drugs (N04), additional antipsychotic drugs (N05A, excluding lithium preparations), selective serotonin reuptake inhibitor (SSRI), and serotonin-noradrenaline reuptake inhibitor (SNRI) (zimeldine, fluoxetine, citalopram, paroxetine, sertraline, alaproclate, fluvoxamine, etoperidone, escitalopram, duloxetine, venlafaxine, milnacipran, desvenlafaxine, levomilnacipran), and iron preparation (drug combinations with iron in B03A).

Analyses of the IBM data were performed using R software. The R package MatchIt was used to perform propensity score matching. The causal relationship between D2R antagonists and the occurrence of akathisia was evaluated by analyzing the sequence symmetry[10] and incidence rate (IR) over an observation period of 3 years. After each D2R antagonist cohort was divided into two groups (with and without acetaminophen), 1:1 propensity score matching was performed to eliminate the deflections in the number of patients who had risk factors.[11] The propensity score-matched pairs were created by matching two groups using the nearest-neighbor method with a 0.01 caliper width.[12] Using the matched cohort pairs, the daily and cumulative doses and administration periods of D2R antagonists and acetaminophen were quantified and compared. The acetaminophen combination group was defined as a case where acetaminophen was prescribed after D2R antagonist, and the IRs between the combination group and the D2R antagonist alone group were compared. The chi-squared test and Fisher's exact test were used to compare population characteristics between propensity score-matched groups. The IR was calculated as follows:

$$\mathrm{IR} = \frac{n1}{\mathrm{T1}} \text{ or } \frac{n2}{\mathrm{T2}}$$

where $n1$ refers to the number of individuals who received the drug of interest (i.e., haloperidol, aripiprazole, or acetaminophen) before exhibiting akathisia and $n2$ refers the number of individuals who did not receive the drug of interest before exhibiting akathisia. T1 refers to the sum of the follow-up periods of all patients who received the drug of interest and T2 refers to the sum of the follow-up periods of all patients who did not receive the drug of interest.

2.3. Animals

All animal experiments were approved by the Kyoto University Animal Research Committee in accordance with the ethical guidelines. Male Wistar rats (9 weeks old, 200–250 g) were purchased from Japan SLC (Shizuoka, Japan). All animals were housed at a constant ambient temperature (22 ± 2 °C) on a 12-h light/dark cycle, with free access to food and water.

2.4. Drugs and reagents

Haloperidol was purchased from Tokyo Chemical Industry (Tokyo, Japan); acetaminophen, (2-hydroxypropyl)-β-cyclodextrin, and methanesulfonic acid was purchased from Nacalai Tesque (Kyoto, Japan); isoflurane was purchased from Pfizer, Inc. (New York, USA). Haloperidol was dissolved by gentle heating in isotonic hydroxypropyl-β-cyclodextrin acidified with methanesulfonic acid to ensure complete dissolution of the compound. The pH of the solution was then adjusted with NaOH.[13] Haloperidol was administered by constant infusion using osmotic mini-pumps (model 2ML4, 28-day delivery; Alzet, Cupertino, USA). Acetaminophen (200 mg/kg) was suspended in 0.5% carboxymethyl cellulose.

2.5. Mini-pump implantation

Rats were anesthetized with 3% isoflurane, and their anesthetized state was maintained with 2% isoflurane using a face mask. An osmotic mini-pump containing either vehicle or haloperidol was implanted under anesthesia. In each rat, a 1.5 cm-wide incision was made in the lower back, and the mini-pump was inserted to lie on the left side of the scapulae, with the flow moderator pointed away from the incision. The incision was closed using 9-mm surgical staples and cleaned with 70% ethanol.[14]

2.6. Treatment protocol

An osmotic mini-pump containing vehicle or haloperidol was implanted in each rat, and then drug administrations were started. The mini-pumps were removed on the 21st day and new pumps were implanted in the same manner. The new mini-pumps were removed on the 42nd day, and akathisia-like behavior was evaluated on the 47th day, i.e., on the 5th day following treatment cessation. The open field test was performed on the 7th day after

withdrawal, and decapitation for immunohistochemical analysis was performed on the 13th or 14th day.

2.7. Akathisia-like behavior evaluation

Akathisia-like behaviors of rats were scored using adapted versions of the applicable human rating scales, i.e., the Barnes Akathisia Rating Scale (BARS) and Hillside Akathisia Scale (HAS). The BARS assesses patient awareness of restlessness as well as characteristic motor phenomena such as fidgety movements of the limbs and an inability to remain still.[1] The HAS is used to rate the severity of akathisia by characterizing the degree of restlessness and the frequency and magnitude of akathisia.[15] In this study, the frequency and severity of the movements captured in the HAS were expressed as the amount of time during which the rats exhibited akathisia-like behaviors. Observable behaviors described in the BARS have already been tested in a primate study.[16] Six akathisia-like behaviors were scored in the study: purposeless movements/stereotypies, rocking from foot to foot/walking on spot, shifting body position, abnormal limb movements, inability to sit still, and agitation. In addition, a subjective overall restlessness degree is assessed considering all scores. We have adapted several of these behaviors to the rodent model. In other words, stereotyped behavior was measured with total time of grooming, rearing, and rotation and immobility time represented overall restlessness. On the day of the experiment, after 20 min of oral treatment with acetaminophen or the vehicle, rats were individually placed in clear-walled cages (10 × 20 × 30 cm) and their behaviors in a 60-min period were recorded. During the observation, two cameras were placed on both sides of the cage to monitor the behavior. The first 10 min were excluded from the analysis because the symptoms could not be correctly assessed due to spontaneous seeking behavior; data were analyzed for stereotyped behavior and immobility time during the remaining 50 min.

2.8. Open field test

After 30 min of acetaminophen or vehicle administration, the rats were placed in the corner of an open field chamber (90 × 90 cm × 40 cm) and monitored for 30 min. The total distance traveled was analyzed using a video tracking system (ANY-maze version 4.99, Stoelting, Wood Dale, IL, USA).

2.9. Immunohistochemistry

Immunohistochemistry was performed as previously described.[7] Briefly, after 90 min of acetaminophen or vehicle administration, the rats were anesthetized with pentobarbital and transcardially perfused with 4% paraformaldehyde (Nacalai Tesque) in phosphate buffer. The brains were cryosectioned into 30 μm-thick coronal sections with a cryostat (Leica CM3050S, Leica Biosystems, Nussloch, Germany) and stored at −80 °C. For c-Fos immunohistochemistry, the sections were incubated overnight at room temperature with mouse monoclonal anti-c-Fos antibody (1:500, NBP2-50037, Novus Biologicals, Fontana, USA) and rabbit monoclonal anti-preproenkephalin (ppENK) antibody (1:500; RA14124, Neuromics, Edina, MN, USA), followed by incubation with Alexa Fluor 594-labeled donkey anti-mouse IgG (1:200, Invitrogen, Waltham, MA, USA), and Alexa Fluor 488-labeled donkey anti-rabbit IgG (1:200, Invitrogen) for 1.5 h at room temperature in the dark. Images were captured using a confocal fluorescence microscope (Fluoview FV10i, Olympus, Tokyo, Japan). The number of $ppENK^+$ or $c\text{-}Fos^+$/$ppENK^+$ cells in a 0.045 mm^2 field of the ventral striatum, 0.3 mm anterior to the bregma, was counted. Counting was performed in the four regions.

2.10. Statistics

Statistical analyses of the animal experiments using two-way ANOVA and unpaired *t* test were performed using Prism v9.4.0 (GraphPad Software, San Diego, CA, USA). For post hoc analyses, Tukey's multiple comparisons tests were used. *P* values of <0.05 were considered indicative of statistically significant findings. Data are presented as mean ± SEM.

3. Results

3.1. Analysis of FAERS data

First, we investigated the association between the use of drugs and incidence of akathisia in FAERS data using disproportionality analysis by calculating each reporting odds ratio (ROR) and its *Z* score. Many D2R antagonists were strongly associated with akathisia onset (Fig. 1A and Supplementary Table 2). We chose typical antipsychotic haloperidol and atypical antipsychotic aripiprazole for further analyses as they were both associated with high ROR values and *Z* scores. ROR values and *Z* scores of antiemetic metoclopramide were also high. However, it should be noted that a black box warning on metoclopramide for tardive dyskinesia was applied in 2009[17]; the akathisia risk associated with metoclopramide may have been overestimated because more reports of similar symptoms may have been filed with the FAERS after the black box warning was applied compared to that before the warning. Therefore, we did not select metoclopramide for the analysis. Moreover, other atypical antipsychotics were not selected for the analysis because they are associated with strong metabolic adverse effects that may bias ROR values for akathisia.

In a population of haloperidol (Fig. 1B) or aripiprazole (Fig. 1C) users, acetaminophen decreased the ROR of akathisia (Supplementary Table 3). There was a negative correlation between the use of acetaminophen and the rates of D2R antagonist-induced akathisia.

3.2. Analysis of IBM data

To investigate the causal relationship between the use of D2R antagonist and akathisia, we analyzed the IBM data. Regarding the time distribution of the first event after enrollment in the IBM (Supplementary Fig. 1), the number of patients who were initially diagnosed with akathisia or prescribed haloperidol or aripiprazole was much higher during the first 3 months and became stable after the 4th month. These results suggest that patients who received a diagnosis of akathisia or were prescribed haloperidol or aripiprazole within 3 months after enrollment may exhibit akathisia before or just after enrollment for receiving health insurance. Therefore, patients who received a diagnosis of akathisia or prescription of haloperidol or aripiprazole during the 0–3-month run-in period were removed from the study cohort.

In sequence symmetry analysis, significant causal associations between haloperidol or aripiprazole use and akathisia onset were detected with an adjusted sequence ratio of 2.00 (95% CI: 1.23–3.72) (Fig. 2A) or 2.60 (95% CI: 2.11–3.29) (Fig. 2B), respectively. Further, we divided the cohort into two populations that did or did not receive a D2R antagonist and estimated the IR of akathisia. The use of haloperidol and aripiprazole was associated with high IR values; the values associated with haloperidol were higher than those associated with aripiprazole (Table 1).

The following characteristics were considered in 1:1 propensity score matching (Supplementary Table 4): older age, female sex, antiparkinsonian drug use, additional antipsychotic use, SSRI and SNRI use, iron preparation use, alcohol and substance abuse/

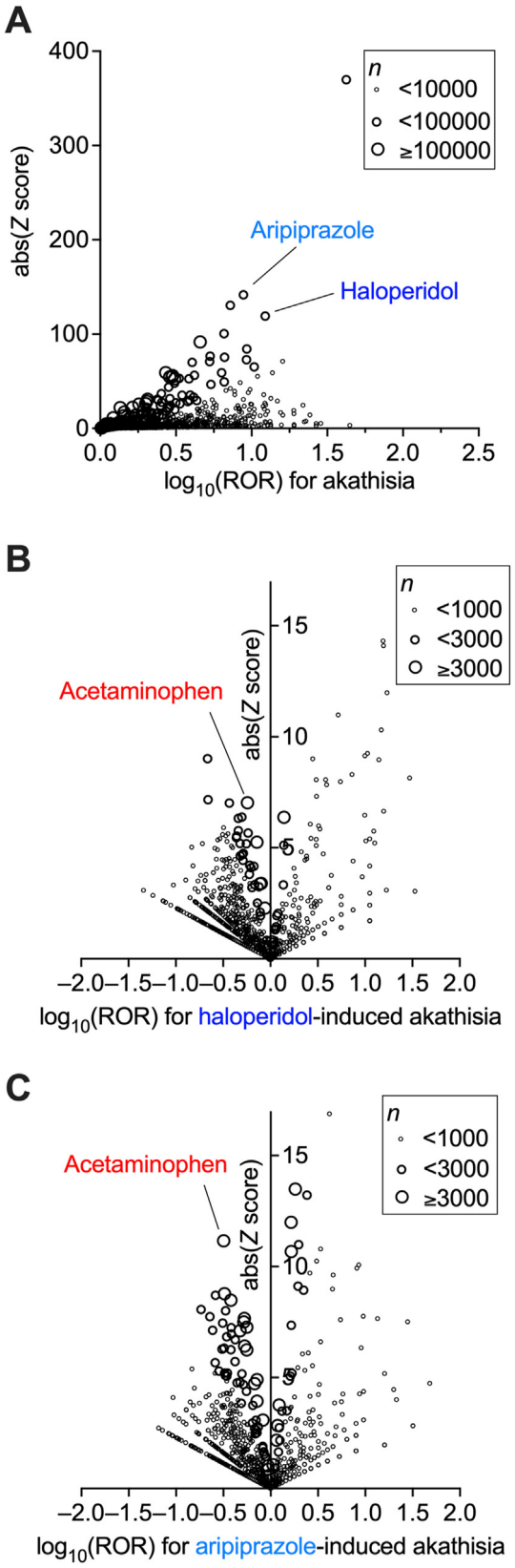

Fig. 1. Increased reporting odds ratio (ROR) of akathisia with the use of dopamine D_2 receptor (D2R) antagonists and confounding effects of acetaminophen on drug-induced akathisia according to the FAERS data.
Volcano plots of ROR values on a log scale and the corresponding absolute *Z* scores are shown. Each circle indicates an individual drug, and the size of the circle reflects the number of patients taking the drug. (A) Strong and significant increases in the ROR of akathisia were seen in patients using D2R antagonists, such as aripiprazole and haloperidol. (B, C) Within the population taking each of the D2R antagonists, the confounding effects of concomitantly used drugs on the ROR of drug-induced akathisia are shown. All drug names, ROR values, and *Z* scores are shown in Supplementary Tables 2 and 3

dependence, and diagnosis of mood disorders, diabetes mellitus, or hepatic diseases.[4,7] In these matched cohorts, daily and cumulative doses and the administration period of haloperidol were equivalent in each pair with or without acetaminophen. Similarly, the daily dose of aripiprazole was equivalent in each pair with or without acetaminophen, while the cumulative dose and administration period of aripiprazole were higher and longer, respectively, in the group with acetaminophen than in that without acetaminophen (Supplementary Table 5).

In the propensity score-matched cohorts, we investigated the effect of acetaminophen combination on D2R antagonist-induced IR (Table 2). Among patients taking haloperidol, 15 of 32 patients treated without acetaminophen developed akathisia, while the patients treated with acetaminophen did not exhibit akathisia. Among patients taking aripiprazole, 160 of 718 patients treated without acetaminophen developed akathisia. However, none of the patients treated with acetaminophen developed akathisia despite the high cumulative dose and long administration period of aripiprazole, as noted earlier. Therefore, the IR values of akathisia turned to 0 in the acetaminophen group, indicating that concomitant use of acetaminophen completely inhibited antipsychotic-induced akathisia.

3.3. Effects of acetaminophen on haloperidol-induced hyperactivity models in rats

To determine whether the inhibitory effect of acetaminophen on D2R antagonist-induced akathisia is reproduced in animals, we established a rat model of akathisia through long-term treatment with haloperidol. Aripiprazole was not used because haloperidol was associated with a relatively higher incidence of akathisia (Table 1). To reflect the clinical presentation of tardive akathisia, the protocol involved 6 weeks of continuous drug administration via an osmotic mini-pump. Furthermore, since tardive akathisia may occur after drug withdrawal, behavioral tests were performed 5–7 days after the osmotic pump was removed (Fig. 3A).

A marked decrease in immobility time and increase in stereotyped behavior rate were observed after the 6-week treatment with haloperidol, suggesting that long-term administration of haloperidol may increase akathisia-like hyperactivity. Moreover, when acetaminophen (200 mg/kg, p.o.) was administered to the haloperidol-treated rats, the immobility time significantly increased (Fig. 3B, two-way ANOVA; haloperidol: $F_{1,28} = 16.37$, $P < 0.001$; acetaminophen: $F_{1,28} = 7.56$, $P = 0.10$; interaction: $F_{1,28} = 19.69$, $P < 0.001$) and the stereotyped behavior rate decreased (Fig. 3C, two-way ANOVA, haloperidol: $F_{1,28} = 22.84$, $P < 0.001$; acetaminophen: $F_{1,28} = 22.89$, $P < 0.001$; interaction: $F_{1,28} = 10.39$, $P < 0.01$).

The clinical manifestation of akathisia is similar to that of restless legs syndrome (RLS).[18] Previous reports showed that locomotor activity in the rodent models of RLS is increased,[19] and behavioral assessments of the RLS model may be adapted to other RLS-related hyperactivity disorders such as akathisia and attention deficit hyperactivity disorder.[20] We then evaluated the change in locomotor activity by performing the open field test. A significant increase in the total distance travelled was observed in rats treated with haloperidol for 6 weeks. Meanwhile, acute treatment with acetaminophen significantly suppressed haloperidol-induced hyperlocomotion (Fig. 3D, two-way ANOVA; haloperidol: $F_{1,36} = 14.23$, $P < 0.001$; acetaminophen: $F_{1,36} = 17.75$, $P < 0.001$; interaction:

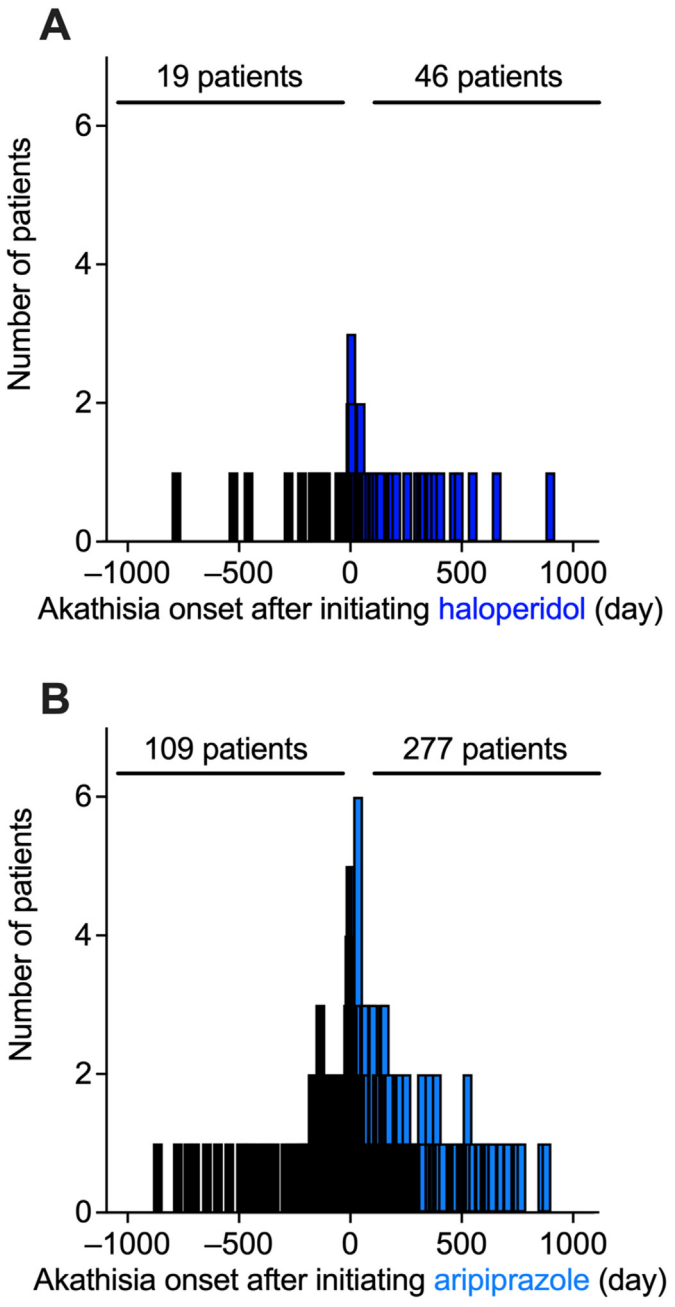

Fig. 2. Time trends in the onset of akathisia in the cohorts prescribed D2R antagonists according to the IBM data. (A) Sequence symmetry analysis showing the causal relationship with an adjusted sequence ratio of 2.00 (95% CI: 1.23–3.72) between the start of haloperidol treatment and the onset of akathisia in an observation period of ±3 years ($n = 65$). (B) Sequence symmetry analysis showing the causal relationship with an adjusted sequence ratio of 2.60 (95% CI: 2.11–3.29) between the start of aripiprazole treatment and the onset of akathisia in an observation period of ±3 years ($n = 386$).

Table 1
Incidence rate (IR) of drug-induced akathisia in the IBM MarketScan Research Database (IBM) data.

Haloperidol	Cases with akathisia	Cases without akathisia	IR/(person-year)
+	46	8259	6.52×10^{-3}
−	2336	39,579,725	3.22×10^{-5}
Aripiprazole	**Cases with akathisia**	**Cases without akathisia**	**IR/(person-year)**
+	278	123,500	2.25×10^{-3}
−	1987	39,377,763	2.75×10^{-5}

$F_{1,36} = 0.60$, $P = 0.44$). These results suggest that acetaminophen may acutely inhibit akathisia-like hyperactivity.

3.4. Acetaminophen improves haloperidol-induced decrease in iMSN activity

Although the pathophysiology of tardive akathisia remains unclear, previous reviews suggest that drug-induced akathisia can be attributed to dopamine signaling in the ventral striatum,[21] and that chronic administration of D2R antagonists may cause a decrease in indirect-pathway medium spiny neuron (iMSN) activity or dysfunction of the neurons through hypersensitization of D2Rs.[22] Therefore, we investigated the effects of continuous haloperidol administration on iMSNs by evaluating the immunoreactivity of ppENK, a marker for iMSNs. We further evaluated the effect of long-term treatment with haloperidol on ppENK$^+$ cells but found no significant change in the number of ppENK$^+$ cells in the ventral striatum upon haloperidol treatment compared with that upon control treatment (Fig. 4A, unpaired t test, $P = 0.15$).

We have previously demonstrated that the number of c-Fos$^+$ppENK$^+$ cells was significantly decreased in mice dorsal striatum after 21 days of haloperidol administration.[7] However, changes in c-Fos$^+$ppENK$^+$ cells in the ventral striatum of rats after 6 weeks of haloperidol treatment have not been reported. Therefore, we investigated the activity of iMSNs via ppENK and c-Fos staining in the ventral striatum. In the control group, c-Fos signals were detected in some of the ppENK$^+$ cells (Fig. 4B), and the number of c-Fos$^+$ppENK$^+$ cells was significantly decreased in the ventral striatum after haloperidol treatment, suggesting a reduction in iMSN activity. The decrease was reversed 90 min after an oral administration of 200 mg/kg acetaminophen (Fig. 4C, two-way ANOVA; haloperidol: $F_{1,30} = 7.51$, $P < 0.05$; acetaminophen: $F_{1,30} = 5.05$, $P < 0.05$; interaction: $F_{1,30} = 4.62$, $P < 0.05$). These results suggest that acetaminophen facilitates the neural activity of iMSNs in the ventral striatum.

4. Discussion

To our knowledge, this study is first to show that antipyretic analgesic acetaminophen mitigates akathisia-like symptoms induced by the long-term use of D2R antagonists. This finding was supported by evidence from two independent human data sources and by *in vivo* studies involving a rodent model of haloperidol-induced hyperactivity.

Our previous study involving FAERS analysis revealed a positive correlation between D2R antagonist use and dyskinesia onset.[7] In this study, the association between D2R antagonist use and akathisia onset was robust due to the large sample size. In addition, ROR values of dyskinesia and D2R antagonist-induced akathisia were significantly decreased by acetaminophen, which has not previously proven to be effective in rodent models or human patients with akathisia.[3]

Chronological analysis of the IBM data helped obtain precise IRs of D2R antagonist-induced akathisia. Akathisia is generally associated with typical antipsychotics. For example, a meta-analysis reported that akathisia risk is higher for typical antipsychotics than for atypical antipsychotics.[23] In fact, our data showed that the akathisia IR of haloperidol is higher than that of aripiprazole, supporting previous findings. A retrospective comparison of matched cohorts demonstrated that the administration of acetaminophen reduced 3-year incidence of D2R antagonist-induced akathisia without decreasing cumulative D2R antagonist doses. In this analysis, many of the patients who were prescribed acetaminophen were using drug combinations with opioids. Opioid preparations (drug combinations with acetaminophen) are effective against

Table 2
Effect of concomitant use of acetaminophen on IR of D2R antagonist-induced akathisia in the IBM data.

Matched haloperidol cohort		Cases with akathisia	Cases without akathisia	IR/(person-year)
Acetaminophen	+	0	32	0
	–	15	17	1.09
Matched aripiprazole cohort		**Cases with akathisia**	**Cases without akathisia**	**IR (/person-year)**
Acetaminophen	+	0	718	0
	–	160	558	0.75

akathisia.[24] Therefore, given the involvement of opioids in akathisia, drug combinations of acetaminophen and opioids were excluded from the analysis. Nevertheless, acetaminophen markedly decreased the IR of haloperidol- and aripiprazole-induced akathisia, indicating that the involvement of opioids is relatively small.

Several animal models have been developed to investigate the pathophysiology of akathisia. These models include the rat defecation model, lesions of the ventral tegmental area and medial prefrontal cortex that can produce locomotor responses that mimic drug-induced akathisia, SSRI-induced restlessness model, and hyperkinesia dog model (see review by Salem et al.[3]). Proposed akathisia models of non-human primates are a dopamine D_1 receptor agonist-induced grooming model[25] and short-term administration of haloperidol and aripiprazole in a phosphodiesterase 10A inhibitor-induced model of stereotypies including teeth grinding, restless limb movements, and hyperlocomotion.[16] However, these models mimic acute akathisia rather than tardive akathisia; in fact, a tardive akathisia model remains lacking. Therefore, to reflect the clinical manifestation of tardive akathisia, haloperidol was administered longitudinally and subsequently withdrawn from rats. The haloperidol-treated rats showed akathisia-like behavior such as an increase in locomotor and stereotyped behavior. Thus, the protocol described in this study may represent a new rodent model of tardive akathisia. Nevertheless, it should be noted that tardive akathisia is accompanied by both hyperactivity and psychiatric symptoms such as anxiety, which is why further studies on this subject are necessary.

Tardive akathisia, like tardive dyskinesia, is associated with long-term use of D2R antagonists.[4] Although the pathophysiological mechanism of tardive syndrome remains unclear, the hypothesis of dopaminergic supersensitivity psychosis is widely accepted.[22] The popular hypothesis is that chronic inhibition of

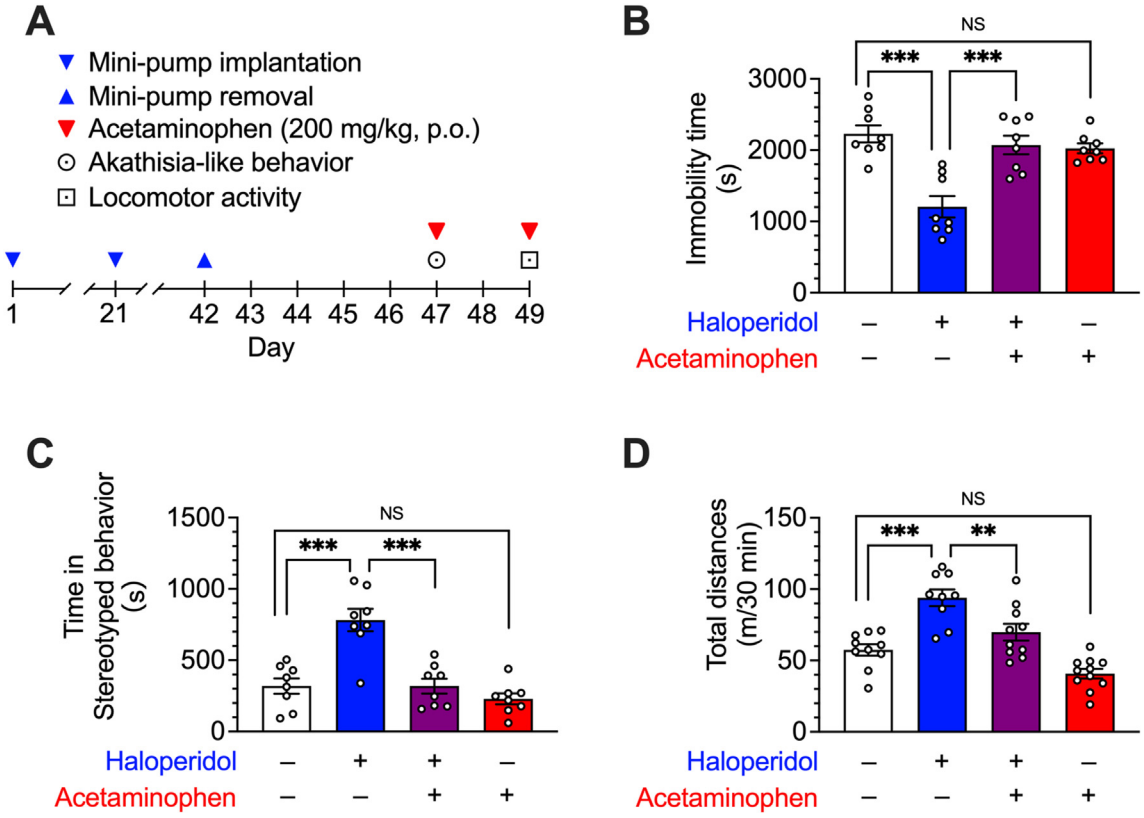

Fig. 3. Effects of acetaminophen on haloperidol-induced decrease in immobility time and increase in stereotyped behavior and locomotor activity in rats. (A) Rats were treated with haloperidol (1 mg/kg/day) for 6 weeks through continuous administration using an osmotic mini-pump, and akathisia-like behavior test and locomotor activity test were performed on days 5 and 7, respectively, after the osmotic pump was removed. (B, C) Rats ($n = 8$ per group) were placed in a cage 20 min after oral administration of acetaminophen (200 mg/kg) or vehicle, and their behavior was recorded for 60 min. The immobility time and stereotyped behavior time were measured for 50 min after excluding the first 10 min of recording. (D) Rats ($n = 9$–10 per group) were orally administered with acetaminophen (200 mg/kg) or vehicle. The locomotor activity of the rats was measured for 30 min, beginning 30 min after acetaminophen administration. Individual data are shown as mean ± SEM. Statistical significance was tested using two-way ANOVA with post hoc multiple comparisons. $^{**}P < 0.01$; $^{***}P < 0.001$; NS, not significant.

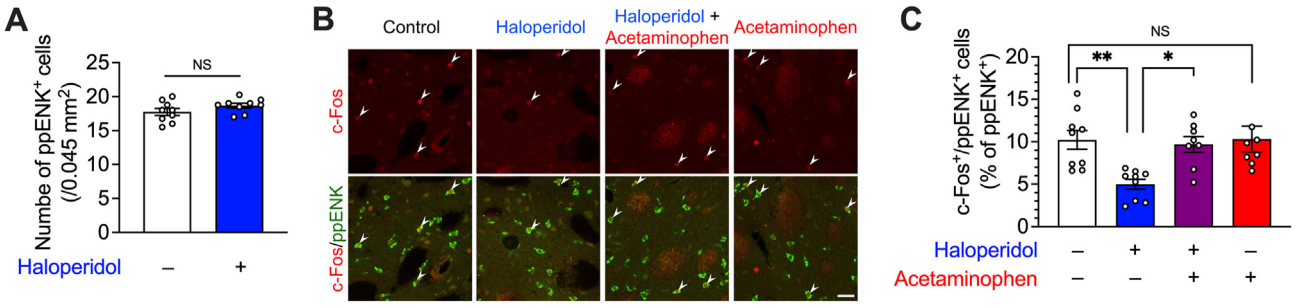

Fig. 4. Effects of acetaminophen on haloperidol-induced decrease in iMSN activity in rats.
Rats ($n = 8–9$ per group) were treated with haloperidol (1 mg/kg/day) for 6 weeks by continuous administration via an osmotic mini-pump, and immunohistological analysis was conducted on day 13 or 14 after the osmotic pump was removed. These rats were orally administered acetaminophen (200 mg/kg) or vehicle. After 90 min, coronal sections containing the ventral striatum were prepared, stained with anti-c-Fos and anti-ppENK antibodies, and imaged using confocal microscopy. (A) The numbers of ppENK$^+$ cells in the haloperidol- and vehicle-treated rats were counted to determine the total number of iMSNs in each group. Individual data are shown as mean ± SEM. Statistical significance was tested using unpaired *t* test. NS, not significant. (B, C) The number of c-Fos$^+$ppENK$^+$ cells (shown by arrowheads) were counted and are presented as percentages of the number of ppENK$^+$ cells. Scale bars: 30 μm. Individual data are shown as mean ± SEM. Statistical significance was tested using two-way ANOVA with post hoc multiple comparisons. $*P < 0.05$; $**P < 0.01$; NS, not significant.

dopamine D2Rs leads to the upregulation and hypersensitization of D2Rs expressed in the iMSNs of the striatum, resulting in their hypoactivity. This observation is supported by the evidence demonstrating the upregulation of *DRD2* expression in the human brain associated with antipsychotics[26] and the upregulation of D2Rs in the striatum alongside an increase in locomotor activity and the onset of dyskinesia by chronic administration of D2R antagonists in animals.[27–29] In the present study, acute acetaminophen use ameliorated the haloperidol-induced increases in locomotor and stereotyped behavior and the decrease in c-Fos signaling in ventral striatal iMSNs. The mechanism of action of acetaminophen is not completely clear, but investigations involving transient receptor potential vanilloid 1 (TRPV1) agonists, cannabinoid type 1 (CB_1) receptors agonists, transient receptor potential ankyrin 1 (TRPA1) agonists, and inhibition of the cyclooxygenase (COX) has been considered.[30] TRPA1 and COX are involved in the antipyretic effect of acetaminophen. Previously, we had reported that acetaminophen inhibits haloperidol-induced dyskinesia and increases c-Fos signaling in dorsostriatal iMSNs; these effects are abolished in TRPV1 knockout mice.[7] Considering that tardive akathisia and tardive dyskinesia may share similar mechanisms in the neighboring brain areas, these data indicate that TRPV1 may be involved in the inhibition of hyperactivity and activation of iMSNs by acetaminophen in rats. However, because hyperactivity induced by the D_2/D_3 receptor agonist is attenuated by inhibitors of endocannabinoid degradation[31] and considering that concentrations of acetaminophen in this study were higher than those in our previous study, the contribution of CB_1 receptors cannot be excluded from the effects of acetaminophen. Future research is required to determine whether specific antagonists alter the therapeutic effects of acetaminophen in rats or in a mouse model of tardive akathisia developed using the gene knockout technique.

In conclusion, our findings demonstrate that acetaminophen is effective in decreasing D2R antagonist-induced akathisia in both human retrospective analysis and experimental animal models. Acetaminophen might be a new therapeutic agent for tardive akathisia in humans and is potentially suitable for use with other hyperactivity disorders with similar symptoms and mechanisms.

Author contributions

Ko.N. performed clinical data analysis and animal experiments. Ka.N. and H.S. provided materials and technical advice. Ko.N. and S.K. designed the project, analyzed the data, and wrote the manuscript.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

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