Usefulness of Olanzapine as an Adjunct to Opioid Treatment and for the Treatment of Neuropathic Pain


ABSTRACT

Background: The use of opioids for pain management is often associated with nausea and vomiting. Although conventional antipsychotics are often used to counter emesis, they can be associated with extrapyramidal symptoms. However, chronic pain can induce sleep disturbance. The authors investigated the effects of the atypical antipsychotic olanzapine on morphine-induced emesis and the sleep dysregulation associated with chronic pain.

Methods: A receptor binding assay was performed using mouse whole brain tissue. The emetic response in ferrets was evaluated by counting retching and vomiting behaviors. Catalepsy in mice was evaluated by placing both of their forepaws over a horizontal bar. Released dopamine was measured by an in vivo microdialysis study. Sleep disturbance in mice in a neuropathic pain-like state was assayed by electroencephalogram and electromyogram recordings.

Results: Olanzapine showed high affinity for muscarinic M1 receptor in brain tissue. Olanzapine decreased morphine-induced nausea and vomiting in a dose-dependent manner.

Conclusion: These findings suggest that olanzapine may be useful for the treatment of morphine-induced emesis and as an adjunct for the treatment of neuropathic pain associated with sleep disturbance.

What We Already Know about This Topic
- Chronic pain is often associated with sleep disturbances
- Severe side effects of opioids given for pain treatment include nausea and vomiting

What This Article Tells Us That Is New
- In ferrets, olanzapine, an atypical thienobenzodiazepine antipsychotic drug, suppressed morphine-induced emesis and improved pain-related sleep disturbances

However, olanzapine at a dose that had an antiemetic effect (0.03 mg/kg) did not induce catalepsy or hyperglycemia. In addition, olanzapine at this dose had no effect on the morphine-induced release of dopamine or inhibition of gastrointestinal transit. Finally, olanzapine inhibited thermal hyperalgesia and completely alleviated the sleep disturbance induced by sciatic nerve ligation.

The World Health Organization has stated that morphine is the “gold standard” for the treatment of moderate to severe pain caused by cancer. However, the use of morphine for this purpose is often associated with distressing side effects, such as drowsiness, constipation, emesis, and delirium. Many clinicians consider that dopamine receptor antagonists, including prochlorperazine, are the preferred drugs for combating opioid-induced nausea and vomiting. However, these drugs often produce adverse effects, including extrapyramidal symptoms. Therefore, new approaches are needed to prevent opioid-induced emesis, as is a better understanding of the mechanism of drug action.

Nausea and vomiting are controlled by the “vomiting center” in the medulla oblongata, which receives signals from the chemoreceptor trigger zone (CTZ) in the area postrema, the gastrointestinal tract, the vestibular apparatus in the temporal lobe, and the cerebral cortex. Opioids have emetogenic effects by stimulating the CTZ and the vestibular apparatus and by inhibiting gut motility. Although stimu-
lation of the CTZ by opioids involves opioid μ and δ receptors,8 signals from the CTZ to the vomiting center mainly involve dopamine D2 and serotonin (5-HT3) receptors in the former. However, opioid-induced stimulation of the vestibular apparatus and subsequent sensory input to the vomiting center have both been suggested to involve histamine H1 and muscarinic acetylcholine pathways.9

Atypical antipsychotic medications treat the positive symptoms of schizophrenia, such as hallucinations and delusions, more effectively than the negative symptoms, such as lack of motivation and social withdrawal. Olanzapine is a newer atypical antipsychotic that blocks dopaminergic, serotonergic, adrenergic, histaminergic, and muscarinic receptors for multiple neurotransmitters. Because it affects neurotransmitters that are associated with nausea, it may have potential as an antiemetic medication.10

In addition, patients with chronic pain commonly experience sleep disturbance11–13 and may benefit from its treatment.13 Sleep problems and daytime sleepiness seem to be related to depression and the severity of pain.14 It has been suggested that olanzapine may improve sleep disturbance.15

The primary endpoint of the study was to investigate whether olanzapine at doses lower than those that would induce catalepsy could suppress morphine-induced emesis with few side effects. We also examined if olanzapine could improve sleep dysregulation under a neuropathic pain-like state.

Materials and Methods

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals at Hoshi University, as adopted by the Committee on Animal Research of Hoshi University (Tokyo, Japan). Every effort was made to minimize the numbers and suffering of animals used in the following experiments.

The observer was not blinded in all of the experiments.

Animals

In the present study, male Institute of Cancer Research mice (20–25 g) (Tokyo Laboratory Animals Science, Tokyo, Japan), male C57BL/6J mice (25–30 g) (CLEA Japan, Tokyo, Japan), and Sprague-Dawley rats (200–300 g) (Tokyo Laboratory Animals Science) were used. Animals were housed in a room maintained at 22°C with a 12-h light–dark cycle. Food and water were available ad libitum. Each animal was used only once. Male ferrets weighing 1–1.5 kg were obtained from Marshall Research Labs (North Rose, NY) and housed individually in a room kept at 23°C ± 1°C under a 12-h light–dark cycle (lights on 8:00 AM-8:00 PM). They were given a standard cat diet (70–80 g/animal, Oriental Yeast Co., Ltd., Chiba, Japan) and allowed free access to water.

Receptor Binding Assay

Mouse whole brain was treated as described previously,16 and the resulting pellet was resuspended and used as the membrane fraction. The binding assay was performed in triplicate with [3H]clozapine (specific activity, 70–87 Ci/mmol; American Radiolabeled Chemicals, St. Louis, MO) at 0.2 nM, [3H]ketanserin hydrochloride (specific activity, 67 Ci/mmol; PerkinElmer, Waltham, MA) at 0.5 nM, [3H]BRL-43694 (granisetron) (specific activity, 85.3 Ci/mmol; PerkinElmer) at 0.5 nM, [3H]GR113808 (specific activity, 78.3 Ci/mmol; PerkinElmer) at 0.5 nM, [3H]pyrilamine (specific activity, 30 Ci/mmol; PerkinElmer) at 0.5 nM, and [3H]pirenzepine (specific activity, 72.8 Ci/mmol; PerkinElmer) at 0.5 nM, in a final volume of 500 ml that contained 50 mM Tris-HCl buffer, pH 7.4, and 200 ml homogenized membrane fraction. Ninety to 140 mg membrane proteins were used in each assay. Specific binding was defined as the difference in binding observed in the absence and presence of 1 mM unlabeled clozapine, ketanserin, granisetron, or GR113808, 10 mM unlabeled pyrilamine, or 100 mM unlabeled pirenzepine, respectively. All samples were incubated as described previously,16 and radioactivity in the samples was determined with a liquid scintillation analyzer. All receptor binding curves were fitted using Prism software (version 5.0a; GraphPad Software, La Jolla, CA).

Evaluation of the Emetic Response

Emesis in ferrets after the administration of morphine (0.6 mg/kg, subcutaneous injection) was evaluated by counting the number of retching or vomiting behaviors as described elsewhere,17 where retching was defined as a rhythmic abdominal contraction without expulsion and vomiting was the oral expulsion of solid or liquid from the gastrointestinal tract. Emesis was assessed for 30 min after the injection of morphine.18 To determine the effect of olanzapine on morphine-induced emesis, groups of ferrets were pretreated with olanzapine 30 min before the injection of morphine.

An interval of at least 7 days was allowed between testing for each animal to allow for drug washout and to minimize the development of tolerance.

Horizontal Bar Test for the Evaluation of Catalepsy

Catalepsy19,20 was evaluated using the horizontal bar test as described previously.21 Briefly, animals were placed so that both forepaws were over a horizontal bar 5 cm above the floor, and the amount of time (s) the animal maintained this position was recorded for as long as 60 s. Catalepsy was considered to have finished when a forepaw touched the floor or when the mouse climbed on the bar. Scores were assigned based on the duration of the cataleptic posture (score 1: 15 to 29 s, score 2: 30 to 59 s, score 3: 60 s or more).
In vivo Microdialysis Study and Quantification of Dopamine and Its Metabolites

After 3 days of habituation to the main animal colony, all of the rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal administration) for surgery as described previously. Briefly, the anesthetized animals were placed in a stereotaxic apparatus, the skull was exposed, and a small hole was made using a dental drill. A guide cannula (AG-8; Eicom, Kyoto, Japan) was implanted into the nucleus accumbens (from the bregma: anterior, +4.0 mm; lateral, −0.8 mm; ventral, −6.8 mm; angle 16 degrees) according to the atlas of Paxinos and Watson and fixed to the skull with cranioplastic cement. Three to 5 days after surgery, microdialysis probes (A1-8–02; 2 mm membrane length; Eicom) were slowly inserted into the nucleus accumbens through guide cannulas during anesthesia with diethyl ether, and the rats were placed in experimental cages (30 cm wide × 30 cm deep × 30 cm high). The probes were perfused continuously (2 ml/min) with artificial cerebrospinal fluid: 0.9 mM MgCl₂, 147.0 mM NaCl, 4.0 mM KCl, and 1.2 mM CaCl₂. Outflow fractions were collected every 20 min. After three baseline fractions were collected from the rat nucleus accumbens, rats were given olanzapine (0.3 mg/kg, intraperitoneal administration), vehicle (5% dimethyl sulfoxide [DMSO]); 1 ml/kg, intraperitoneal administration) or saline (1 ml/kg, intraperitoneal administration) 30 min before treatment with morphine (0.7 mg/kg, subcutaneous injection) or vehicle (5% DMSO) 30 min before treatment with morphine (10 mg/kg, intraperitoneal administration). Dialysis samples were collected for 180 min after treatment and analyzed by high-performance liquid chromatography (Eicom) with electrochemical detection (Eicom). Dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenyl acetic acid, were separated by column chromatography and identified and quantified by the use of standards, as described previously.

Gastrointestinal Transit

In the study of gastrointestinal transit, Institute of Cancer Research mice were fasted for 12 h before the experiments. Groups of mice were pretreated with olanzapine (0.03–1 mg/kg, subcutaneous injection) or vehicle (5% DMSO) 30 min before the administration of morphine (0.7 mg/kg, subcutaneous injection) or saline, and ink (0.3 ml/mouse) was orally administered 20 min after the injection of morphine or saline. Twenty minutes after the administration of ink, the animal was killed by cervical dislocation, and the small intestine was removed. The percentage inhibition of gastrointestinal transit was calculated as follows: (distance traveled by the ink/length from the pylorus to the cecum) × 100.

Blood Glucose Measurement

C57BL/6J mice were administered olanzapine (0.03–1 mg/kg, subcutaneous injection) or vehicle (5% DMSO) once a day for 1 week. At 60 min after the final injection, the tail was cut and blood was collected. Blood glucose was measured using a self-monitoring blood glucose meter (Medisafe-Mini; Terumo, Tokyo, Japan). The Medisafe-Mini system is based on the optoelectric colorimetry method.

Neuropathic Pain Model

C57BL/6J mice were anesthetized with 3% isoflurane. A partial sciatic nerve ligation model was made by tying a tight ligature with 8–0 silk suture around approximately one third to one half the diameter of the sciatic nerve on the right side (ipsilateral side) under a light microscope (SD30; Olympus, Tokyo, Japan). In sham-operated mice, the nerve was exposed without ligation.

Measurement of Thermal Thresholds

The sensitivity to thermal stimulation was measured as described previously. Briefly, the right plantar surface of mice was exposed to a well-focused radiant heat light source (model 33 Analgesia Meter; IITC/Life Science Instruments, Woodland Hills, CA) that had been adjusted so that the average baseline latency of paw withdrawal in naive mice was approximately 8–10 s. Only quick movements of the hind paw away from the stimulus were considered to be a withdrawal response: paw movements associated with locomotion or weight shifting were not counted as a response. The paws were measured alternating between left and right with an interval of more than 3 min between measurements. Before testing, mice were placed in a clear acrylic cylinder (15 cm high and 8 cm in diameter) for at least 30 min. The data represent the average latency of paw withdrawal for the right hind paw.

Electroencephalogram and Electromyogram Recordings

Electroencephalogram and electromyogram recordings were obtained as described previously. Briefly, electroencephalogram signals were monitored with two stainless-steel electroencephalogram recording screws 1 mm anterior to the bregma or A, both 1.5 mm lateral to the midline, and electromyogram activity was monitored by stainless steel, non-stick-coated wires placed bilaterally into both trapezius muscles. Electroencephalogram and electromyogram signals were amplified, filtered (0.5–30 Hz and 20–200 Hz, respectively), digitized at a sampling rate of 128 Hz, and recorded using SleepSign software (Kissei Comtec, Nagano, Japan), which was also used to automatically classify vigilance over 4-s epochs as wakefulness, rapid eye movement (REM) sleep, or non-REM sleep using standard criteria. Finally, defined sleep–wake stages were examined visually and corrected, if necessary. For each epoch, the electroencephalogram power density in the δ (0.75–4.0 Hz) and θ bands (6.25–9.0 Hz) and the integrated electromyogram value were displayed on a computer monitor. Electroencephalogram and electromyogram activities were monitored for 24 h at 7 days after sciatic nerve ligation. Recordings were started at 8:00 PM. Vehicle
(5% DMSO) or olanzapine (0.06 mg/kg, intraperitoneal administration) was injected every day at 8:00 AM.

**Drugs**
The drugs used in the current study were morphine hydrochloride (Daiichi-Sankyo, Tokyo, Japan), prochlorperazine maleate (Sigma–Aldrich, St. Louis, MO), clozapine (Wako Pure Chemical Industries, Osaka, Japan), olanzapine (Toronto Research Chemicals, Toronto, Ontario, Canada), telenzepine dihydrochloride hydrate (Sigma–Aldrich), ritanserin (Tocris Biotechnology, Ellisville, CA), pyrilamine maleate salt (Sigma–Aldrich), ketanserin tartrate (Wako Pure Chemical Industries), granisetron (Sigma–Aldrich), GR113808 (Sigma–Aldrich), haloperidol (Sigma–Aldrich), L745870 (Research Biochemicals International, Natick, MA), raclopride (Santa Cruz Biotechnology, Santa Cruz, CA), pirenzepine (Toronto Research Chemicals), and DL-trihexyphenidyl hydrochloride (Sigma–Aldrich).

**Statistical Analysis**
Data are expressed as the mean with SEM. The statistical significance of differences between the groups was assessed with one-way and two-way ANOVA followed by the Bonferroni multiple comparisons test or Student t test (unpaired, two-tailed). The concentration of the test compound that caused 50% inhibition of specific binding (IC$_{50}$ value) was determined from each concentration-response curve. All statistical analyses and IC$_{50}$ values were calculated by Prism software (version 5.0a, GraphPad Software). A P value of <0.05 was considered to reflect significance.

**Results**

**Binding Properties of Clozapine**
In mouse brain membranes, we determined the competitive displacement binding of [$^3$H]clozapine with graded concentrations ($10^{-11}$–$10^{-4}$ M) of unlabeled clozapine, olanzapine, telenzepine, ritanserin, pyrilamine, ketanserin, GR113808, granisetron, haloperidol, L745870, and raclopride. The binding of [$^3$H]clozapine was displaced by olanzapine in a concentration-dependent manner (fig. 1A). In addition, the binding of [$^3$H]clozapine was partially displaced by telenzepine (M$_1$), ritanserin (5-HT$_2A$), pyrilamine (H$_1$), ketanserin (5-HT$_2C$), GR113808 (5-HT$_4$), granisetron (5-HT$_3$), haloperidol (D$_2$), L745870 (D$_3$), and raclopride (D$_3$) (fig. 1B).

**Binding Properties of Olanzapine with 5-HT$_2A/2D$, 5-HT$_3$, 5-HT$_4$, H$_1$, and M$_1$ Receptors**
In mouse brain membranes, we determined the competitive displacement binding of [$^3$H]ketanserin, [$^3$H]BRL-43694 (granisetron), [$^3$H]pyrilamine, [$^3$H]GR113808, and [$^3$H]pirenzepine with graded concentrations ($10^{-11}$–$10^{-4}$ M) of unlabeled ketanserin, granisetron, pyrilamine, GR113808, telenzepine, pirenzepine and olanzapine. The binding of [$^3$H]ketanserin and [$^3$H]pirenzepine was displaced by olanzapine in a concentration-dependent manner (fig. 2, A and B). The binding of [$^3$H]pyrilamine, [$^3$H]BRL-43694, and [$^3$H]GR113808 was partially displaced by olanzapine (fig. 2, C, D, and E).

**Suppression of Morphine-induced Emesis by Olanzapine or Prochlorperazine**
Pretreatment with either olanzapine (0.03 mg/kg, subcutaneous injection) 30 min before the injection of morphine (0.6 mg/kg, subcutaneous injection) or prochlorperazine (0.3 mg/kg and 1.0 mg/kg, subcutaneous injection) 60 min before the injection of morphine reduced the number of retching and vomiting behaviors induced by morphine (fig. 3).

**Effects of Antipsychotics on Catalepsy**
The results from the horizontal bar test as a measure of catalepsy were obtained at 15, 30, 45, and 60 min after the subcutaneous injection of vehicle, prochlorperazine (0.3–1 mg/kg), haloperidol (0.03–0.3 mg/kg), risperidone (0.01–0.1 mg/kg), or olanzapine (0.03–0.3 mg/kg). Although the catalepsy scores were not affected by a single subcutaneous injection of olanzapine (0.03–0.3 mg/kg), catalepsy was observed with the other antipsychotics within this dose range (fig. 4).

**Effects of Olanzapine on the Morphine-induced Increase in the Concentrations of Dopamine and Its Metabolites in Dialysate**
In the microdialysis study, the concentrations of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenyl acetic acid in dialysate from the rat nucleus accumbens were markedly increased by the intraperitoneal administration of morphine at 10 mg/kg compared with those under the administration of saline. The increased concentrations of dopamine, 3,4-dihydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenyl acetic acid in the nucleus accumbens after the administration of morphine were not affected by olanzapine at 0.3 mg/kg (olanzapine-morphine vs. vehicle-morphine: F(1,77) = 0.1516, P = 0.7086 fig. 5A; F(1,77) = 0.06326, P = 0.8086 fig. 5B; F(1,77) = 1.851, P = 0.2158 fig. 5C).

**Effect of Olanzapine on the Morphine-induced Inhibition of Gastrointestinal Transit**
Morphine slowed gastrointestinal transit, and this effect was not significantly altered by the coadministration (subcutaneous injection) of olanzapine at 0.03–1 mg/kg (fig. 6A). Olanzapine itself did not slow gastrointestinal transit at doses of 0.03 and 0.1 mg/kg but significantly inhibited gastrointestinal transit at 0.3 and 1 mg/kg (fig. 6B).

**Effects of Olanzapine on Blood Glucose**
Blood glucose was measured after long-term treatment with olanzapine, saline, or vehicle (5% DMSO) in mice. Hypergly-
cemia was not observed under treatment with olanzapine at 0.03, 0.1, or 0.3 mg/kg (subcutaneous injection) (fig. 7).

**Thermal Hyperalgesia Induced by Sciatic Nerve Ligation in Mice**

Sciatic nerve ligation markedly decreased the latency of paw withdrawal in response to a thermal stimulus on the ipsilateral side. This state of persistent pain caused by partial ligation of the sciatic nerve was suppressed by treatment with olanzapine at 0.06 mg/kg (fig. 8).

**Changes in Sleep Vigilance in a Neuropathic Pain-like State Using Electroencephalogram and Electromyogram Recordings**

We next investigated the changes in sleep patterns in sciatic nerve-ligated mice. Vigilance was classified automatically offline as wakefulness, REM sleep, or non-REM sleep. Mice with sciatic nerve ligation showed a statistically significant increase in wakefulness ($P = 0.0009$ vs. sham operated mice with vehicle, fig. 9A) and a decrease in non-REM sleep ($P = 0.0067$ vs. sham-operated mice with vehicle, fig. 9C) during the light phase. REM sleep during the light phase was not affected by sciatic nerve ligation ($P = 0.2896$ vs. sham-operated mice with vehicle, fig. 9B). On the other hand, there was no statistically significant difference in the sleep conditions during the dark phase between the two groups (wakefulness: $P = 0.6170$ vs. sham operated mice with vehicle, fig. 9D; REM: $P = 0.3936$ vs. sham operated mice with vehicle, fig. 9E; non-REM: $P = 0.5479$ vs. sham operated mice with vehicle, fig. 9F).
Changes in the Hypnotic Effects of Olanzapine in a Neuropathic Pain-like State Using Electroencephalogram and Electromyogram Recordings

To ascertain the hypnotic effect of olanzapine in a neuropathic pain-like state, we performed electroencephalogram and electromyogram recordings. The increased wakefulness and decreased non-REM during the light phase in nerve-ligated mice were significantly attenuated compared with those in sham-operated mice by the intraperitoneal administration of olanzapine (wakefulness: $P = 0.0006$ vs. nerve-ligated mice).

Fig. 2. Displacement of the binding of the serotonin (5-HT)2A/C receptor ligand [3H]ketanserin (A), the muscarinic M1 receptor ligand [3H]pirenzepine (B), the H1 receptor ligand [3H]pyrilamine (C), the 5-HT3 receptor ligand [3H]BRL-43694 (granisetron) (D), or the 5-HT4 receptor ligand [3H]GR113808 (E) in membranes of mouse brain without the cerebellum by ketanserin, pirenzepine, telenzepine, pyrilamine, granisetron, GR113808, or olanzapine. Experiments were performed in the presence of [3H]ketanserin (0.5 nM), [3H]BRL-43694 (0.5 nM), [3H]pyrilamine (0.5 nM), [3H]GR113808 (0.5 nM), or [3H]pirenzepine (0.5 nM) and increasing concentrations of ketanserin, granisetron, pyrilamine, GR113808, pirenzepine, telenzepine, or olanzapine. The data represent the mean ± SEM of three to four samples. IC50 values were determined using an analysis of variance and linear regression techniques. To calculate the IC50 values, at least six drug doses were used, and three samples were used for each dose. Values in parentheses indicate the 95% confidence range.

Usefulness of Olanzapine for Opioid Management

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ligated mice with vehicle, fig. 9A; non-REM: \( P < 0.001 \) vs. nerve-ligated mice with vehicle, fig. 9C).

**Discussion**

The use of opioids for pain management is often associated with nausea and vomiting. Opioids induce emesis by stimulating the CTZ in the brainstem and by enhancing vestibular sensitivity.\(^{25,26}\) Although several compounds are known to act on receptors in the CTZ, opioid-induced nausea and vomiting are attributable primarily to the transmission of dopamine. Many clinicians consider that typical antipsychotics such as prochlorperazine and haloperidol, which mainly act as dopamine D\(_2\) receptor antagonists, are the drugs of choice for preventing the nausea and vomiting induced by morphine.\(^{27-29}\) However, such compounds often produce extrapyramidal symptoms.\(^{4}\)

Olanzapine is an atypical thienobenzodiazepine antipsychotic that is clinically indicated for schizophrenia and mania.\(^{30}\) It blocks multiple neurotransmitters, including dopaminergic, serotoninergic, adrenergic, histaminergic, and muscarinic receptors.\(^{31}\) In the current binding study, olanzapine showed the highest affinity for muscarinic M\(_1\) receptors. To understand its affinity in greater detail, we investigated the affinity of olanzapine toward serotonin 5-HT\(_{2A/2C}\), 5-HT\(_3\), histamine H\(_1\), dopamine D\(_2\), dopamine D\(_4\), and 5-HT\(_4\) receptors. Olanzapine also showed affinity for each of these receptors. Because of its effect on several neurotransmitters that are associated with nausea and vomiting, we expected that olanzapine would have potential as an antiemetic medication. In a behavioral study, we found that morphine-induced nausea and vomiting were decreased in a dose-dependent manner by pretreatment with olanzapine at a dose that was almost 1/200 of that at which an antipsychotic effect is observed,\(^{32}\) whereas olanzapine at a dose that had antiemetic effects failed to induce catalepsy. However, although the dopamine D\(_2\) receptor antagonist prochlorperazine suppressed morphine-induced nausea and vomiting, it did so at a dose that caused a dose-dependent increase in the expression of catalepsy. Furthermore, olanzapine had no effect on the morphine-induced release of dopamine in the nucleus accumbens. Muscarinic M\(_1\) receptors have been suggested to be responsible for the opioid-induced stimula-

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**Fig. 3.** Effects of olanzapine on subcutaneous injection morphine-induced retching (A, C) and vomiting (B, D) in ferrets. Groups of ferrets were pretreated with olanzapine (0.01 and 0.03 mg/kg, subcutaneous injection) (A, B), prochlorperazine (0.3 and 1.0 mg/kg, subcutaneous injection) (C, D), or vehicle before the administration of morphine (0.6 mg/kg, subcutaneous injection). Animals were observed for 30 min after subcutaneous injection of morphine. Each column represents the mean ± SEM of 9–10 ferrets. Statistical analyses were performed using one-way ANOVA followed by the Bonferroni multiple comparisons test: \( F_{(3,39)} = 20.41, P < 0.0001 \) (A); \( F_{(3,39)} = 11.29, P < 0.0001 \) (B); \( F_{(3,37)} = 15.13, P < 0.0001 \) (C); \( F_{(3,37)} = 13.70, P < 0.0001 \) (D). *** \( P < 0.001 \) versus vehicle-saline; ### \( P < 0.001 \); ## \( P < 0.01 \) or # \( P < 0.05 \) versus vehicle-morphine.
tion of the vestibular apparatus. In addition, sensory input from the vestibular apparatus to the vomiting center follows muscarinic M1 receptor pathways. Taken together with the fact that olanzapine showed the highest affinity toward muscarinic M1 receptors, these findings suggest that, although the exact mechanism by which olanzapine suppresses morphine-induced emesis remains unclear, muscarinic M1 receptors may be a critical target for morphine-induced emesis.

To prove our hypothesis, we next investigated whether the selective muscarinic M1 receptor antagonist trihexyphenidyl could affect morphine-induced nausea and vomiting. Trihexyphenidyl significantly suppressed morphine-induced retching and vomiting in ferrets (data not shown), which indicates that M1 receptors play an important role in the opioid-sensitive emetic pathway. However, trihexyphenidyl significantly enhanced the morphine-induced increase in the release of dopamine in the nucleus accumbens (data not shown). If we consider the risk of the overexcitation of dopamine transmission with the use of drug combinations, a specific M1 receptor antagonist might not be a better choice as an adjunct agent in combination with opioids. Because olanzapine acts not only on muscarinic M1 receptors, but also partly on histamine H1 and dopamine D2 receptors as an antagonist, it is likely that olanzapine at a dose lower than that at which it has antipsychotic effects could be useful for strongly preventing opioid-induced emesis without severe side effects.

Constipation is another adverse effect of treatment with morphine. In the current study, olanzapine at doses that had antiemetic effects had no effect on the morphine-induced...
inhibition of gastrointestinal transit. This may be attributable to the high central transitivity of olanzapine.

Long-term treatment with olanzapine is most commonly associated with increased weight gain, obesity, and diabetes mellitus. Therefore, we evaluated the effect of chronic treatment with olanzapine on blood glucose. As a result, hyperglycemia was not observed during treatment with olanzapine at a dose at which it had an antiemetic effect (0.03 mg/kg). However, long-term treatment with olanzapine at a dose of 1 mg/kg was associated with a slight but statistically significant increase in blood glucose concentrations. These findings support the idea that olanzapine may have a wide margin of safety when used as an antiemetic.

With regard to sleep disturbance in severe pain, we confirmed that mice with sciatic nerve ligation showed a statistically significant increase in wakefulness and a decrease in non-REM sleep during the light phase. Under the current conditions, treatment with olanzapine at doses at which the pain threshold was restored improved the sleep disturbance after sciatic nerve ligation. Histamine and serotonin are key neurotransmitters that regulate wakefulness, and their receptors are the ultimate targets of many wakefulness- and sleep-promoting drugs. In particular, histamine H1 receptor antagonist and serotonin 5-HT2A/2C receptor antagonist are known to shift one’s arousal state from hyperactivity to sleep. Therefore, the improvement of sleep disturbance during treatment with olanzapine may result from the

Fig. 6. Effect of pretreatment with olanzapine on the morphine-induced (A) inhibition of gastrointestinal transit and the effect of olanzapine itself (B). Each column represents the mean ± SEM of six mice. Ink was administered orally 20 min after the injection of morphine (0.7 mg/kg, subcutaneous injection) or saline, respectively. Groups of mice were pretreated with olanzapine (0.03–1 mg/kg, subcutaneous injection) at 30 min before the administration of morphine. Gastrointestinal transit was evaluated at 20 min after the oral administration of ink. Statistical analyses were performed with one-way ANOVA followed by the Bonferroni multiple comparisons test: F(5,35) = 15.99, P < 0.0001 (A); F(4,29) = 5.778, P = 0.0020 (B). * P < 0.05, *** P < 0.001 versus vehicle.

Fig. 7. Blood glucose concentrations after chronic treatment with olanzapine. Hyperglycemia was not observed in treatment with olanzapine (0.03, 0.1, or 0.3 mg/kg subcutaneous injection) for 7 days, whereas the glucose concentration was significantly increased by subcutaneous injection of olanzapine at 1 mg/kg. Values are expressed as a percentage of the control. Each column represents the mean ± SEM of four mice. Statistical analyses were performed with one-way ANOVA followed by the Bonferroni multiple comparisons test. * P < 0.05 versus vehicle.

Fig. 8. Effect of olanzapine on thermal hyperalgesia induced by nerve ligation in mice. Groups of mice were injected with olanzapine (0.06 mg/kg, intraperitoneal administration) or vehicle at 7 days after sciatic nerve ligation or sham operation. Thermal hyperalgesia was measured 1 h after a single intraperitoneal administration of olanzapine or vehicle. Each column represents the mean ± SEM of six to eight mice (number of mice: sham-vehicle, n = 6; ligation-vehicle, ligation-olanzapine, n = 8). Statistical analyses were performed with Student t test. ** P = 0.0017 versus sham-vehicle. # P = 0.031 versus nerve ligation-vehicle.
Overall, the current results suggest that olanzapine may be useful for the treatment of morphine-induced emesis, reducing neuropathic pain, and improving pain-related sleep disturbance. Against a background of increasing concern about “polypharmacy,” olanzapine can be used as a single adjunct agent and can be given in a state-dependent dose, which should improve the quality of life for patients while greatly reducing the side effects of opioids.

In conclusion, we propose that treatment with olanzapine may lead to a new strategy for controlling emesis when patients are given opioid medications.

In addition, the current study provides evidence that olanzapine may be a useful agent for improving the sleep disturbance caused by neuropathic pain that is observed in some patients with cancer.

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