We investigated the effects of olanzapine on cisplatin-induced pica (the consumption of non-nutrient materials such as kaolin) and glucose homeostasis in rats to clarify the effects of olanzapine when used as an anti-emetic drug. Rats were injected intraperitoneally (i.p.) with either 5 mg/kg cisplatin or saline. Additionally, 2 or 10 mg/kg olanzapine were administered i.p. to the rats 10 min before the administration of cisplatin and subsequently administered every 24 h for 3 d. Kaolin and food intake was measured using an automatic monitoring apparatus. Plasma glucose levels were measured by an enzyme electrode method. The plasma levels of insulin and intact proinsulin were measured by enzyme-linked immunosorbent assay (ELISA). The proinsulin-to-insulin (P/I) ratio was calculated. Cisplatin significantly increased kaolin intake, but decreased food intake and body weight up to 72 h. Olanzapine had no effect on these parameters. Neither olanzapine nor cisplatin alone had a significant effect on the plasma levels of glucose, insulin, or proinsulin. However, a combination of olanzapine and cisplatin significantly decreased plasma insulin levels, but increased plasma intact proinsulin levels and the P/I ratio. Our results suggest that an additive deterioration of insulin-secreting beta-cell function and disturbance of glucose homeostasis should be considered during treatment of patients with olanzapine for cisplatin-induced nausea and vomiting.

Key words  chemotherapy-induced nausea and vomiting; olanzapine; cisplatin; pica; glucose homeostasis
MATERIALS AND METHODS

Drugs and Reagents  Cisplatin was obtained from Nippon Kayaku Co., Ltd. (Tokyo, Japan). Olanzapine was obtained from Tokyo Kasei Industry Co., Ltd. (Tokyo, Japan). The other reagents used in this study were of special grade and purchased from local suppliers, unless stated otherwise.

Animals  Male Wistar rats weighing 180–200 g were purchased from Sankyo Laboratory Service Co., Ltd. (Shizuoka, Japan). They were housed under constant conditions at a room temperature of 22±2°C and humidity of 50±10% with a regular 12-light (8:00–20:00)–dark (20:00–8:00) cycle and free access to water and food. The animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals by the Animal Research Committee of Health Sciences University of Hokkaido.

Rats were injected intraperitoneally (i.p.) with a single dose of either 5 mg/kg cisplatin or physiological saline (control). Additionally, 2 mg/kg olanzapine, 10 mg/kg olanzapine, or physiological saline was administered i.p. to the rats 10 min before the administration of cisplatin (at 13:00) and subsequently administered every 24 h for 3 d. Then, 72 h after the administration of cisplatin, the rats were killed at 13:00 by exsanguination under light anesthesia. Blood samples were collected in plastic tubes containing 500 KIU aprotinin and ethylenediaminetetraacetic acid (EDTA) disodium. Plasma was prepared from blood samples by centrifugation at 1600×g for 20 min, and the plasma levels of glucose, insulin, and intact proinsulin were measured.

Measurement of Kaolin and Food Intake  The intake of kaolin and food was measured with an automatic feeding monitoring apparatus (FDM700SW; Melquest Ltd., Toyama, Japan), as described previously. This apparatus consists of a housing cage, two containers for kaolin pellets (PMI Nutrition International, Richmond, IN, U.S.A.) and normal chow pellets (MF, Oriental Yeast, Tokyo, Japan), and a controller equipped with two weight sensors. The kaolin and food pellets were provided in their respective containers facing the housing cage. Kaolin and food intake was measured hourly to the nearest 0.01 g and the data were analyzed by computer. The results are reported as cumulative daily amounts (g) per 24-h period up to 72 h after administration.

Metabolic Measurements  Plasma glucose levels (mmol/L) were measured using an enzyme electrode method with the Precision Xceed system (Abbott, Tokyo, Japan). Plasma insulin levels (pmol/L) were measured using a commercially available rat enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Uppsala, Sweden). Specific intact proinsulin levels in plasma (pmol/L) were measured using a commercially available mouse/rat proinsulin ELISA kit (Shibayagi, Gunma, Japan). The proinsulin-to-insulin (P/I) ratio was calculated as: (intact proinsulin level/insulin level×100).29)

Statistical Analysis  Statistical analysis was performed using one-way or two-way ANOVA followed by Dunnett’s test for multiple comparisons. The p values less than 0.05 were considered significant.

RESULTS

Effects of Olanzapine on Cisplatin-Induced Changes in Kaolin, Food and Water Intake, and Body Weight  As shown in Fig. 1A, a single administration of cisplatin (5 mg/kg...
kg, i.p.) caused a significant acute (within 24 h) increase of kaolin intake. Delayed (24–72 h) kaolin intake was also observed. Corresponding to this, total kaolin intake (0–72 h) was significantly increased by cisplatin administration (Fig. 2A). Although olanzapine (2 and 10 mg/kg, i.p.) decreased the mean value of cisplatin-induced kaolin intake, the change was not significant (Figs. 1A, 2A). Cisplatin tended to decrease food and water intake in a time-dependent manner (Figs. 1B, C). In fact, total food intake (0–72 h) was significantly decreased by cisplatin administration (Fig. 2B). Total water intake (0–72 h) also tended to decrease following cisplatin administration (Fig. 2C). Olanzapine had no significant effect on water intake alone or in either the absence or presence of cisplatin (Figs. 1C, 2C). Olanzapine alone also had no significant effect on these parameters.

Cisplatin caused a slight, but significant, decrease in body weight up to 72 h, whereas body weight increased gradually in the control group (Fig. 3). Olanzapine alone tended to decrease body weight, but it was not significant. Olanzapine also had no significant effect on the cisplatin-induced decrease of body weight.

Effects of Olanzapine on the Cisplatin-Induced Change of Glucose Homeostasis As we were interested in plasma glucose, insulin, and proinsulin levels, we first analyzed food intake at 24 h before taking blood samples at 24 h before being killed. The rats were administered saline, cisplatin (5 mg/kg), olanzapine (2 mg/kg and 10 mg/kg), or the combination of cisplatin and olanzapine. Each column represents the mean±S.E.M. (n=23).

Fig. 2. Effects of Cisplatin (5 mg/kg, i.p.), Olanzapine (2 and 10 mg/kg, i.p.), and Their Combination on Total Kaolin (A), Food (B), and Water (C) Intake for 3 d in Rats

Each column represents the mean±S.E.M. (n=8 for control; n=6 for olanzapine 2 mg/kg alone, cisplatin+olanzapine 2 mg/kg, and cisplatin+olanzapine 10 mg/kg; n=4 for olanzapine 10 mg/kg alone; n=10 for cisplatin alone). *p<0.05 vs. untreated control.

Fig. 3. Effects of Cisplatin (5 mg/kg, i.p.), Olanzapine (2 and 10 mg/kg, i.p.), and Their Combination on Body Weight Changes in Rats at 72 h after a Single Administration of the Drugs or saline

Body weight at 0 h is considered to be 100%. Each column represents the mean±S.E.M. (n=8 for control; n=6 for olanzapine 2 mg/kg alone, cisplatin+olanzapine 2 mg/kg, and cisplatin+olanzapine 10 mg/kg; n=4 for olanzapine 10 mg/kg alone; n=10 for cisplatin alone). *p<0.05, **p<0.01 vs. control.

Fig. 4. Time–Course Analysis of Food Intake in Rats for 24 h before Being Killed

The rats were administered saline, cisplatin (5 mg/kg), olanzapine (2 mg/kg and 10 mg/kg), or the combination of cisplatin and olanzapine. Each column represents the mean±S.E.M. (n=23).
21:00 and 10:00, whereas almost no food intake occurred during the 3 h before blood samples were taken (i.e., at 13:00) (Fig. 4).

No significant differences in glucose levels between these groups were observed (Fig. 5A). Although either cisplatin or olanzapine alone decreased mean insulin levels, the changes were not significant (Fig. 5B). However, the combination of cisplatin and olanzapine significantly decreased insulin levels (Fig. 5B). Neither cisplatin nor olanzapine alone had an effect on plasma intact proinsulin levels (Fig. 5C). When the rats were treated with olanzapine in addition to cisplatin, intact proinsulin levels were significantly increased in a dose-dependent manner (Fig. 5C).

Finally, the P/I ratio was calculated using the intact proinsulin and insulin values. Olanzapine significantly increased the P/I ratio in a dose-dependent manner only in cisplatin-treated rats (Fig. 6).

**DISCUSSION**

In the present study, we first investigated the effects of olanzapine, an antiemetic drug, on pica behavior. Pica in rats is recognized to be analogous to emesis. A single administration of cisplatin, which is categorized as a highly emetogenic chemotherapeutic agent, caused an increase in kaolin intake in the acute and delayed phases. This is consistent with previous studies from our and other laboratories. Yamamoto et al. showed that 6 mg/kg cisplatin-induced pica was inhibited by both granisetron, a 5-HT 3 receptor antagonist, and aprepitant, a NK 1 receptor antagonist. Furthermore, we confirmed that 5 mg/kg cisplatin-induced pica is inhibited by dexamethasone (unpublished data). Moreover, we reported recently that methotrexate caused an increase in kaolin intake in the delayed, but not acute, phase. Therefore, pica behavior in rats is related to the clinical emetogenic response induced by chemotherapeutic agents. In contrast to pica behavior, cisplatin caused a gradual decrease in food and water intake as well as body weight lasting up to 72 h, suggesting impairment of quality of life.

The administration of 2 and 10 mg/kg olanzapine to rats had no effect on kaolin, food, and water intake and body weight in the absence or presence of cisplatin. The same dose of olanzapine (10 mg/daily) is used for patients with schizophrenia or those developing CINV. Although there is no report of the use of olanzapine for pica in rats, Shobo et al. showed...
that olanzapine induces a dose-dependent antipsychotic effect on amphetamine-induced hyperlocomotion in rats, with an ED$_{50}$ value of 2.2 mg/kg, i.p. Therefore, two doses of olanzapine, 2 and 10 mg/kg, i.p., were chosen in the present study. In fact, in the present study, the administration of olanzapine was also observed to cause immediate and transient sedation in a dose-dependent manner. However, it is unlikely that this sedation affects the pica behavior observed in the present study, because treatment with olanzapine had no effect on food and water intake or body weight. Sejima et al.\(^4\) reported that chronic treatment with olanzapine (2 mg/kg) for 2 weeks significantly increased both the total gain of body weight and food intake. The reason why no change was observed in body weight by the administration of olanzapine alone in the present study may be due to the shorter experimental period used compared to that used when its antipsychotic effects were examined.\(^{32,33}\) Taken together, our results suggest that treatment with olanzapine alone has no effect on cisplatin-induced pica behavior.

Olanzapine antagonizes multiple receptors such as dopamine D$_2$, serotonin 5-HT$_{2C}$, and 5-HT$_3$ receptors. Blockade of these receptors is thought to be important for its anti-emetic properties.\(^34\) The dopamine D$_2$ receptor antagonist domperidone and a therapeutic dose of metoclopramide have been shown to have no effect on cisplatin-evoked emesis in ferrets.\(^35\) Moreover, Naravi et al.\(^36\) showed that olanzapine is significantly better than metoclopramide in the control of breakthrough emesis and nausea in patients receiving HEC. Therefore, the relative contribution of these receptors to the antiemetic properties of olanzapine for better therapeutic control of CINV remains to be clarified. Tan et al.\(^12\) also showed that the addition of olanzapine to the combination of azasetron, a serotonin 5-HT$_3$ receptor antagonist, and dexamethasone significantly improved delayed CINV in patients receiving HEC as well as moderately emetogenic chemotherapy. Furthermore, olanzapine in combination with palonosetron, a 5-HT$_3$ receptor antagonist, and dexamethasone improved delayed CINV in patients receiving HEC more than in patients receiving aprepitant instead of olanzapine.\(^31\) Therefore, it is speculated that the pharmacological characteristics of olanzapine enhance the effect of the combination of a serotonin 5-HT$_3$ antagonist and dexamethasone on delayed CINV rather than decreasing delayed CINV by itself.

Olanzapine is known to induce significant weight gain\(^36,37\) and the deterioration of glucose homeostasis in schizophrenia patients.\(^38\) This deterioration in glucose homeostasis was accompanied by an increase in insulin resistance.\(^38,39\) Although the plasma levels of glucose and insulin were not fasting levels in the present study, we confirmed that there was almost no food intake during at least 3 h before collecting the blood samples in rats, probably due to the time at which we collected the samples (i.e., 13:00, in the middle of the sleeping phase of rats). Increased plasma intact proinsulin levels and P/I ratio were observed with the combination of cisplatin and olanzapine. These results suggest that the deterioration of beta-cell function is accelerated by this combination, since elevated intact proinsulin levels and P/I ratio are indicators of beta-cell dysfunction and predictors of the progression of insulin resistance.\(^40,41\)

Cisplatin is known to inhibit glucose uptake in various cells.\(^42,43\) In fact, cisplatin rapidly decreases the function of glucose transporter 1 by changing its intracellular localization in ovarian cancer cells.\(^44\) This cisplatin-dependent acute decrease in the removal of glucose from the blood system might increase the risk of hyperglycemia by the administration of olanzapine. Thus, sustained disturbances in insulin secretion might induce further increments in glucose levels during the administration of olanzapine with cisplatin. On the other hand, regardless of the deterioration of beta-cell function caused by the combination of olanzapine and cisplatin in the present study, this combination did not cause a significant impact on glucose levels. In phase III trials, Tan et al.\(^12\) and Navari et al.\(^13\) showed that olanzapine had no effect on blood glucose levels. However, the detailed effects of olanzapine on beta-cell function are unknown. Increased intact proinsulin levels may be one reason for the unchanged glucose levels observed in the present study, since intact proinsulin still has 10–20% of the glucose-lowering effect of insulin.\(^45\) Thus, it might be difficult to observe the assumed beta-cell dysfunction induced by the combination of cisplatin and olanzapine by examining changes in plasma glucose levels. However, it can be speculated that the administration of olanzapine at a high dose or for more than 72 h causes an increase in glucose levels. It is unknown whether the impaired glucose tolerance observed in the present study is reversible, which raises a concern about the use of olanzapine as an antiemetic for diabetic patients who receive cisplatin. Further studies are required to elucidate the effects of olanzapine and cisplatin on glucose homeostasis using diabetic rat models and tumor-bearing rats to establish the safe use of olanzapine as an antiemetic for CINV. Taken together with these considerations, it should still be considered that the administration of olanzapine for cisplatin-induced nausea and vomiting can cause deterioration in the function of insulin-secreting beta-cells and disturbances in glucose homeostasis, accompanied by a change in glucose values.

In conclusion, the administration of olanzapine alone had no effect on cisplatin-induced pica behavior in rats. Further studies are required to clarify the precise mechanism of the suppression of CINV by olanzapine. The combination of olanzapine and cisplatin caused a deterioration of beta-cell function. Additive deterioration of insulin-secreting beta-cells and disturbances in glucose homeostasis should be considered during the treatment of patients with olanzapine for cisplatin-induced nausea and vomiting.

**Conflict of Interest** The authors declare no conflict of interest.

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