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The anti-cancer drug-induced pica in rats is related to their clinical emetogenic potential

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Abstract

Cancer chemotherapy is frequently accompanied by severe emesis. The anti-cancer drugs are classified according to their clinical emetogenic potential. We have already found that kaolin ingestion behavior "pica" is analogous to emesis in rats. The aim of this study was to examine the effects of the clinical emetogenic potential of anti-cancer drugs on the induction of the pica in rats. Rats were housed in individual cages with free access to food and kaolin pellets and the daily food and kaolin intakes were measured for 3 days after the intraperitoneal administration of anti-cancer drugs (cisplatin, cyclophosphamide, actinomycin D, 5-fluorouracil and vincristine). The drugs with high potential for inducing emesis, such as cisplatin and cyclophosphamide, induced pica in all animals on the day of administration and the behavior lasted during the observation period. The drugs with moderate emetogenic potential, i.e. actinomycin D and 5-fluorouracil, also induced pica on the first and second day after the drug administration but the kaolin intake was less than that of the drugs with high potential. Vincristine, a drug with low emetogenic potential, slightly increased the kaolin intake in rats on the only first day of the administration. Cyclophosphamide, actinomycin D and vincristine induced anorexia and decreased their body weight during the observation period. These results suggested that the both amounts of kaolin intake and duration of behavior in the anti-cancer drug-induced pica are related to the clinical emetogenic potential of the drugs and the incidence of the anorexia is not related to their emetogenic potential.

Keywords: Anti-cancer drugs; Emetognic potential; Emesis; Pica; (Rat)

1. Introduction

During the course of cancer chemotherapy, patients are distressed by severe emesis and insufficient control of this troublesome symptom impairs the patients' quality of life and leads their refusing the continuation of the therapy.

Anti-cancer drugs-induced emesis is divided into two classes: (1) acute emesis, which occurs within 24 h following therapy, and (2) delayed emesis, which persists for several days after therapy (Jordan et al., 2005; Kris et al., 1998; Veyrat-Follet et al., 1997; ASHP Therapeutic Guidelines, 1999). The pharmacological management of this symptom should be based on the underlying etiology.

Not all of the anti-cancer drugs have the same potential to induce the acute and delayed emesis, and the emetogenic potential of each drug is determined by the incidence and severity of the drug-induced emesis. Cisplatin induces severe acute emesis in more than 95% of patients who do not receive prophylaxis agents, and the emesis lasts for a few days. On the other hand, vincristine induces emesis in less than 10% of patients (Hesketh et al., 1997; Grunberg et al., 2005; Schnell, 2003). To assess the emetogenic potential of the anti-cancer drugs, animals that vomit in response to the drugs, such as ferrets, piglets, cats, *Suncus murinus* and pigeons, have been generally utilized in preclinical studies (King, 1990; Rudd and Naylor, 1996; Milano et al., 1995; Matsuki et al., 1988; Tanihata et al., 2000). Rats, one of the most common laboratory animal species, are known to be a species that does not vomit, but Takeda et al. and we have previously reported that kaolin ingestion behavior "pica" in rats was analogous to gastrointestinal discomfort, such as emesis

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(Takeda et al., 1993; Yamamoto et al., 2002, 2004). We also have reported that cisplatin actually induces acute and delayed pica in rats (Rudd et al., 2002).

In this study, we selected five anti-cancer drugs with different emetogenic potentials, cisplatin and cyclophosphamide as drugs with high, actinomycin D and 5-fluorouracil as drugs with moderate, and vincristine as a drug with a low emetogenic potential, and compared their potential to induce acute and delayed pica in rats.

2. Materials and methods

2.1. General procedure

Male Wistar rats (200–270 g) were obtained from Charles River Japan (Yokohama, Japan) and were housed in individual home cages (25 cm × 20 cm × 20 cm) in a room with a regular light/dark cycle (lights on 7:00–19:00) at a constant temperature (approximately 24 °C) and humidity (approximately 50%). They were allowed free access to water, standard laboratory chow (MF, Oriental Yeast, Osaka, Japan) and kaolin pellets during the habituation period. At the end of the experiment, the animals were sacrificed by an intraperitoneal (i.p.) injection of an excessive dose of sodium pentobarbital. Animals were not used more than once. Kaolin pellets were prepared according to a previously reported method (Yamamoto et al., 2004). Briefly, pharmaceutical grade kaolin (hydrated aluminum silicate) was mixed with 1% (w/w) gum Arabic in distilled water to form similar size to chow pellets and then these pellets were completely dried at room temperature. The kaolin and food pellets were provided in their respective stainless steel containers (5 cm × 5 cm × 10 cm) placed in the home cage.

On the day of the experiment, the rats received an i.p. injection of cisplatin (1 and 3 mg/kg), cyclophosphamide (60 and 120 mg/kg), actinomycin D (0.13 and 0.25 mg/kg), 5-fluorouracil (10 and 35 mg/kg) or vicristine (0.1 and 1 mg/kg) at 1830 h. The higher doses of these anti-cancer drugs were selected from half the 50% lethal dose of rats. The controls were treated with vehicles in each drug regimen. The number of rats used in each group was 4.

Their daily kaolin intake, food consumption and body weight were measured on the day before and for 3 days after receiving the respective anti-cancer drugs. Spilt kaolin and food were collected and weighed to correct the actual consumption. Food and kaolin consumption were calculated to the nearest 0.01 g. All experiments and protocols were approved and conducted in accordance with the Animal Care Committee of Faculty of Medicine, Osaka University.

2.2. Drugs

Gum Arabic and kaolin were obtained from Sigma–Aldrich Japan (Tokyo, Japan). Cisplatin, cyclophosphamide (Sigma–Aldrich, St. Louis, MO, USA) and vincristine (Wako, Osaka, Japan) were dissolved in physiological saline. Actinomycin D (Wako) and 5-fluorouracil (Sigma–Aldrich) were dissolved in distilled water containing 0.5% (v/v) dimethyl sulfoxide (DMSO; Sigma–Aldrich). Doses are expressed as the free base.

2.3. Statistical analysis

Data are represented as the mean values ± S.E.M. Kaolin and food consumption were compared between the doses of respective chemotherapeutic agents using a two-way repeated-measures analysis of variance (ANOVA) followed by Dunnett multiple comparison tests. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Food and kaolin consumption in vehicle-treated rats

Some rats ate approximately 0.5 g of kaolin on the first day of the habituation period, but none of animals ate any kaolin during the subsequent days. The control animals ate approximately 23 g of food and a small amount of kaolin (range

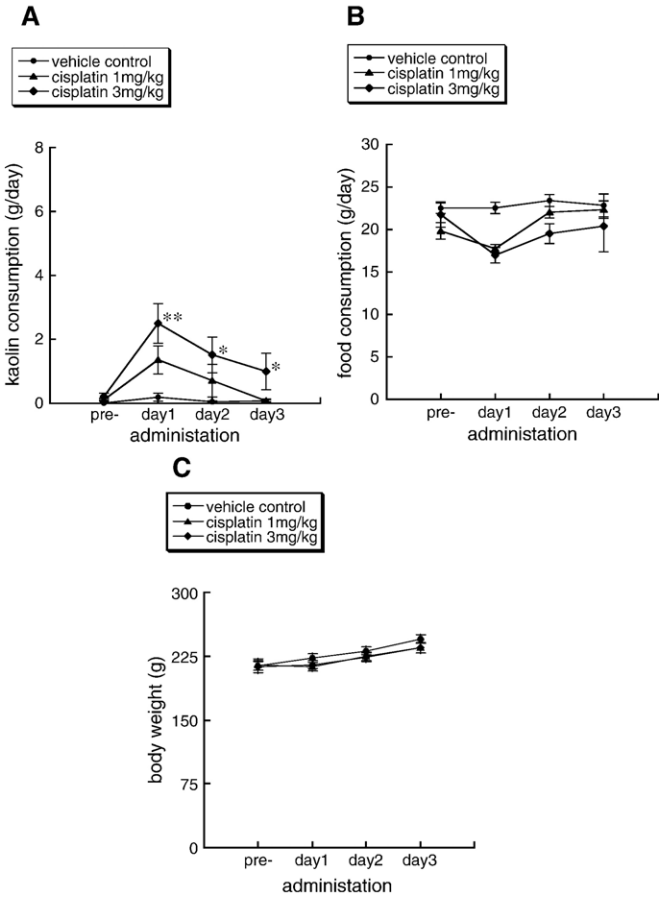

Fig. 1. Effects of cisplatin on (A) kaolin, (B) food intake and (C) body weight in rats. Cisplatin (1 or 3 mg/kg) and vehicle were intraperitoneally administered. Points and bars represent the means ± S.E.M. of the kaolin and food consumption of each day. The data were analyzed for any significant differences using the two-way repeated-measures analysis of the variance (ANOVA), followed by *post hoc* Dunnett multiple comparison tests. *$P<0.05$ and **$P<0.01$ vs. vehicle.

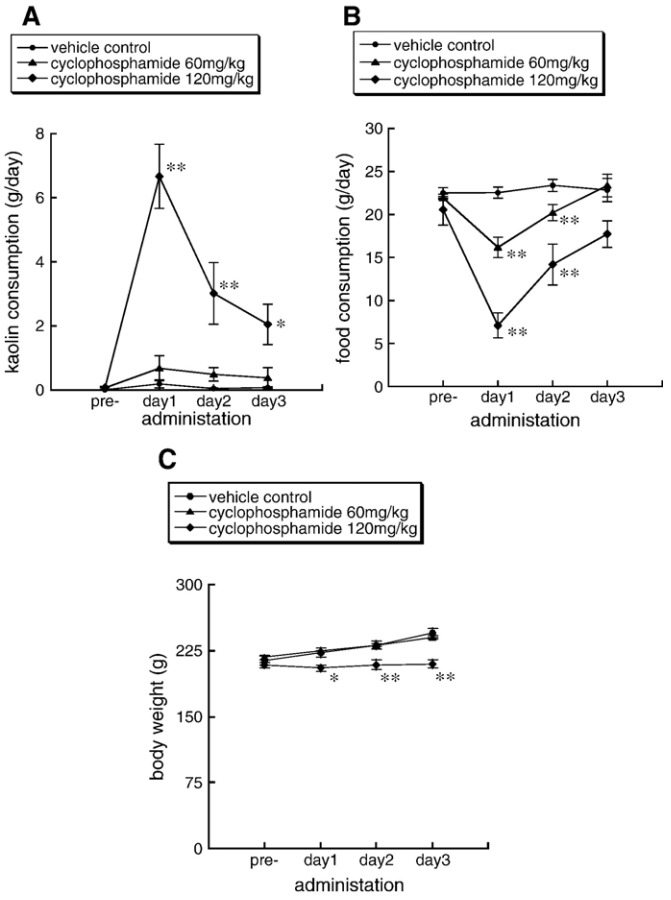

Fig. 2. Effects of cyclophosphamide on (A) kaolin, (B) food intake and (C) body weight in rats. Cyclophosphamide (60 or 120 mg/kg) and vehicle were intraperitoneally administered. Points and bars represent the means±S.E.M. of the kaolin and food consumption of each day. The data were analyzed for any significant differences using the two-way repeated-measures analysis of the variance (ANOVA), followed by *post hoc* Dunnett multiple comparison tests. *$P<0.05$ and **$P<0.01$ vs. vehicle.

0–0.3 g) in the 24 h period after the administration of the respective vehicles.

3.2. Food and kaolin consumption in cisplatin or cyclophosphamide-treated rats

As shown in Fig. 1A, the kaolin consumption after the administration of cisplatin was significantly and dose-dependently increased on the first day of the drug administration ($F(6,27)=4.698$, $P<0.01$). Cisplatin at 1 mg/kg induced the eating of 1.5 g of kaolin in 2 of 4 animals; on the other hand, cisplatin at 3 mg/kg induced the eating of 3 g of kaolin in 3 of 4 animals. The pica induced by both doses of cisplatin lasted throughout the entire observation period. As shown in Fig. 1B, the food consumption was slightly decreased after the administration of both doses of cisplatin, but the consumption was returned to the control level on the third day after the administration ($F(6,27)=0.659$, $P>0.05$). As shown in Fig. 1C, both doses of cisplatin did not affect the body weight of rats for the duration of the observation period ($F(6,27)=2.305$, $P>0.05$).

Cyclophosphamide at a dose of 120 mg/kg induced kaolin intake to more than 3 g and reduced food consumption to less than 10 g in all rats, and the body weight was significantly decreased during the observation period (Fig. 2A, B, C) (kaolin intake: $F(6,27)=15.80$, $P<0.01$; food intake: $F(6,27)=12.73$, $P<0.01$; body weight: $F(6,27)=14.72$, $P<0.01$). The increased kaolin consumption and the decreased food consumption were observed throughout the observation period. The dose of cyclophosphamide of 60 mg/kg had a significant effect of decreasing food consumption but did not change the kaolin intake or body weight (Fig. 2A, B, C).

3.3. Food and kaolin consumption in actinomycin D or 5-fluorouracil-treated rats

The kaolin consumption induced by actinomycin D or 5-fluorouracil was also significantly increased on the first day of the drug administration (actinomycin D: $F(6,27)=2.673$, $P<0.05$; 5-fluorouracil: $F(6,27)=2.573$, $P<0.05$). Three of 4 rats that received actinomycin D at a dose of 0.25 mg/kg (Fig. 3A) and 2 of 4 rats that received 5-fluorouracil at a dose of 35 mg/kg (Fig. 4A) ate 1 g of kaolin during the next 24 h of

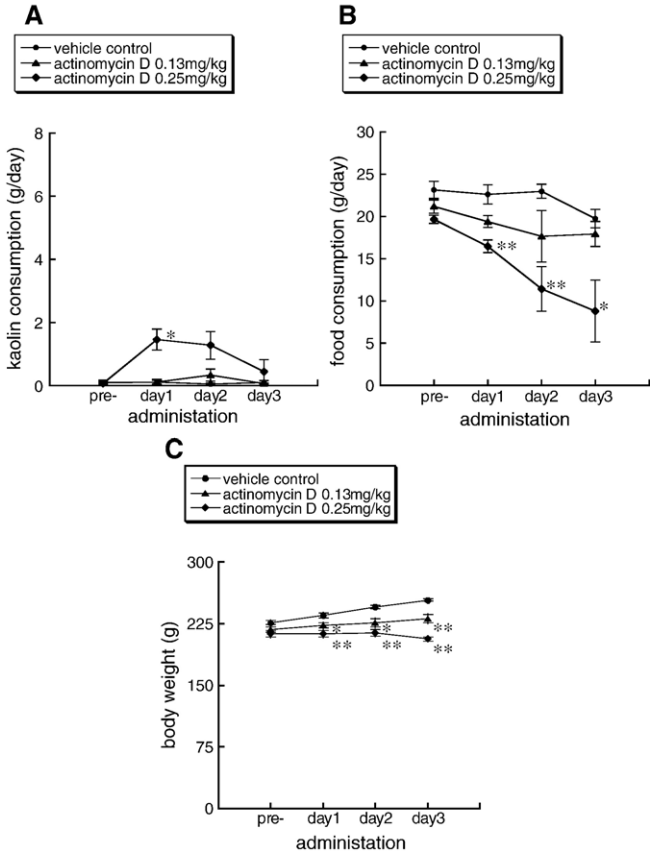

Fig. 3. Effects of actinomycin D on (A) kaolin, (B) food intake and (C) body weight in rats. Actinomycin D (0.13 or 0.25 mg/kg) and vehicle were intraperitoneally administered. Points and bars represent the means±S.E.M. of the kaolin and food consumption of each day. The data were analyzed for any significant differences using the two-way repeated-measures analysis of the variance (ANOVA), followed by *post hoc* Dunnett multiple comparison tests. *$P<0.05$ and **$P<0.01$ vs. vehicle.

administration. Some rats that received actinomycin D but not 5-fluorouracil showed pica on the second day, while none of rats ate any kaolin on the third day.

The lower dose of actinomycin D or 5-fluorouracil did not induce any change of the food consumption or body weight during the observation period (Figs. 3B, C, 4B and C), but the higher dose of actinomycin D induced a significant decrease of food consumption on the second and third day (actinomycin D: $F(6,27)=4.006$, $P<0.01$; 5-fluorouracil: $F(6,27)=1.406$, $P>0.05$). The body weight of rats that received both doses of actinomycin D was significantly reduced during the observation periods, but that of 5-fluorouracil was not affected (actinomycin D: $F(6,27)=12.20$, $p<0.01$; 5-fluorouracil: $F(6,27)=1.479$, $P>0.05$).

3.4. Food and kaolin consumption in vincristine-treated rats

As shown in Fig. 5A, kaolin consumption after the administration of vincristine at the dose of 1 mg/kg slightly increased kaolin consumption in some rats, but no rats ate more than 1 g of kaolin ($F(6,27)=1.239$, $P>0.05$). Food consumption was decreased after administration, but there were no significant differences between the vehicle control animals and the vincristine-treated rats (Fig. 5B) ($F(6,27)=1.906$, $P>0.05$). As shown in Fig. 5C, the higher dose of vincristine significantly induced a decrease of body weight ($F(6,27)=4.326$, $P<0.05$).

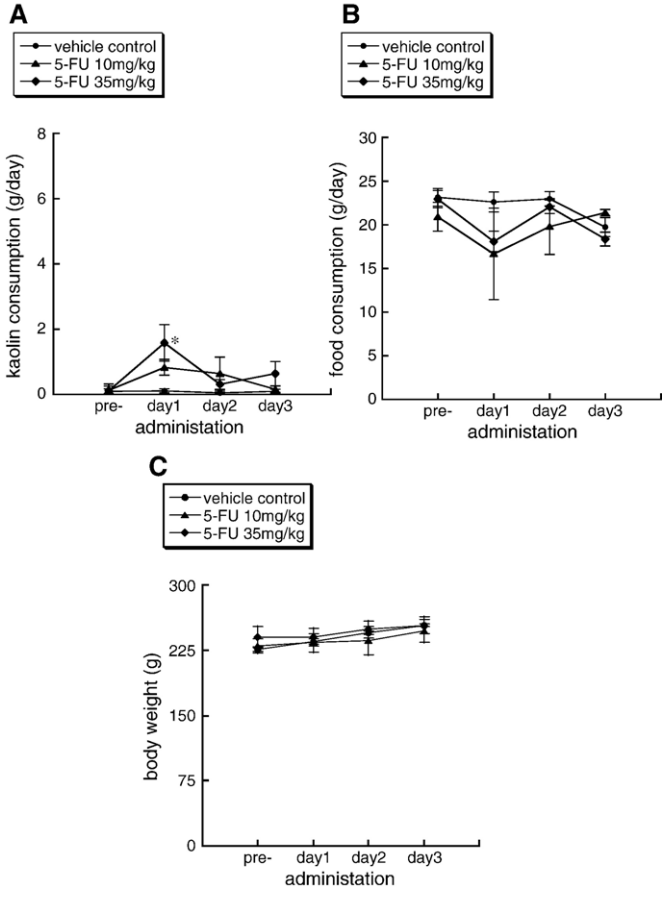

Fig. 4. Effects of 5-fluorouracil on (A) kaolin, (B) food intake and (C) body weight in rats. Five-fluorouracil (10 or 35 mg/kg) and vehicle were intraperitoneally administered. Points and bars represent the means±S.E.M. of the kaolin and food consumption of each day. The data were analyzed for any significant differences using the two-way repeated-measures analysis of the variance (ANOVA), followed by *post hoc* Dunnett multiple comparison tests. *$P<0.05$ vs. vehicle.

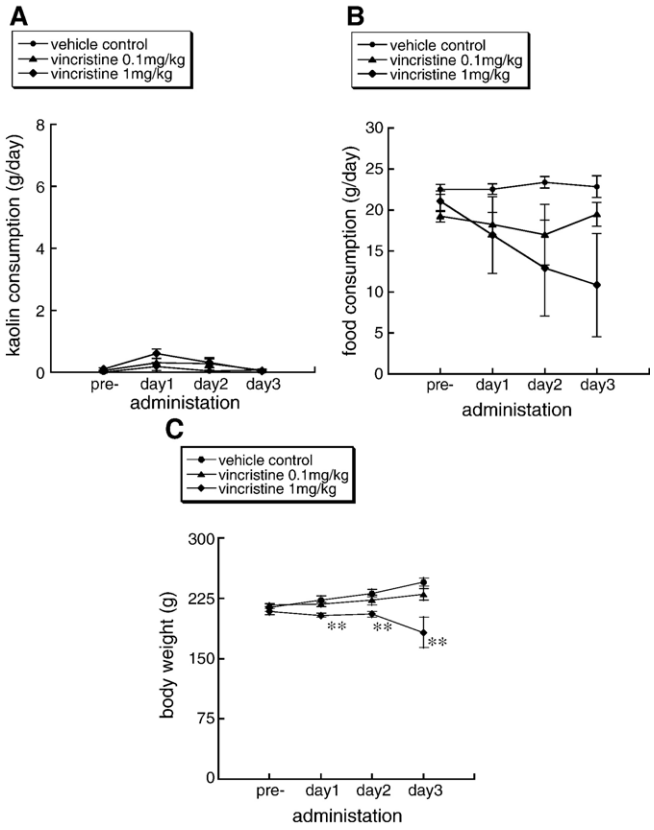

Fig. 5. Effects of vincristine on (A) kaolin, (B) food intake and (C) body weight in rats. Vincristine (0.1 or 1 mg/kg) and vehicle were intraperitoneally administered. Points and bars represent the means±S.E.M. of the kaolin and food consumption of each day. The data were analyzed for any significant differences using the two-way repeated-measures analysis of the variance (ANOVA), followed by *post hoc* Dunnett multiple comparison tests. **$P<0.01$ vs. vehicle.

4. Discussion

Defining the emetogenic potential of the anti-cancer drugs is essential to establish the anti-emetic guidelines in cancer chemotherapy. After the proposal of classification of chemotherapeutic agents according to their clinical emetogenic potential by Hesketh et al. (1997), the chemotherapeutic agents have been divided into several categories of emetogenic levels as follows (Grunberg et al., 2005; Schnell, 2003). The “high” emetogenic agents, including cisplatin, cyclophosphamide, streptozotocin and dacarbazine, produce severe emesis in more than 90% of the patients. The “moderate” emetogenic agents, including actinomycin D, ifosamide and doxorubicin, cause vomiting in 30% to 90% patients. The “low” emetogenic

agents, including etoposide, mitomycin and 5-fluorouracil, induce emesis in 10 to 30% patients. Finally, the "minimal" emetogenic agents, such as vincristine and vinblastine, induce emesis in less than a 10% of patients. In the present study, we demonstrated that the agents with a "high" potential for inducing emesis, cisplatin and cyclophosphamide, induced pica in all animals, while the agent with "minimal" potential, vincristine did not induce it. We also found that the agents with "moderate" and "low" emetogenic potential, actinomycin D and 5-fluorouracil, caused pica in some rats, but the kaolin consumptions were lower than those with cisplatin and cyclophosphamide. The number of rats taking more than 1 g of kaolin was 4, 4, 3, 2 and 0 out of 4 rats that received cisplatin, cyclophosphamide, actinomycin D, 5-fluorouracil and vincristine, respectively. The rank order of the potential of pica in this study is cyclophosphamide ≥ cisplatin > actinomycin D = 5-fluorouracil > vincristin, suggesting that the chemotherapeutic agent-induced pica in rats is closely related to the clinical emetogenic potential of the agents. Previous studies demonstrated that the incidence of cisplatin or cyclophosphamide-induced vomiting is more than that of 5-fluorouracil-induced vomiting in other experimental animal species, such as dogs and *S. murinus* (Fukui et al., 1993; Matsuki et al., 1988). Their results indicated that chemotherapeutic agent-induced pica in rats is comparable to the chemotherapeutic agent-induced emesis in most experimental animals.

The duration of anti-cancer drug-induced emesis is also dependent on the drugs. Most of the drugs induce only acute emesis, defined as occurring within 24 h after the administration (Grunberg et al., 2005). On the other hand, cisplatin and cyclophosphamide induce not only acute emesis, but also delayed emesis, defined as occurring 24 h after treatment (ASHP Therapeutic Guidelines, 1999; Grunberg et al., 2005). The delayed emesis is a less severe event than acute emesis, but there are no satisfactory effective drugs to ameliorate the symptom, and it may impair the quality of life of the patients (Gregory and Ettinger, 1998; Olver et al., 1996). In this study, we demonstrated that the pica induced by cisplatin and cyclophosphamide was observed over the 3 days following the administration, while the behavior induced by actinomycin D and 5-fluorouracil occurred on only the first and second day. These results suggest that the duration of anti-cancer drug-induced pica in rats is also related to the potential to induce the acute and delayed emesis.

The chemotherapy-induced anorexia is generally accompanied by emesis, because anti-emetic drugs were used for the prevention and treatment of the symptom (Efficace et al., 2004; Inoue et al., 2003; Feng et al., 2002). However, the anorexia often occurred in the patients that received the drug with low emetogenic potential (Ishii et al., 1988), suggesting that the incidence of the anorexia is not related to the emetogenicity. In this study, we observed that all the rats, even in rats that received the anti-cancer drugs with "low" emetogenic potential, lower doses of cyclophosphamide, 5-fluorouracil and vincristine, decreased their food consumption. Clinical reports suggested that cancer chemotherapeutic agents induced the gastric motor dysfunction and resulted in the alteration of gastric emptying, dyspepsia, satiety and anorexia (Nelson et al., 2002). Thus, the intraperitoneal administration of the cytotoxic agents might cause bowel irritation and abnormalities in gastrointestinal motility. Further experiments will be needed to elucidate the etiology of anti-cancer drug-induced anorexia.

These results suggested that both amount of kaolin intake and the duration of behavior in the anti-cancer drug-induced pica are related to the clinical emetogenic potential of the drugs. The pica in rats is useful to evaluate the emetogenic potential of drugs in preclinical studies.

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