Establishment of an Animal Model for Radiation-induced Vomiting in Rats Using Pica

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We investigated whether radiation-induced pica, a behavior characterized by the eating of a non-food substance, such as kaolin, can be used as an index of radiation-induced vomiting in rats. Since there was an individual difference in the susceptibility to pica, we selected rats that actually ate kaolin following X-ray irradiation, and used them for the experiment. The total-body irradiation (TBI) increased kaolin consumption in a dose-dependent manner (sham, 0.05 ± 0.03 (SEM) g; 2 Gy, 0.38 ± 0.11 g; 4 Gy, 1.54 ± 0.28 g; 8 Gy, 3.55 ± 0.67 g), and the increased kaolin consumption after 4 Gy of TBI was inhibited by a pretreatment with the serotonin 5-HT³ receptor antagonist ondansetron (2 mg/kg, i.p.) (saline, 1.49 ± 0.33 g; ondansetron, 0.75 ± 0.11 g). Furthermore, 4 Gy of abdominal irradiation was more effective to induce pica than that of head irradiation (abdomen: 0.37 ± 0.05 g, head: 0.06 ± 0.01 g). These findings suggested that peripheral serotonergic pathway is predominantly involved in the development of radiation-induced pica in rats and that the radiation-induced pica could be useful as a behavioral index for the severity of radiation-induced vomiting in rats.

INTRODUCTION

Radiation therapy is well established as one of the most important modalities of cancer therapy. During the course of radiation, patients often experience several kinds of adverse effects. Gastrointestinal symptoms, such as nausea and vomiting, are frequently accompanied by exposure to a dose of radiation greater than 1 Gy¹–³. These symptoms, with a latent period of 40 to 90 min following irradiation, rarely result in lethality, but they may impair the quality of life and lead to a refusal to continue the therapy. Several clinical trials have revealed that patients receiving total body irradiation (TBI) or upper-abdominal irradiation often show vomiting³,⁴), and that serotonin 5-HT³ receptor antagonists, such as ondansetron, granisetron and tropisetron, are effective in both prevention and treatment³,⁵,⁶). These clinical findings indicate that although the peripheral serotonergic pathway is predominantly involved in the development of radiation-induced vomiting, the precise etiology is unknown.

To investigate radiation-induced vomiting in experimental animals, species that vomit in response to radiation have been utilized, such as ferrets, dogs, monkeys, cats and Suncus⁷–¹³). In contrast, rats, which are the most common experimental animal, were not used because they do not vomit. However, Mitchell et al. reported that several stimuli that commonly cause emesis in humans, induced pica, i.e. an eating disorder that involves the satisfaction of a craving by eating a...
non-food substance, such as soil or kaolin, in rats\textsuperscript{14}. We previously reported that pica is an index of emesis in rats, and established a rat model in motion sickness using motion-induced pica\textsuperscript{15–18}. Moreover, recent study has shown that although X-ray irradiation increased kaolin consumption in rats\textsuperscript{19}, there has been no study on whether pica could be used as a behavioral index of radiation-induced vomiting. In the present study, we examined the effect of total-body or regional (abdomen or head) X-ray irradiation on pica and the efficacy of ondansetron on the inhibition of radiation-induced pica.

**MATERIALS AND METHODS**

**Susceptibility to pica in rats**

Eight-week-old male Wistar rats, weighing about 180 g at the beginning of the experiment, were obtained from Japan SLC (n = 36, Shizuoka, Japan). Throughout the experiment, all of the rats were housed in individual home cages (23 cm \( \times \) 23 cm \( \times \) 20 cm) in a room with a 12 h light/12 h dark cycle (light on 7:00–19:00) at a constant temperature (23 ± 1°C) and humidity (50 ± 5%), and had free access to standard laboratory chow pellets (MF, Oriental Yeast, Osaka, Japan), water and kaolin pellets. Kaolin pellets were prepared according to a previously reported method\textsuperscript{16}. Briefly, pharmaceutical-grade kaolin (hydrated aluminum silicate; Katayama Chemical, Osaka, Japan) was mixed with 1% gum arabic (Katayama Chemical) in distilled water to form the same shape of chow pellets, and dried at room temperature. The kaolin and chow pellets were provided in respective stainless-steel containers (7 cm \( \times \) 8 cm \( \times \) 3 cm) placed in a home cage. Between 13:00–16:00 each day the kaolin and food consumption were measured to the nearest 0.01 g. Splinters were collected and weighed to correct the values of these consumptions.

The irradiation schedule for this experiment is shown in Fig. 1. After adaptation to the experimental environment for three days, the rats were transferred to an acrylic cage (14 cm \( \times \) 29 cm \( \times \) 15 cm) and exposed to 4 Gy of 4 MV X-ray irradiation to the total body using a medical linear accelerator (EXL-6SP, Mitsubishi Electric, Tokyo, Japan) to screen the susceptibility to radiation-induced pica (“screening irradiation”). We selected susceptible rats that ate more than 0.15 g of kaolin during the next 24 hours. Six days after the screening irradiation, the susceptible rats (n = 31) were used for experimental irradiation.

**Effects of total body irradiation on pica in rats**

On the day of the experiment, the rats were
restrained individually in an acrylic holder (diameter: 6 cm), and then received TBI at a single 2, 4 or 8 Gy (n = 3: each group) dose of 4 MV X-rays at a dose rate of 1.5 Gy/ min delivered at a depth of 4 cm with a source target distance of 100 cm using a linear accelerator. Sham irradiation (n = 3) as a control was performed by placing the rats in an irradiation room surrounded by 10 cm lead shield bricks. The tissue maximum ratio (TMR) value of this point was determined as to be 0.937. Their daily kaolin and food consumption were measured for the next five days after irradiation.

Effect of ondansetron on radiation-induced pica in rats

The method was identical to that of the 4 Gy of the TBI experiment, except that ondansetron (2 mg/kg; n = 5, GlaxoSmithKline, Tokyo, Japan) was intraperitoneal (i.p.) injected 30 min before irradiation. Control animals (n = 4) received i.p. saline.

Effect of abdominal and head irradiation on pica in rats

The method was the same as that used in the 4 Gy of the TBI experiment, except that the irradiated fields were limited to the abdomen (n = 5) or head (n = 5). Head irradiation was delivered at a depth of 2 cm. The TMR values were determined to be 0.923 and 0.984 for the abdomen and head, respectively.

Data analysis

The data were represented as the mean ± S.E.M. of kaolin and food consumption and analyzed for any significant differences using a two-way analysis of the variance (ANOVA), followed by post hoc Newman-Keuls multiple comparison tests. Values of p < 0.05 were regarded as being significantly different.

RESULTS

Susceptibility of pica in rats

Figure 2 shows the kaolin consumption of the susceptible and non-susceptible rats, respectively. The susceptible rats (31/36) that took more than 0.15 g of kaolin after the screening irradiation ate significant amounts of kaolin after the experimental irradiation (sham, 0.05 ± 0.03 g; 2 Gy, 0.38 ± 0.11 g; 4 Gy, 1.54 ± 0.28 g; 8 Gy, 3.55 ± 0.67 g; F(3,8) = 23.27; p < 0.01) and the increased consumption in 8 Gy irradiated rats lasted for a few days after the experimental irradiation. In contrast, as shown in Fig. 3B, food consumption was significantly decreased after the TBI (sham, 17.08 ± 0.45 g; 2 Gy, 9.46 ± 0.87
and the decreased consumption lasted during the observation period. Sham-irradiated rats did not show any significant changes in kaolin and food consumption during the observation period.

Fig. 3. Effect of total body irradiation (TBI) on (A) kaolin and (B) food consumption in rats (sham (○): n = 3, 2 Gy (△): n = 3, 4 Gy (□): n = 3, 8 Gy (♦): n = 3). The points and bars represent the mean ± S.E.M. of the daily consumption. * p < 0.05, ** p < 0.01 vs. sham irradiation group.

Fig. 4. Effect of ondansetron on (A) kaolin and (B) food consumption after total body irradiation (TBI). Ondansetron (△: 2 mg/kg, n = 5) and saline (○: n = 4) were injected 30 min before the 4 Gy of TBI. The points and bars represent the mean ± S.E.M. of the daily consumptions. * p < 0.05, ** p < 0.01 vs. saline group.
Effect of ondansetron on radiation-induced pica in rats

Susceptible rats administered intraperitoneal saline and ondansetron did not show pica (saline, $0.01 \pm 0.01$ g; ondansetron, $0.01 \pm 0.01$ g). As shown in Fig. 4A, the 4 Gy of experimental TBI-induced pica was significantly suppressed by a pretreatment with ondansetron compared with the control (saline, $1.43 \pm 0.24$ g; ondansetron, $0.75 \pm 0.11$ g; $F(1,7) = 42.00; p < 0.01$). However, the premedication with ondansetron did not affect the decreased food consumption (control, $9.55 \pm 0.81$ g; ondansetron, $10.54 \pm 0.35$ g; $F(1,7) = 0.80; p = 0.40$) (Fig. 4B).

Effect of abdominal and head irradiation on pica in rats

Figure 5A shows that kaolin consumption significantly increased after abdominal irradiation ($0.40 \pm 0.05$ g) compared to that after the head irradiation ($0.06 \pm 0.01$ g; $F(1, 8) = 15.86; p < 0.01$). However, the decreased food consumption was independent of the irradiation fields (abdomen, $9.94 \pm 1.02$ g; head, $11.53 \pm 0.58$ g; $F(1,8) = 0.92; p = 0.37$) (Fig. 5B).

DISCUSSION

In this study, we observed an individual difference in the susceptibility to pica induced by X-ray irradiation in rats (Fig. 2). The non-susceptible rats receiving screening irradiation never ate kaolin after the experimental irradiation. Hasegawa et al. suggested that susceptibility to pica is related to the difference in the sensitivity of the emetic center, because the rats that ate no kaolin after rotation did not show pica upon the administration of apomorphine or copper sulfate$^{20}$. Thus, to evaluate radiation-induced vomiting in rats using pica behavior, the selection of susceptible rats prior to the experiment was needed.

The etiology of radiation-induced vomiting has been postulated to be due to released serotonin (5-HT) from enterochromaffin cells in the mucosa of the upper gastrointestinal tract that activates the vomiting center, such as the area postrema and the nucleus of the solitary tract (NTS), by depolarization of the vagal afferents via 5-HT$_3$ receptors, which initiates an emetic reflex$^{3,21,22}$.
tion-induced emesis is higher in patients receiving TBI or upper-abdominal irradiation than head and neck or breast irradiation\(^{4,23}\). Furthermore, the urinary excretion of 5-hydroxyindoleacetic acid, a metabolite of 5-HT, increases in patients who suffer from radiation-induced emesis\(^{24}\) and 5-HT\(_3\) receptor antagonists, such as ondansetron, granisetron and tropisetron, are effective in the management of the symptom\(^ {5,6}\). In the present study, we demonstrated that TBI caused pica in rats (Fig. 3A), that a pretreatment with ondansetron could suppress the increased kaolin consumption (Fig. 4A) and that abdominal irradiation was more effective to induce pica than head irradiation (Fig. 5A). Pentila \textit{et al}. demonstrated that the 5-HT content in the gastrointestinal tract of rats was decreased by TBI\(^ {25}\). Furthermore, Yamada \textit{et al}. demonstrated that the expression of Fos protein, the product of the \textit{c-fos} gene which is used as a marker of neuronal activity at the single-cell level, was significantly increased in the NTS of rat brain after TBI and abdominal irradiation, and that the increased Fos-positive neurons after TBI were suppressed by vagotomy at the subdiaphragmatic level and by premedication with tropisetron\(^ {20}\). They suggested that the NTS of the total body or abdominal irradiated rats is activated by the vagal afferents via the 5-HT\(_3\) receptors. These findings suggested that pica induced by X-ray irradiation was similar to radiation-induced vomiting in humans with respect to activation of the serotonergic pathway in the gastrointestinal tract.

Because dogs, ferrets, cats, monkeys and \textit{Suncus} also experience an emetic response to radiation, these species have been used as animal models for radiation-induced vomiting\(^ {7–13}\). The sensitivity of radiation-induced vomiting varies among species. For example, in humans, most exposed cases at doses of 4 Gy experience emesis\(^ {2,3}\). King reported that ferrets are more sensitive to radiation because their threshold for vomiting was 0.7 Gy and a single dose of 2 Gy was sufficient to elicit a 100% response\(^ {10}\). In dogs, the dose which caused radiation-induced emesis in 100% of animals was 8 Gy\(^ {8,9}\). Monkeys, \textit{Suncus} and cats are more resistant to radiation than humans. At doses of 5 Gy of TBI emesis occurs in about 50% of monkeys and \textit{Suncus}\(^ {12,13}\), but in only 33% of cats\(^ {7}\). In the present study, the 4 Gy of screening irradiation induced pica in 86% of rats. This suggests that the dose that causes radiation-induced pica in rats is comparable to that of radiation-induced vomiting in most experimental animals.

Anorexia also occurs frequently in patients receiving radiation therapy and experimental animals\(^ {3,26}\) and causes discomfort. In this study, we observed rats exposed to X-rays significantly decreased their food consumption (Fig. 3B, 5B) and that the administration of ondansetron showed no effects on TBI-induced anorexia (Fig. 4B), indicating that anorexia is probably not concerned with the serotonergic pathway. Although previous studies reported that cholecystokinin\(^ {27}\) and thyroid hormones\(^ {28}\) may contribute to radiation-induced anorexia, the etiology is currently uncertain. Further experiments are required to elucidate the exact mechanism of this symptom.

In conclusion, we found that total-body and abdominal irradiation caused pica in rats, and that TBI-induced pica was inhibited by a pretreatment with ondansetron. These findings suggested that serotonergic pathways in the gastrointestinal tract are involved in the development of radiation-induced pica in rats, which is similar to the etiology of radiation-induced vomiting in humans. Thus, rats could be useful for studying radiation-induced vomiting by determining their kaolin consumption as an index of the severity of the symptom. This animal model could facilitate further studies to elucidate the neural mechanism of the symptom and to establish methods for the prevention and treatment of radiation-induced vomiting.

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