INTRODUCTION

Unidentified complaints in the digestive system are often observed as side effects with many medicines and lead to poor treatment compliance. In the present study, we aimed to establish simple methods for predicting nausea and/or emesis in mice, which do not vomit, using drugs and chemicals known to evoke nausea and/or emesis. The gastrointestinal transit test, the liquid gastric emptying by phenol red solution (Phenol red method) and the solid gastric emptying by resin beads (Beads method) were used and the effects of antispasmones (atropine, 0.1-3 mg/kg i.p.; salmon calcitonin, 1-30 units/kg i.m.), nauseants (copper sulfate, 1-30 mg/kg p.o.; apomorphine, 0.01-0.3 mg/kg s.c.) and chemotherapeutics (cisplatin, 0.3-10 mg/kg i.v.; doxorubicin, 0.3-10 mg/kg i.v.) were evaluated. In addition, the effects of ondansetron, a serotonin (5-HT)3 receptor antagonist, on the inhibition of solid gastric emptying induced by salmon calcitonin, copper sulfate, cisplatin and doxorubicin were also assessed. Only the solid gastric emptying method could detect changes of gastric emptying by all drugs and chemicals. We also found that the inhibition of solid gastric emptying induced by cisplatin and doxorubicin was dose-dependently antagonized by ondansetron. However, ondansetron failed to antagonize the salmon calcitonin-induced delay, but exerted only very weak effects with copper sulfate. Solid gastric emptying may be more suitable than gastrointestinal intestinal transit or liquid gastric emptying in mice to predict nausea and/or emesis. Our results also suggest that chemotherapeutic-induced delay of solid gastric emptying mediated via 5-HT3 receptors in mice could also be useful for prediction purposes.

Key words: 5-HT (5-Hydroxytryptamine, serotonin), receptor, Chemotherapeutics, Gastric emptying, Mice, Nausea, Emesis
because it primarily evaluates gastrointestinal motility. We have experienced stomach distension with charcoal solution without any effects on the gastrointestinal transit in rats and mice using some drug candidates, which did tend to cause side effects in the gastrointestinal system in clinical studies.

Several other attempts to estimate drug-induced nausea and emesis in rodents have been reported previously (Mitchell et al., 1976; Yamamoto et al., 2005, 2007; Malik et al., 2007), but the methods need care. For example, pica behavior, the eating of non-nutritive substances such as kaolin, is known to evaluate emetic potential in rodents (Yamamoto et al., 2007). However, emetic assay by pica behavior is difficult to follow pharmacokinetics of test compounds and needs a certain amount of time. If a simple method for predicting emesis in mouse can be established, the amount of test compounds can reduce markedly compared to that for rats and many kinds of mice with malignant tumor and genetic modification are available. In the present investigation, therefore, we focused on compared three different methods, which are often employed in safety pharmacology and pharmacodynamic studies. In addition to gastrointestinal transit testing, liquid gastric emptying assessed with phenol red solution and solid gastric emptying using resin beads were applied with a number of drugs well established to cause nausea and/or emesis. These were anti-spasmodgens (atropine and salmon calcitonin), nauseants (copper sulfate and apomorphine), and chemotherapeutics (cisplatin and doxorubicin). Atropine is known to have emetic actions in human, dogs and pigeons (King, 1990) and salmon calcitonin, a drug for osteoporosis, reported to have nausea and diarrhea as side effects in clinical use (Wüster et al., 1991), and to suppress gastric emptying and gastrointestinal motility in dogs (Nakamura et al., 1995). Copper sulfate stimulates the terminals of the visceral afferents innervating the stomach wall in humans, dogs, cats, and ferrets (King, 1990) and apomorphine acts via the chemoreceptor trigger zone (CTZ) in the area postrema in the floor of the forth ventricle and induce vomiting through dopamine D₂ receptors (Takeda et al., 1993, 1995a). Two chemotherapeutics, cisplatin and doxorubicin release serotonin through enterochromaffin cells in the upper gastrointestinal tract which stimulates the gastrointestinal visceral afferents via 5-HT₃ receptors to induce vomiting (Andrews et al., 1988; Yoshikawa et al., 2001). In addition, we examined the involvement of 5-HT₃ receptors on the tested drugs/chemicals-induced delay in gastric emptying using 5-HT₃ antagonist ondansetron-coadministered mice. Doses and administration routes of the drugs were determined by clinical usage and previous studies.

**MATERIALS AND METHODS**

**Animals**

All experiments were performed according to the Guidance for care and use of laboratory animals at the test site and the Japanese Pharmacological Society. Male ddY mice (4-7 week-old) were purchased from Japan SLC (Shizuoka, Japan) and housed 5 mice to a cage on hardwood chip laboratory bedding (Japan SLC) in a room with a 12-hr light/12-hr dark cycle (lights on between 7:00 and 19:00) at a constant temperature (22 ± 3°C) and humidity (55 ± 20%). Pelleted food (MF, Oriental Yeast, Osaka, Japan) and water were available ad libitum. After quarantine and acclimation for at least 3 days, mice (17.2 -30.0 g) in good condition were used. Mice were fasted overnight in wire-mesh cages individually to prevent coprophagy before experiments.

**Drugs and chemicals**

The following drugs and chemicals were purchased: copper sulfate (Kanto Chemical, Osaka, Japan), apomorphine (Sigma, St. Louis, MO, USA), cisplatin (Maruko, Aichi, Japan), doxorubicin (Mercian, Tokyo, Japan), atropine (Wako Pure Chemical, Osaka, Japan), and ondansetron (Zofran® injection, GlaxoSmithKline, Tokyo, Japan), charcoal (Wako Pure Chemical), gum Arabic (Junsei Chemical, Tokyo, Japan), phenol red (Wako Pure Chemical), carboxymethyl cellulose sodium (Wako Pure Chemical) and trisodium phosphate (Wako Pure Chemical). Salmon calcitonin was synthesized at Mitsubishi Chemical Corporation, the forerunner of Mitsubishi Tanabe Pharma Corporation.

**Gastrointestinal transit**

Each mouse was orally given 5% of charcoal suspension (0.2 ml/mouse, in 5% gum arabic solution), and the small intestine was removed at 30 min post-dosing to assess gastrointestinal transit with the following formula; gastrointestinal transit (%) = (the distance from the pylorus to the leading edge of the charcoal meal) / (the distance from the pylorus to the distal edge of the ileum) × 100. The charcoal suspension was given immediately after administration of test drugs except in the cisplatin case, where a 1 hr delay was applied.

**Liquid gastric emptying by phenol red solution (Phenol red method)**

Each mouse was orally given 0.2 w/v% of phenol red solution (0.05 ml/mouse, in 0.5% carboxymethyl cellulose
sodium solution). The stomach was removed at 1 hr post-dose and the contents were suspended in 20 ml of 2.75% trisodium phosphate solution. Following centrifugation at 3,000 rpm for 15 min, the concentration of phenol red in an aliquot of supernatant was measured with absorbance at 450 nm (Spectra MAX 250, Molecular Devices, Sunnyvale, CA, USA). The gastric emptying rate was calculated as the following formula; gastric emptying (%) = (1 - (Amount of phenol red remaining in the stomach / (Mean amount of phenol red in the stomach immediately after phenol red administration measured from the satellite group))) × 100. Phenol red solution was given immediately after administration of drugs except with cisplatin, when a 1 hr delay was applied.

**Solid gastric emptying by resin beads (Beads method)**

Each mouse was orally given distilled water (0.5 ml/mouse) containing 40 resin beads (diameter: 0.6 mm, DIAION SA10A, Mitsubishi Chemical, Tokyo, Japan) via a plastic feeding tube. The resin beads used these studies were comparable to beads made with metals or glass in size, hardness and weight. The stomach was removed at 1 hr post-dose and cut along the greater curvature. The contents were washed out into a petri dish and the number of beads in it was carefully counted. The gastric emptying rate was calculated with the following formula; gastric emptying (%) = (40 - the number of beads in the stomach) / 40 × 100. Beads were given immediately after administration of the drugs except with cisplatin, when there was a 1 hr delay.

**Interaction of ondansetron with other chemicals with reference to solid gastric emptying**

Interactions of ondansetron with salmon calcitonin, copper sulfate, cisplatin and doxorubicin effects on solid gastric emptying were investigated by the beads method. Ondansetron was injected from tail vein 15 min before the administration of the beads.

**Statistical analysis**

Data presented as mean ± S.E.M. values from 7 or 8 mice were compared between the controls and the drug groups by one-way analysis of variance followed by Dunnett multiple comparison tests. In the interaction study, statistical analysis between the controls and the drug groups were conducted by one-way analysis of variance followed by Dunnett multiple comparison tests, if a comparison between vehicle (non-treatment) and control (test drug only) performed by Student’s t-test was statistically significant. If the comparison between vehicle and control was not statistically significant, further comparison between controls and the drug groups was not performed. A value of P < 0.05 (double-sided) was considered statistically significant.

**RESULTS**

**Antispasmodenics**

Atropine inhibited not only gastrointestinal transit, but also gastric emptying by the phenol red and beads methods (Fig. 1). Gastrointestinal transit was significantly inhibited from 1 mg/kg or more, and gastric emptying in both methods by 0.1 mg/kg or more. Salmon calcitonin did not affect gastrointestinal transit, but gastric emptying was significantly inhibited from 10 units/kg with the phenol red method and from 3 units/kg with the beads method (Fig. 1).

**Nauseants**

Copper sulfate significantly enhanced gastrointestinal transit from 10 mg/kg, but significantly inhibited gastric emptying by the phenol red method at 30 mg/kg and by the beads method from 3 mg/kg (Fig. 2). Apomorphine did not affect gastrointestinal transit or gastric emptying by the phenol red method, but significantly enhanced gastric emptying by the beads method at 0.3 mg/kg (Fig. 2).

**Chemotherapeutics**

Cisplatin did not affect gastrointestinal transit or gastric emptying by the phenol red method, neither, but significantly inhibited gastric emptying by the beads method from 1 mg/kg (Fig. 3). Doxorubicin did not affect gastrointestinal transit or gastric emptying by the phenol red method, but significantly inhibited gastric emptying by the beads method from 3 mg/kg (Fig. 3).

**Interaction of ondansetron with other chemicals with reference to solid gastric emptying**

Ondansetron itself did not affect the gastric emptying and only weakly antagonized copper sulfate-induced gastric emptying at 100 μg/kg (Fig. 4). It also failed to influence salmon calcitonin-induced gastric emptying and only weakly antagonized copper sulfate-induced gastric emptying at 100 μg/kg (Fig. 4). On the other hand, it significantly and dose-dependently antagonized cisplatin-induced and the doxorubicin-induced gastric emptying from 10 μg/kg (Fig. 4).

**DISCUSSION**

The present study provided clear evidence that the solid gastric emptying method has advantages over both liq-
Fig. 1. Effects of atropine (0.1-3 mg/kg) and salmon calcitonin (1-30 units/kg) on gastrointestinal transit, liquid gastric emptying and solid gastric emptying in mice. Administration was intraperitoneal for atropine and intramuscular for salmon calcitonin. Data are mean ± S.E.M. values from 7 or 8 mice per group. Statistical significant differences from control are indicated as *P < 0.05, **P < 0.01.

Fig. 2. Effects of copper sulfate (1-30 mg/kg) and apomorphine (0.01-0.3 mg/kg) on gastrointestinal transit, liquid gastric emptying and solid gastric emptying in mice. Administration was per os for copper sulfate and subcutaneous for apomorphine. Data are mean ± S.E.M. values from 8 mice per group. Statistical significant differences from control are indicated as *P < 0.05, **P < 0.01.

Fig. 3. Effects of cisplatin (0.3-10 mg/kg) and doxorubicin (0.3-10 mg/kg) on gastrointestinal transit, liquid gastric emptying and solid gastric emptying in mice. Administration was intravenous for the both drugs. Data are mean ± S.E.M. values from 8 mice per group. Statistical significant differences from control are indicated as *P < 0.05, **P < 0.01.
uid emptying and gastrointestinal transit for determination of influence of various categories of agents causing nausea and emesis. Atropine, a positive control drug for digestive system studies, strongly inhibited gastrointestinal transit and gastric emptying, validating the experimental methods and techniques. However, the inhibitory dose was lowest for gastric emptying, and salmon calcitonin only inhibited gastric emptying, and not gastrointestinal transit. Emptying of solids from the stomach has been reported to be much later than that of liquid in dogs (Hinder and Kelly, 1977). Although the stomach of rats and mice is anatomically different from that in dogs, the

Fig. 4. Effects of ondansetron (3-100 µg/kg) alone and coadministered with salmon calcitonin (10 units/kg), copper sulfate (10 mg/kg), cisplatin (3 mg/kg) and doxorubicin (10 mg/kg) on solid gastric emptying in mice. Administration was intravenous for ondansetron and the same as in the previous experiments for the other drugs. Data are mean ± S.E.M. values from 8 mice per group. Statistical significant differences from vehicle and control are indicated as *P < 0.05, **P < 0.01.
emptying rates for solid and liquid meals appear to be the same, at least in mice. Copper sulfate, which evokes vomiting via stimulation of the terminals of the visceral afferents innervating the stomach wall in humans, dogs, cats, and ferrets (King, 1990), enhanced gastrointestinal transit but inhibited the gastric emptying in mice. Visceral afferents innervating the stomach wall have also been observed in mice (Green and Dockray, 1988) and rats (Green and Dockray, 1988; Takeda et al., 1993) suggesting that the inhibition of the gastric solid emptying probably occurred by the same physiological mechanisms as observed in non-rodents. The opposite results observed between gastrointestinal transit and the gastric emptying can be explained by the fact that the gastrointestinal transit test mostly depends on gastrointestinal motility. In other words, if there are only small amounts of charcoal extract from stomach and gastrointestinal motility is not inhibited, the transit rate will be normal. Enhancement in gastric transit seemed to occur by hypersecretion of intestinal juice in the copper sulfate group. Emesis induced by copper sulfate in dogs, Suncus murinus and ferrets has been observed at 3 mg/kg (Nakamura et al., 1995), 120 mg/kg (Yamamoto et al., 2004) and 25 mg/kg or less (Costall et al., 1990), respectively, while delay of gastric emptying was observed in mice at 3 mg/kg or more by the bead methods in the present studies. Oral administration of copper sulfate induces kaolin consumption, analogous to emesis in rats, from 4 mg/kg or more (Takeda et al., 1993). These results suggest that copper sulfate-induced emesis is predictable in mice, which do not vomit, if the gastric emptying rate by the bead method is used as the index.

Apomorphine acts on the CTZ, which also exists in rats (Takeda et al., 1995a), and evokes emesis in humans, dogs, cats, and ferrets (King, 1990). Copper sulfate and apomorphine are both nauseaants in non-rodents, but opposite effects were observed between apomorphine-induced and copper sulfate-induced solid gastric emptying. Although emesis and/or nausea-like behavior in mice may differ in drug sites of action, further investigations are needed to clarify the mechanism of action in mice.

Cisplatin and doxorubicin cause release of serotonin from enterochromaffin cells in the upper gastrointestinal tract and this then stimulates gastrointestinal visceral afferents via 5-HT_{3} receptors, resulting in vomiting (Andrews et al., 1988). Here, only the solid gastric emptying test detected gastric functional disorders with these two chemotherapeutic drugs. Intravenous doses of cisplatin with which emesis is consistently observed in experiments are 3.0 mg/kg for dog, 7.8 mg/kg for ferret and 1.25 mg/kg for human (King, 1990), respectively. The degree apparently increased from 10 mg/kg of intravenous administration in ferrets (Costall et al., 1990). These reports suggest that gastric emptying by the bead method in mice is suitable to predict emesis and/or nausea at comparable doses to those in the dog and ferret.

Although ondansetron itself did not affect gastric solid emesis in mice, it dose-dependently antagonized the cisplatin- and the doxorubicin-induced inhibition of gastric emptying, while failing to antagonize that of salmoxacin and antagonizing very weakly that of copper sulfate, in agreement with a previous report in rats (Takeda et al., 1995b) and in ferrets (Bhandari and Andrews, 1991), respectively. This suggests that gastric emptying in mice is mediated via 5-HT_{3} receptors and can be used to predict chemotherapeutic drug-induced emesis and/or nausea. Cisplatin-induced kaolin consumption in rats was earlier found to be inhibited by ondansetron (Takeda et al., 1995b).

In conclusion, the beads method may be more suitable to predict nausea and emesis than the gastrointestinal transit test and the phenol red method, because it proved the most sensitive with all types of drug tested here. Although detail mechanisms of gastric emptying in mice remain to be clarified, delayed emptying produced by cisplatin and doxorubicin in mice appears to be partly mediated by 5-HT_{3} receptors. Therefore, the bead method in mice can be recommended as a simple and suitable approach to predict drug-induced emesis and/or nausea, especially with chemotherapeutics.

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