Jpn. J. Pharmacol. 81, 292–297 (1999)

Effects of SK-951, a Benzofuran Derivative, as a Prokinetic Agent in Rats and Dogs

Motohiro Takeda, Katsura Tsukamoto, Masaya Yamano and Hiroshi Uesaka

Pharmaceutical Laboratory, Sanwa Kagaku Kenkyusho Co., Ltd., Shiosaki 363, Mie 511-0406, Japan

Received July 5, 1999 *Accepted August 24, 1999*

ABSTRACT—The gastrokinetic activity of SK-951 ((−)4-amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-5-chloro-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxamide hemifumarate), a benzofuran derivative with 5-hydroxytryptamine (5-HT)$_4$-receptor agonist activity, was studied in rats and dogs. The effects of SK-951 were also investigated in a model of vagotomy-induced gastroparesis in comparison with cisapride. In rats, both SK-951 and cisapride enhanced gastric emptying of liquids (phenol red) at a dose of 1–100 mg/kg, p.o. Gastric emptying of liquid (acetaminophen) in fasted beagle dogs was enhanced significantly by SK-951 (1.0 mg/kg, i.v.), whereas the effect of cisapride (0.2–1.0 mg/kg, i.v.) was not statically significant. Similar results were found when radiopaque markers were given with standard meal to dogs with vagotomy-induced gastroparesis. The delayed gastric emptying of radiopaque markers by vagotomy was reversed by SK-951 (1.0 mg/kg, i.v.), whereas cisapride showed no effect at doses from 0.1 to 1.0 mg/kg, i.v. These results indicated that oral and intravenous administration of SK-951 accelerates gastric emptying of both liquids and solids in animal models. Thus, SK-951 may be a highly potent and useful prokinetic agent in comparison to cisapride.

Keywords: SK-951, 5-HT$_4$ receptor, Gastric emptying, Vagotomy, Gastroparesis

Prokinetic benzamides with 5-hydroxytryptamine (5-HT)$_4$-receptor agonist activity such as metoclopramide and cisapride are widely used in the treatment of gastrointestinal tract disorders (1). It has been reported that cisapride and metclopramide may be useful in patients with idiopathic gastric stasis (2), vagotomized gastroparesis (3) and diabetic gastroparesis (2, 4). Among the benzamide derivatives examined to date, cisapride (5) and renzapride (6) have been shown to stimulate gastrointestinal motor activity through enhancement of cholinergic transmission in experiments with isolated guinea pig gastrointestinal preparations (5, 7). Stimulation of 5-HT$_4$ receptors has been shown to evoke the release of acetylcholine (ACh) from the guinea pig stomach (8) and to cause cholinergic and non-cholinergic contraction of rat gastric fundus (9). The contractile 5-HT$_4$ receptors appear to be mainly located on the excitatory neurons such as the cholinergic neurons (8, 10, 11). To obtain gastrointestinal prokinetic drugs with greater potency than the benzamide derivatives commonly in use, we synthesized and evaluated a series of novel test compounds. We obtained SK-951 ((−)4-amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-5-chloro-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxamide hemifumarate), a benzofuran derivative, that is structurally similar to benzamides, has 5-HT$_4$-receptor agonist properties, and has a gastrokinetic effect similar to that of cisapride (12).

The present study was designed to compare the prokinetic effects of SK-951 on gastric emptying with those of cisapride in rats and dogs, particularly in the dogs with vagotomy-induced gastroparesis.

MATERIALS AND METHODS

Gastric emptying of phenol red in rats

The experiment was performed according to the method reported by Yokochi et al. (13). Male Wistar rats, weighing 200–230 g, were fasted for 24 h before the experiments. SK-951 (0.01–100 mg/kg) and cisapride (0.01–100 mg/kg) in a 5% solution of arabic gum were orally administered in a volume of 5 ml/kg. Thirty minutes after drug administration, 0.01% phenol red solution (1 ml/rat) was administered orally. Each rat was sacrificed 15 min after phenol red administration except those sacrificed immediately for recovery of the whole amount of phenol red as controls, and the stomach was

removed. The stomach was cut into several pieces in 10 ml of 0.1 M Na_2HPO_4, and then 1 ml of the rinsed solution was added to 2 ml of 0.1 M Na_2HPO_4 (S1). The residual rinsed solution was added to 1 ml of 0.01% phenol red solution. The solution was mixed and then diluted tenfold with 0.1 M Na_2HPO_4 (S2). Both S1 and S2 were centrifuged (3,000 rpm) for 10 min, and then the absorbance of each supernatant was measured as OD1 and OD2 at a wavelength of 560 nm with a spectrophotometer (model 220; Hitachi, Tokyo), respectively. Gastric emptying was calculated as follows:

Gastric emptying (%) = $100 - (A / B) \times 100$

A: The amount of phenol red remaining in the stomach (μg)
$= 100 - 3 \times OD1 / (10 \times OD2 / 3 \times OD1 - 1)$

B: The whole amount of phenol red recovered from the stomach immediately after phenol red administration

Gastric emptying of liquid test meal in dogs

Three to four beagle dogs of either sex, weighing 8–11 kg, were used. Animals were treated several times with a minimum 3-day resting period between treatments. The experiment was performed according to the method reported by Harasawa et al. (14). Each dog was fasted for 24 h before the experiment. The dogs were orally given a liquid test meal (5 ml/kg, Sun-et A; Sanwa Kagaku Kenkyusho, Nagoya) containing acetaminophen (2 mg/ml). The test meal (1 kcal/ml) contained 65.9% carbohydrate (corn dextrin), 18.8% protein (casein), and 15.5% fat (soybean oil). SK-951 (0.04–1 mg/kg) and cisapride (0.04–1 mg/kg) in a solution containing 0.1% tartaric acid were injected intravenously into the sephenous vein immediately after test meal administration. Blood samples were obtained at 0, 15, 30, 45, 60, 90 and 120 min after test meal administration, and the plasma concentration of acetaminophen was measured by high-performance liquid chromatography (HPLC) according to a slight modification of the method of Mizuta et al. (15). Briefly, 0.02 ml of 0.02% caffeine as an internal standard, 0.2 ml of 0.3 N $Ba(OH)_2$ and 0.2 ml of 7% $ZnSO_4$ were added to 0.4 ml of plasma, and then the mixture was shaken for 30 s. After centrifugation at 15,000 rpm for 10 min, 0.4 ml of the supernatant was added to 0.2 ml 1 N HCl and 2.4 ml of ethylacetate, and the mixture was shaken for 30 s. After centrifugation at 3,000 rpm for 10 min, 2.0 ml of the organic phase was collected, dried under a stream of N_2 gas at 37℃, and the residue was dissolved in 60 μl of methanol. This specimen (10 μl) was used for HPLC analysis. The chromatographic system consisted of a UV absorbance detector at 244 nm (UV-8020; Tosoh, Tokyo) and a TSKgel ODS 80Ts column (4.6×250 mm i.d., particle size: 5 μm; Tosoh). The mobile phase used had the following composition: 50 mM phosphate buffer, pH 2.5 : methanol = 4 : 1. The flow rate was maintained at 1 ml/min.

Gastric emptying in vagotomized dogs

Three male beagle dogs approximately 2-year-old, weighing 10 to 15 kg, were used. Assays were performed with animals fasted for 24 h. The animals received 50 barium sulfate spheroids (1.9 mm in diameter, 1.59 g /cm^3 density) with a standard meal (60 g: Lab Ration #4360; Purina, Tokyo). Gastric emptying was measured by means of X-ray location (VPX-20, 80 kV, 160 mA, 0.03 s; Toshiba, Tokyo). Passage of the markers was monitored every 2 h. Following control recording (Normal: prevagotomized dog), each animal was subjected to thoracic vagotomy. The animals were anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg, i.v.; Dainabot, Osaka) and placed in the supine position. Under aseptic conditions, the thoracal vagus nerve on the esophagus was exposed by making a fifth and sixth intercostal incision in the right chest, carefully lifted from the esophagus with a pair of fine forceps, and approximately 1.5 cm of the vagus nerve was removed with fine scissors. Then, the incision was closed. For three days after surgery, 500 ml of KN (Otsuka Pharmaceutical, Tokushima) fluid in which 0.5 g of ceftriaxone sodium (Rocephin; Rosch, Tokyo) for injection was dissolved was infused at a rate of 500 ml/day. The dogs were given pasty meal and water during the recovery period after surgery. The experiments were started at least 14 days after surgery.

To examine the effects of SK-951 and cisapride on vagotomy-induced gastroparesis, test compounds were dissolved in a solution containing 0.1% tartaric acid and intravenously injected into dogs immediately after oral ingestion of 50 barium sulfate spheroids with a standard meal (60 g). Animals were treated several times, with a minimum 3-day resting period between treatments. Gastric emptying of controls (postvagotomized dogs) was recorded every week.

Calculations and statistics

All values are expressed as means±S.E.M. Data were analyzed by one-way analysis of variance followed by Fisher's protected least significant difference procedure.

Drugs and chemicals

The drugs and chemicals used were as follows: SK-951 (Sanwa Kagaku Kenkyusho Co., Ltd.); cisapride (Quimica Sintetica S.A., Madrid, Spain); phenol red Na (Tokyo Kasei, Tokyo); acetaminophen (Nacalai Tesque, Kyoto). Other chemicals used were of reagent grade.

RESULTS

Effects of SK-951 on gastric emptying of phenol red in rats

SK-951 (1−10 mg/kg, p.o.) enhanced gastric emptying of phenol red in a dose-dependent manner with potency similar to that of cisapride (Fig. 1). Maximal enhancement was observed at a dose of 10 mg/kg. Higher doses of both SK-951 and cisapride decreased the enhancing effect. Both drugs significantly enhanced gastric emptying, but the dose-response relationship seemed to be bell-shaped.

Effects of SK-951 on gastric emptying of liquid test meal in dogs

The plasma acetaminophen concentration in a control study was increased time-dependently and peaked at 45 min (3.26±0.82 μg/ml) after acetaminophen administration (Fig. 2). Therefore, the plasma acetaminophen concentration at 15 or 30 min after test meal ingestion appeared to be a good parameter of gastric emptying. SK-951 (0.04−1.0 mg/kg, i.v.) increased the plasma acetaminophen level in a dose-dependent manner (Table 1). Cisapride (0.04−0.2 mg/kg, i.v.) tended to increase the plasma acetaminophen level, but this effect was not significant. Higher doses of cisapride decreased the plasma concentration of acetaminophen.

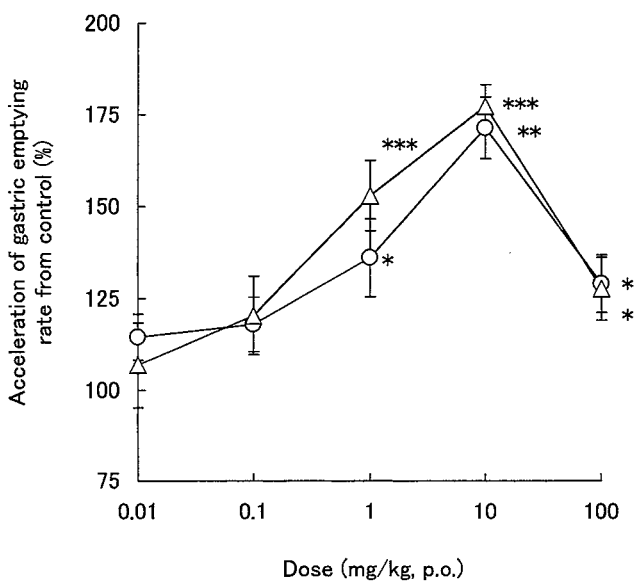

Fig. 1. Effects of SK-951 (○) and cisapride (△) on gastric emptying in rats. The gastric emptying rate was measured by the phenol red method. Each drug and 5% gum arabic as a control were orally administered. At 30 min after administration, 0.01% phenol red solution was orally administered. Gastric emptying was measured at 15 min after phenol red administration. Each point and vertical bar represents the mean±S.E.M. from 9 to 10 rats. *P<0.05, **P<0.01, ***P<0.001: significantly different from the control by one-way ANOVA followed by Fisher's PLSD.

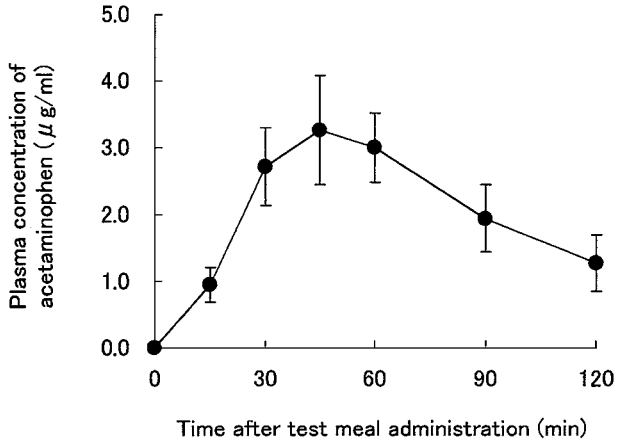

Fig. 2. Time course of changes in plasma acetaminophen concentration after test meal administration. After a 24-h fast, the dogs were orally administered acetaminophen (10 mg/kg) with liquid test meal (5 ml/kg). Each point and vertical bar represents the mean± S.E.M. from 4 dogs.

Effects of SK-951 on gastric emptying of solid radiopaque markers in vagotomy-induced gastroparesis dogs

In normal (prevagotomized) dogs, the gastric emptying of radiopaque markers was 82.7±3.3% at 6 h after solid meal ingestion. The gastric emptying of radiopaque markers in the post-vagotomized dogs was 12.2±5.2% at 6 h and was significantly delayed compared with the rate in normal (prevagotomized) dogs at 4, 6 and 8 h after solid meal ingestion (Fig. 3).

SK-951 (1 mg/kg, i.v.) significantly enhanced gastric emptying of solid radiopaque markers compared with the

Table 1. Effects of SK-951 and cisapride on plasma concentration of acetaminophen after liquid meal ingestion in fasted beagle dogs

Treatment	Dose (mg/kg)	n	Plasma concentration of acetaminophen (μg/ml)	
			15 min	30 min
Control (0.1% tartaric acid)		4	0.95±0.26	2.71±0.58
SK-951	0.04	3	2.43±0.22	3.72±0.34
	0.2	4	3.03±0.45	4.28±0.47
	1	4	3.30±0.30*	4.27±0.40**
Cisapride	0.04	3	1.65±0.46	4.02±0.52
	0.2	3	2.72±0.86	3.83±0.37
	1	4	1.98±0.48	3.25±0.37

SK-951 and cisapride were administered intravenously immediately after the test meal. Each value represents the mean±S.E.M. *P<0.05, **P<0.01: significantly different from the control (one-way ANOVA followed by Fisher's PLSD).

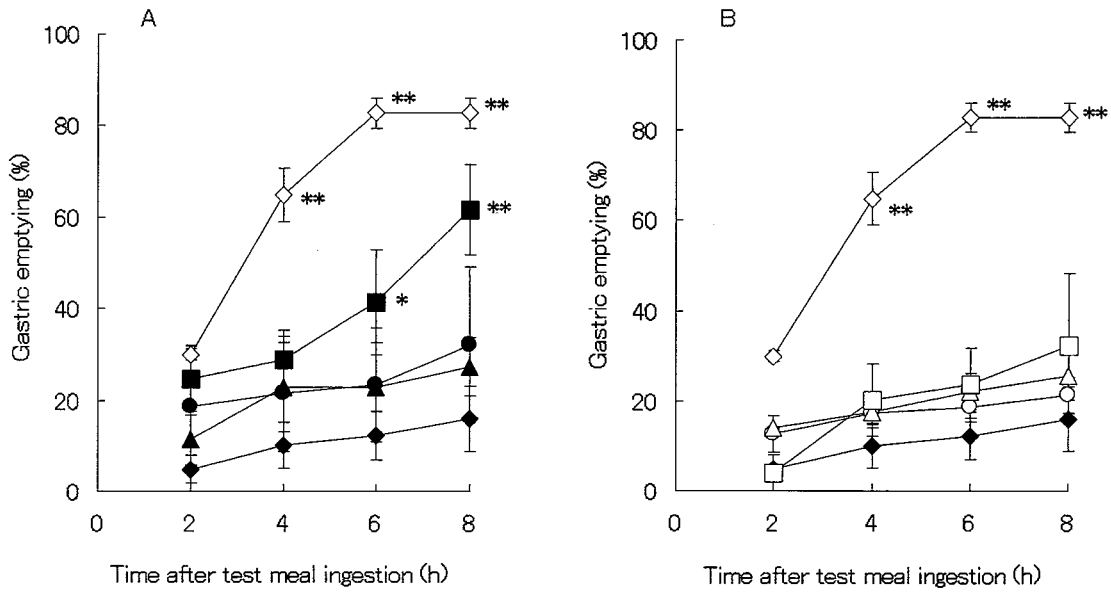

Fig. 3. Effects of SK-951 (A) and cisapride (B) on the vagotomy-induced gastroparesis in beagle dogs. Test compounds were intravenously injected into dogs immediately after oral administration of barium sulfate spheroids. Each point and vertical bar represents the mean±S.E.M. from three dogs. *$P<0.05$, **$P<0.01$: significantly different from control by one-way ANOVA followed by Fisher's PLSD. ◇: normal (prevagotomized dog); ◆: control (postvagtomized dog); ●: SK-951, 0.1 mg/kg, i.v.; ▲: SK-951, 0.3 mg/kg, i.v.; ■: SK-951, 1.0 mg/kg, i.v.; ○: cisapride, 0.1 mg/kg, i.v.; △: cisapride, 0.3 mg/kg, i.v.; □: cisapride, 1.0 mg/kg, i.v.

controls (Fig. 3A). Cisapride only tended to increase gastric emptying (Fig. 3B).

DISCUSSION

Prokinetic drugs such as cisapride clearly stimulate gastric and intestinal contractions in dogs and normal healthy human subjects, but gastric emptying may not be enhanced (16, 17). It was therefore a matter of interest to induce gastroparesis to examine the effects of prokinetic drugs on gastric emptying. The present study showed that SK-951 accelerates gastric emptying not only under physiological conditions in rats and dogs but also under conditions of vagotomy-induced gastroparesis in dogs.

Both SK-951 and cisapride facilitated gastric emptying of liquid meal in normal rats. SK-951 also enhanced gastric emptying of liquid meal in normal dogs, whereas cisapride only produced a weak effect that was not statistically significant. Moreover, SK-951 significantly reversed the delayed gastric emptying of solid meals in vagotomy-induced dogs, but cisapride had no effect.

The prokinetic benzamides such as cisapride and zacopride act as 5-HT_4-receptor agonists and 5-HT_3-receptor antagonists (18). In vitro studies have shown that SK-951 is a 5-HT_4-receptor agonist and a weak 5-HT_3-receptor antagonist (12). Stimulation of the 5-HT_4 receptor accelerates the motility of the gastrointestinal tract and gastric emptying in vivo (19, 20). 5-HT_3-receptor antagonists are able to promote gastric emptying in rats but not in dogs or in humans (21–23). 5-HT_3-receptor antagonists such as granisetron neither stimulate nor inhibit gastric emptying in dogs (24). Cisapride was more potent than SK-951 as an antagonist at 5-HT_3 receptors in the guinea pig ileum (data not shown). SK-951 enhanced gastric emptying in both animal species, while cisapride was more effective in rats, but lacked any apparent effect on gastric emptying in dogs. The reason why cisapride was only effective in rats may be due to a more potent 5-HT_3-receptor antagonist.

The present study was performed to estimate the effects of the agents on the vagotomy-induced canine gastroparesis model using barium sulfate spheres with a standard solid meal. Radiopaque markers have been used in dogs and humans to study gastric emptying since this is a simple and highly sensitive procedure for evaluating gastric motor dysfunction and the effects of prokinetic agents (25–27). It has been reported that gastric emptying of indigestible solids is influenced by particle size, and the markers showed gastric emptying at the same rate as food when sphere diameter was 1.6 mm to 2.4 mm (28). The vagus nerve markedly influences the contractile activity of the stomach. The multiple effects of vagotomy on gastric motility include alterations of the pressure-volume relationship of the fundus (29, 30), weakening and disorientation of antral contraction (31), and disruption in the normal frequency and coupling patterns of the myo-

electric slow waves (30, 32).

SK-951 significantly reversed gastroparesis, while cisapride showed no effect on gastric emptying in vagotomized dogs. The differences in the effects of SK-951 and cisapride on gastric emptying in rats and dogs may be due to different emptying patterns. The type of meal influences the gastric emptying of a meal; a solid meal is emptied slowly from the stomach, but a liquid meal is quickly emptied since liquid emptying is regulated by tonic contractions of the proximal part of the stomach, whereas the peristaltic activity of the distal part (antrum) is responsible for solid emptying (33). 5-HT_4 receptors have a contractile function in the human gastric fundus, corpus and antrum (34); rat gastric fundus (9); and guinea pig gastric corpus (8) and antrum (35). Therefore, differences in the effects of SK-951 and cisapride on gastric emptying may reflect the activity of 5-HT_4-receptor agonism rather than differences in the test meal and 5-HT_3-receptor antagonism. The maximum amount of ACh released by SK-951 was more than that by cisapride, as observed in the 5-HT_4-receptor mediated contractile response of the guinea pig ileum (12). Thus, the observation that the enhancement of gastric emptying in dogs in response to SK-951 was greater than that induced by cisapride may reflect the intrinsic activities of these agents.

In conclusion, SK-951 enhances gastric emptying of both solids and liquids in different animal models. SK-951 may be useful for treatment of gastrointestinal motor disorders as a gastrointestinal prokinetic drug.

Acknowledgment

We would like to thank Prof. K. Taniyama (Nagasaki University School of Medicine) for helpful discussions.

REFERENCES

1 Harrington RA, Hamilton CW, Brogden RN, Linkewich JA, Romankiewicz JA and Heel RC: Metoclopramide. An updated review of its pharmacological properties and clinical use. Drugs **25**, 451–494 (1983)
2 Champion MC: Management of idiopathic, diabetic and miscellaneous gastroparesis with cisapride. Scand J Gastroenterol **24**, Suppl **165**, 44–53 (1989)
3 Stadaas JO and Aune S: Clinical trial of metoclopramide on postvagotomy gastric stasis. Arch Surg **104**, 684–686 (1972)
4 Mchugh S, Lico S and Diamant NE: Cisapride vs metoclopramide: an acute study in diabetic gastroparesis. Dig Dis Sci **37**, 997–1001 (1992)
5 Schuurkes JAJ, Van Nueten JM, Van Daele PGH, Reyntjens AJ and Janssen PAJ: Motor-stimulating properties of cisapride on isolated gastrointestinal preparations of the guinea pig. J Pharmacol Exp Ther **234**, 775–783 (1985)
6 Sanger GJ: Increased gut cholinergic activity and antagonism of 5-hydroxytryptamine M-receptors by BRL 24924: potential clinical importance of BRL 24924. Br J Pharmacol **91**, 77–87 (1987)
7 Buchheit KH, Costall B, Engel G, Gunning SJ, Naylor RJ and Richardson BP: 5-Hydroxytryptamine receptor antagonism by metoclopramide and ICS 205-930 in the guinea pig leads to enhancement of contractions of stomach muscle strips induced by electrical field stimulation and facilitation of gastric emptying in vivo. J Pharm Pharmacol **37**, 664–667 (1985)
8 Matsuyama S, Sakiyama H, Nei K and Tanaka C: Identification of putative 5-hydroxytryptamine$_4$ (5-HT_4) receptors in guinea pig stomach: the effect of TSK159, a novel agonist, on gastric motility and acetylcholine release. J Pharmacol Exp Ther **276**, 989–995 (1996)
9 Amemiya N, Hatta S, Takemura H and Ohshika H: Characterization of the contractile response induced by 5-methoxytryptamine in rat stomach fundus strip. Eur J Pharmacol **318**, 403–409 (1996)
10 Taniyama K, Nakayama S, Takeda K, Matsuyama S, Shirakawa J, Sano I and Tanaka C: Cisapride stimulates motility of the intestine via the 5-hydroxytryptamine receptors. J Pharmacol Exp Ther **258**, 1098–1104 (1991)
11 Kilbinger H and Wolf D: Effect of 5-HT_4 receptor stimulation on basal and electrically evoked release of acetylcholine from guinea pig myenteric plexus. Naunyn Schmiedebergs Arch Pharmacol **345**, 270–275 (1992)
12 Takeda M, Tsukamoto K, Misutani Y, Suzuki T and Taniyama K: Identification of SK-951, a novel benzofuran derivative, as an agonist to 5-HT_4 receptors. Jpn J Pharmacol **79**, 203–212 (1999)
13 Yokochi E, Kohno S and Ohata K: Pharmacological studies on the clathrate compound of mobenzoxamine with beta-cyclodextrin. (I). Effects on the digestive system. Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi) **92**, 297–310 (1988) (in Japanese with English abstract)
14 Harasawa S, Tani N, Suzuki S, Miwa M, Sakita R, Nomiyama T and Miwa T: Gastric emptying in normal subjects and patients with peptic ulcer: a study using acetaminophen method. Gastroenterol Jpn **14**, 1–10 (1979)
15 Mizuta H, Kawazoe Y and Ogawa K: Gastrointestinal absorption of chlorothiazide: evaluation of a method using salicylazosulfapyridine and acetaminophen as the marker compounds for determination of the gastrointestinal transit time in the dog. Chem Pharm Bull (Tokyo) **38**, 2810–2813 (1990)
16 Wulschke S, Ehrlein HJ and Tsiamitas C: The control mechanisms of gastric emptying are not overridden by motor stimulants. Am J Physiol **251**, G744–G751 (1986)
17 Edwards CA, Holden S, Brown C and Read NW: Effect of cisapride on the gastrointestinal transit of a solid meal in normal human subjects. Gut **28**, 13–16 (1987)
18 Briejer MR, Akkermans LMA and Schuurkes JAJ: Gastrointestinal prokinetic benzamides: the pharmacology underlying stimulation of motility. Pharmacol Rev **47**, 631–651 (1995)
19 Gullikson GW, Virina MA, Loeffler RF, Yang DC, Goldstin B, Wang SX, Moummi C, Flynn DL and Zabrowski DL: SC-49518 enhances gastric emptying of solid and liquid meals and stimulates gastrointestinal motility in dogs by a 5-hydroxytryptamine$_4$ receptor mechanism. J Pharmacol Exp Ther **264**, 240–248 (1993)
20 Hegde SS, Wong AG, Perry MR, Ku P, Moy TM, Loeb M and Eglen RM: 5-HT_4 receptor mediated stimulation of gastric emptying in rats. Naunyn Schmiedebergs Arch Pharmacol

351, 589–595 (1995)

21 Nielson OH, Hvid-jacobsen K, Lund P and Langohlz E: Gastric emptying and subjective symptoms of nausea: lack of effect of a 5-hydroxytryptamine$_3$ antagonist ondansetron on gastric emptying in patients with gastric stasis syndrome. Digestion **46**, 89–96 (1990)

22 Schiavone A, Volonté M and Micheletti R: The gastrointestinal motor effect of benzamide derivatives is unrelated to 5-HT$_3$ receptor blockade. Eur J Pharmacol **187**, 323–329 (1990)

23 Eglen RM, Lee CH, Smith WL, Johnson LG, Whiting RL and Hedge SS: RS 42358-197, a novel and potent 5-HT$_3$ receptor antagonist, in vitro and in vivo. J Pharmacol Exp Ther **266**, 535–543 (1993)

24 Gullikson GW, Loeffler RF and Virina MA: Relationship of serotonin$_3$ receptor antagonist activity to gastric emptying and motor-stimulating actions of prokinetic drugs in dogs. J Pharmacol Exp Ther **258**, 103–109 (1991)

25 Feldman M and Smith HJ: Effect of cisapride on gastric emptying of indigestible solids in patients with gastraparesis diabeticorum. A comparison with metoclopramide and placebo. Gastroenterology **92**, 171–174 (1987)

26 Kawagishi T, Nishizawa Y, Okuno Y, Sekiya K and Morii H: Effect of cisapride on gastric emptying of indigestible solids and plasma motilin concentration in diabetic autonomic neuropathy. Am J Gastroenterol **88**, 933–938 (1993)

27 García-Garayoa E, Monge A, Roca J, Del Río J and Lasheras B: VB20B7, a novel 5-HT-ergic agent with gastrokinetic activity. II. Evaluation of the gastroprokinetic activity in rats and dogs. J Pharm Pharmacol **49**, 66–73 (1997)

28 Meyer JH, Dressman J, Fink A and Amidon G: Effect of size and density on canine gastric emptying of nondigestible solids. Gastroenterology **89**, 805–813 (1985)

29 Stadaas J and Aune S: Intragastric pressure-volume relationship before and after vagotomy. Acta Chir Scand **136**, 611–615 (1970)

30 Wilbur BG and Kelly KA: Effect of proximal gastric, complete gastric, and truncal vagotomy on canine gastric electric activity, motility, and emptying. Ann Surg **178**, 295–303 (1973)

31 Ludwick JR, Wiley JN and Bass P: Pyloroplasty and vagotomy. Early effects on antral and duodenal contractile activity. Arch Surg **99**, 553–559 (1969)

32 Kelly KA and Code CF: Effect of transthoracic vagotomy on canine gastric electrical activity. Gastroenterology **57**, 51–58 (1969)

33 Minami H and McCallum RW: The physiology and pathophysiology of gastric emptying in humans. Gastroenterology **86**, 1592–1610 (1984)

34 Schuurkes JAJ, Meulemans AL, Obertop H and Akkermans LMA: 5-HT$_4$ receptors on the human stomach. J Gastrointest Motil **3**, P199 (1991)

35 Tamura T, Sano I, Satoh M, Mizumoto A and Itoh Z: Pharmacological characterization of 5-hydroxytryptamine-induced motor activity (in vitro) in the guinea pig gastric antrum and corpus. Eur J Pharmacol **308**, 315–324 (1996)