Stimulatory Action of Itopride Hydrochloride on Colonic Motor Activity in Vitro and in Vivo

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ABSTRACT
We investigated the effects of itopride hydrochloride (itopride, N-[4-[2-(dimethylamino)ethoxy]benzyl]-3,4-dimethoxybenzamide hydrochloride), a gastroprokinetic agent, on the colonic motor activity in vitro and in vivo, in comparison with benzamides, cisapride hydrate (cisapride), and mosapride citrate (mosapride). Itopride stimulated both peristaltic and segmental motility induced by applying intraluminal pressure to the isolated guinea pig colon. Although cisapride and mosapride enhanced the segmental motility, they markedly reduced the peristaltic motility. In conscious dogs with implanted strain gauge force transducers, itopride stimulated contractile activity in the gastrointestinal tract from the stomach to the colon. Cisapride stimulated contractile activity in the gastric antrum, ileum, and ascending colon. Mosapride stimulated contractile activity only in the gastric antrum and ileum. In guinea pigs and rats, itopride accelerated colonic luminal transit. On the other hand, cisapride and mosapride failed to enhance colonic transit. These results demonstrate that itopride has a stimulatory action on colonic peristalsis, propelling colonic luminal contents, different from that of cisapride and mosapride. Therefore, itopride may be a useful drug for the treatment of functional bowel disorders such as functional constipation.

Itopride hydrochloride (itopride) and mosapride citrate (mosapride) have been used as a gastroprokinetic agent in some countries, including Japan, for the symptomatic treatment of functional dyspepsia. Delayed gastric emptying is considered one of causes of functional dyspepsia. Itopride was reported to enhance gastric emptying in dogs, rats, and humans, and it stimulated canine gastrointestinal motility (Iwanaga et al., 1990, 1991; Harasawa and Miwa, 1993). Mosapride was also reported to stimulate upper gastrointestinal motility in vivo and in vitro (Mine et al., 1997). On the other hand, cisapride hydrate (cisapride) was reported to stimulate motor activity not only in the stomach but also in the colon in conscious dogs (Yoshida et al., 1991). Although cisapride was only prescribed for the treatment of reflux oesophagitis or heartburn in the United States, cisapride was thought to be useful in improving patients with constipation as well as functional dyspepsia. Itopride was thought to be useful in improving patients with constipation because of its side effects, namely, heart rhythm disturbances, including QT prolongation, syncope, and serious ventricular arrhythmias such as torsades de pointes (Barbey et al., 2000). Under this situation, new gastroprokinetic prokinetic agents are required for the remedy of functional bowel disorders such as functional constipation. A novel 5-HT₄ receptor agent, tegaserod, was recently accepted in the United States as an agent for the female irritable bowel syndrome patients complaining of constipation. However, there are little useful data showing the stimulatory action of clinically available gastroprokinetics on colonic motility.

Therefore, we investigated the effects of itopride on colonic motility and in vitro and in vivo transit in comparison with cisapride and mosapride, to determine the possibility of using itopride as a remedy for functional bowel disorders as well as functional dyspepsia.

Materials and Methods

Animals and Housing Conditions. Male Hartley guinea pigs (Japan SLC, Inc., Hamamatsu, Japan), weighing 329 to 804 g; beagle dogs of either sex (Oriental Yeast Co., Ltd., Tokyo, Japan), weighing 7 to 14 kg; and male Sprague-Dawley rats (Charles River Japan Inc., Hino, Japan), weighing 213 to 281 g, were used. Before the experimental phase, animals were housed under standard controlled environmental conditions at 20–26°C and 30 to 70% humidity, with 12-h light/dark cycles and food and water available ad libitum. Guinea pigs and rats were allowed at least 1 week to acclimate to the laboratory conditions before the experiments were performed, and dogs were allowed at least 2 weeks. Animals with normal appetite and stool consistency were used in the experiments. All experiments were conducted in accordance with the guideline established by the
Contractile Activity in Isolated Guinea Pig Colon. Guinea pigs were stunned by a blow on the head and exsanguinated. Segments (8 cm) of proximal colon in the distance of 5 cm from the ileo-cecal junction and distal colon in the distance of 5 cm from the anus were dissected, and the luminal contents were washed out. The preparations were horizontally mounted in a 50-ml organ bath containing Krebs’ solution (composition 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 2.6 mM CaCl2, 25.0 mM NaHCO3, and 11.1 mM glucose) bubbled with a mixture of 95% O2 and 5% CO2. Spontaneous contractions were evoked according to a modified method of Bulbring and Lin (1958).

The oral and aboral ends of the colonic segment were fastened over to the respective tubes in the organ bath. The oral end was connected to a syringe pump (STC-525, TERUMO, Tokyo, Japan). The intraluminal pressure was measured at the aboral side by connecting to a pressure transducer (TP-400T, Nihon Kohden Co., Tokyo, Japan) and recorded in the computer system. The intraluminal pressure was applied to the colon by infusing Krebs’ solution into the colon with the use of the syringe pump at the speed of 2 ml/min. When long-lasting peristalsis was triggered as the visible contractions composed of peristaltic and segmental contractions, intraluminal pressure was considered to reach the threshold pressure and then the infusion was stopped to maintain the threshold pressure. The mean threshold pressure was about 3 cm H2O. Macroscopically, high- and low-amplitude contractions could be seen representing peristaltic and segmental motility, respectively. After this regular peristalsis was confirmed in the preparations, a single concentration of test drug was applied to the serosal side of the segment. For quantitative analysis, the contractions whose amplitudes were greater than 50 or were 10 to 50% of the highest contractions observed for the 15 min before application of the drugs were defined as peristaltic contractions or segmental contractions, respectively. The number of each contraction was counted using computer software (Eight Star; Star Medical, Tokyo, Japan), and the increase in frequency was expressed as the percentage of change of the frequency over the 15-min period after application of the drug compared with that in the presubstitution period.

Contractile Activity in Conscious Dogs. Dogs were deprived of food 18 h before surgery with free access to water. Under general anesthesia with a mixture of nitrous oxide, oxygen, and enflurane (Ethrane; Abbott Laboratories, Chicago, IL), eight strain gauge force transducers (type 1215; Star Medical) were implanted into the serosal side of the gastric body, gastric antrum, duodenum, upper and middle jejunum, ileum, and ascending and descending colon, in a direction that made it possible to measure circular muscle contractions. The sites of the implantation of the gastric body, gastric antrum, duodenum, upper and middle jejunum, ileum, and ascending and descending colon were 5 cm distal to the cardia, 5 cm proximal to the pylorus, 7 cm distal to the pylorus, 50 cm distal to the pylorus, in the middle of small intestine, 15 cm proximal to the cecum, 10 cm distal to the cecum, and nearest to the anus, respectively. The free end of the transducer was brought out through a skin incision between the scapulae and protected with a jacket. A silicon tube (2.0-mm o.d.; Kaneka Medix Corporation, Osaka, Japan) was inserted into the external jugular vein and secured onto the adjacent skin as a route for the intravenous injection of the test drugs. The animals were allowed to recover for at least 10 days after this surgery before the commencement of the experiments.

The free end of the strain gauge force transducer was connected to an amplifier (FA-01; Star Medical) and contractile activity was recorded on a pen-writing recorder (WR-3701; GRAPHTEC, Tokyo, Japan) and simultaneously stored in a computer for quantitative analysis. The test drugs or solvent was given intravenously via the indwelling silicon tube after postprandial gastrointestinal motility became stable (more than 2 h after feeding). Drug was administered only once per day. Data for each contractile motor activity were stored continuously and then analyzed by means of a gastrointestinal motility measuring system (FA-01, FS-08M, and FB-01; Star Medical). The motor index was calculated by integrating the area under the contractile waves every 30-min period and expressed as the percentage of change from the preadministration period.

Colonic Luminal Transit in Guinea Pigs and Rats. Colonic luminal transit in guinea pigs and rats was examined with a slight modification of the reported method (Ueda et al., 1969). Each animal was anesthetized with pentobarbital sodium at 50 mg/kg i.p. and the cecum was exposed by laparotomy. A polyethylene tube (1.2 mm o.d.; Natsume Seisakusho Co., Ltd., Tokyo, Japan) was inserted from the small incision in the cecum to the beginning of the colon. The other end of the tube came out through the back. The animals were allowed to adapt to individual cages for more than 3 days.

Animals were deprived of food overnight before experiment. Test drugs were given orally. A marker [barium sulfate, 60% (w/v), 0.5 ml/animal] was administered through the colonic cannula 30 min later for the guinea pigs but immediately for the rats. The guinea pigs and rats were euthanized by cervical dislocation at 30 and 60 min, respectively, after administration of the marker. The colon was removed, and the length from the colo-cecal junction to the front traveling edge of the barium sulfate was measured. Colonic luminal transit was expressed as the percentage of distance traversed to the total length of the colon.

Gastric Emptying in Rats. A solution of 0.05% (w/v) phenol red in aqueous carboxymethyl cellulose [4.5% (w/v)] was used as a test meal. After an 18 h fast, test drug was administered orally and then the test meal was given 30 min later. Each rat was sacrificed at 15 min after the test meal administration, and the stomach was removed immediately.

The removed stomach containing residual phenol red solution was incised in 20 ml of distilled water and shaken for 10 min. The pieces of stomach were rinsed and discarded, and then the recovered phenol red solution was made up to a total volume of 40 ml with distilled water. The recovered phenol red solution was centrifuged at 3000 rpm for 10 min, and 3 ml of the supernatant was added to 2 ml of 1 M NaOH to develop the color. The absorbance at 558-nm wavelength of the solution was measured with a spectrophotometer (U-2000; Hitachi, Tokyo, Japan).

The gastric emptying (G.E.) for each rat was calculated according to the following formula: G.E. (%) = (1 - (amount of residual phenol red recovered 15 min after test meal administration)/average amount of phenol red present in the stomach immediately after test meal administration) × 100.

Drugs. Itopride, cisapride, and mosapride were synthesized by Abbott Japan Co., Ltd. Barium sulfate and phenol red were purchased from Wako Pure Chemicals (Osaka, Japan). Carboxymethyl cellulose was from Nakalai Tesque, Inc. (Kyoto, Japan).

Itopride was dissolved in saline or distilled water. Cisapride and mosapride were dissolved in a solution containing 1% lactic acid.

Statistical Analyses. All results are presented as means ± S.E.M. Statistical analysis of the in vivo data were performed with Williams’ multiple range test. For the in vitro experiments, Student’s t test was used to test the significance of any differences. Probability values less than 0.05 were considered statistically significant.

Results

Effects on Peristaltic and Segmental Motility in the Isolated Guinea Pig Colon. Stable contractions were induced by applying the threshold pressure to the isolated guinea pig colon. Figure 1 shows the typical effects of itopride, cisapride, and mosapride at 10 μM on the colonic contractions. Constrictions with higher amplitude were considered to be peristaltic contractions that started from the oral end and migrated to the aboral end. Constrictions with lower amplitude and higher frequency were considered to be...
segmental contractions that did not migrate. Itopride significantly increased the frequency of peristaltic and segmental contractions in the proximal and distal colon in a concentration-dependent manner (Figs. 1A and 2). The amplitude of peristaltic contractions was not changed by itopride (Fig. 1A). On the other hand, cisapride and mosapride reduced the frequency of peristaltic contractions in the proximal and distal colon (Figs. 1B and C, and 2). The inhibitory effects were significant at 10 μM. With respect to segmental motility, itopride significantly increased the frequency of contractions with enhancement of amplitude (Figs. 1A and 2B). Itopride and mosapride produced similar responses in the proximal and distal colon (Figs. 1A and C, and 2B) but cisapride exerted distinct effects on segmental motility between the proximal and distal colon. Cisapride increased the frequency of segmental contraction in the distal colon only at the highest concentration of 10 μM. In contrast, cisapride significantly increased the frequency of segmental contraction in the proximal colon up to 1 μM but significantly decreased the frequency at 10 μM (Figs. 1B and 2B).

Effects on Gastrointestinal Motility in Conscious Dogs. Figure 3 shows the stimulatory effects of itopride (10 mg/kg i.v.), cisapride (0.3 mg/kg i.v.), and mosapride (3 mg/kg i.v.) on the postprandial gastrointestinal motor activity in conscious dogs. As shown in Figs. 3A and 4A, itopride dose dependently stimulated gastrointestinal motility from the stomach through the colon, although the stimulatory effect on the small intestine was weaker than for other regions. Itopride enhanced contractile activities in the gastric antrum, duodenum, and upper jejunum significantly at 3 mg/kg (Fig. 4A). In the middle jejunum, ileum, ascending colon, and descending colon, itopride stimulated contractile activities significantly at 10 mg/kg (Fig. 4A). Cisapride enhanced antral, ileal, and colonic motility significantly at 0.03, 0.1, and 0.3 mg/kg, respectively (Figs. 3B and 4B). On the other hand, mosapride did not enhance colonic motility up to 3 mg/kg, although it significantly stimulated antral and ileal motility at 1 and 3 mg/kg, respectively (Figs. 3C and 4C). In addition, itopride produced giant migrating contractions, which were high-amplitude, rapidly migrating contractions that propelled colonic contents to the rectum (Sarna et al., 1984; Karaus and Sarna, 1987), followed by defecation in some dogs at 10 mg/kg, but this was not seen with cisapride nor mosapride. Throughout this experiment, no behavioral changes were observed.

Colonic Transit in Guinea Pigs and Rats, and Gastric Emptying in Rats. Itopride accelerated colonic transit dose dependently in both guinea pigs and rats and significant acceleration was observed at 10 mg/kg p.o. in both animals (Figs. 5A and 6A). However, cisapride did not affect colonic transit significantly up to 10 mg/kg in guinea pigs and rats.
Moreover, mosapride slightly delayed colonic transit in rats, the delay being statistically significant at a dose of 1 mg/kg and above (Fig. 6C).

In rats, itopride, cisapride, and mosapride all enhanced gastric emptying dose dependently (Fig. 7), with statistically significant effects being observed at doses of 10, 1, and 1 mg/kg, respectively. Therefore, itopride exerted stimulatory effects on gastric emptying and colonic transit at the same dose, whereas cisapride and mosapride were found not to accelerate colonic transit at 10 mg/kg p.o., a dose that was 10 times higher than that required to accelerate gastric emptying.

**Discussion**

Itopride has antiacetylcholinesterase (AChE) activity as well as dopamine D2 receptor antagonist activity and is used for the symptomatic treatment of functional dyspepsia (Iwanaga et al., 1990, 1994). It is well established that the M₃ receptor exists on the smooth muscle layer throughout the whole gut and that acetylcholine released from the enteric nerve endings stimulates the contraction of smooth muscle through the M₃ receptor. Therefore, we predicted that itopride might have a colonic prokinetic action, but there was limited data with respect to the effects of itopride on colonic motility. In this study, we evaluated the effects in vitro and in vivo, in comparison with the benzamide derivatives cisapride and mosapride, which both have a gastroprokinetic action with a 5-HT₄ receptor agonist effect.

The in vitro study exhibited that cisapride and mosapride increased segmental motility but inhibited the peristaltic motility in the isolated guinea pig colon. Buchheit and Buhl (1991, 1993) and Buchheit et al. (1992) reported that benzamides induced the inhibition of circular muscle contraction and an increase in longitudinal muscle activity via 5-HT₄ receptor activation. Peristalsis is known to consist of the circular muscle contraction at oral side and the relaxation at aboral side in the intestine. Although longitudinal muscle contraction during the preparatory phase of peristalsis precedes circular muscle contraction, the main propulsive drive in the peristaltic reflex comes from the aborally directed contraction of the circular muscle during the emptying phase (Kosterlitz et al., 1956). Therefore, the peristalsis requires not only coordination between contraction and relaxation in circular muscle but also coordination between circular muscle and longitudinal muscle. Cisapride and mosapride might collapse the coordinated peristalsis composed of peristaltic and segmental motility due to their relaxant effects on the smooth muscles. This could be responsible for their failure to have a stimulatory action on colonic transit in guinea pigs.
and rats. Cisapride and mosapride both decreased peristaltic motility in the isolated guinea pig colon, but mosapride increased segmental motility in a concentration-dependent manner. Enhancement of segmental motility not accompanied by peristaltic motility may interfere with colonic transit in vivo. Stimulatory effect of mosapride on the segmental motility seems stronger than that of cisapride and this explains that mosapride did not accelerate colonic transit. In guinea pigs, cisapride tended to accelerate colonic transit despite the in vitro inhibitory effect on the peristaltic motility. The stimulatory effect of cisapride on the colonic transit may be ascribed to the enhancement of gastric emptying and intestinal motility. On the other hand, itopride enhanced both the peristaltic and segmental motility in the isolated guinea pig colon. It is true that stimulatory effects on the isolated gastrointestinal motility do not always produce the propulsion of the luminal contents, but it was confirmed that the stimulatory effects of itopride successfully increased colonic transit in guinea pigs and rats.

In vivo studies using dogs and rats indicate gastrointestinal region selectivity of the prokinetic action of each agent. As a result, cisapride and mosapride selectively stimulated upper gastrointestinal motility, compared with itopride. In conscious dogs, cisapride significantly stimulated antral motility and the effective dose for antral motility was 10 times less than that for colonic motility. Furthermore, mosapride failed to stimulate colonic contractions. Our findings of the selectivity of cisapride and mosapride for the stomach are consistent with the study by Mine et al. (1997). Gastrointestinal region selectivity for cisapride and mosapride was also demonstrated in rats. Cisapride and mosapride had no stimulatory effect on the colonic transit at the dose that was effective to enhance the gastric emptying. On the contrary, itopride had stimulatory effects on all sites of the canine gastrointestinal tract from the stomach through the colon. The effective dose of itopride stimulating the canine colon was not more than 3 times greater than that stimulating antral motility. In addition, itopride enhanced gastric emptying and colonic transit at the same dose, 10 mg/kg, in rats.

Although stimulation of gastrointestinal motility by itopride is ascribed to activation of the cholinergic drive based on D₂ receptor blocking and anti-AChE activity (Iwanaga et al., 1990, 1994), the stimulatory action on colonic motility seems mainly due to anti-AChE activity. Because gastrointestinal smooth muscle is directly stimulated by ACh through the activation of the M₃ receptor irrespective of the gastrointestinal site and animal species, it is apparent that itopride can stimulate colonic contractions as well as antral contractions in all species. On the other hand, benzamides, such as cisapride and mosapride, stimulate gastrointestinal motility via activation of the 5-HT₄ receptor (Yoshida et al., 1991, 1993; Mine et al., 1997). The 5-HT₄ receptor is located on smooth muscle and the excitatory neurons, which mediate relaxation and contractions, respectively. With regard to

![Fig. 5. Effects of itopride (A), cisapride (B), and mosapride (C) on colonic transit in guinea pigs. Colonic transit was calculated as the percentage of the distance traveled by an administered intracolonic marker relative to the total length of the colon for 30 min after marker administration. Drugs were given orally 30 min before administration of the marker. Each column represents the mean ± S.E.M. of 10 guinea pigs. **p < 0.01 compared with control (vehicle).](image)

![Fig. 6. Effects of itopride (A), cisapride (B), and mosapride (C) on colonic transit in rats. Colonic transit was calculated as the percentage of the distance traveled by an administered intracolonic marker relative to the total length of the colon for 60 min after administration of the marker. Drugs were given orally just before administration of the marker. Each column represents the mean ± S.E.M. of 10 to 12 rats. *p < 0.05; **p < 0.01 compared with control (vehicle).](image)
Stimulation of gastrointestinal motility by itopride is ascribed to the activation of the cholinergic drive based on D₂ receptor blocking and anti-AChE activity, but the stimulatory action on colonic motility seems mainly due to anti-AChE activity. D₂ receptor agonists domperidone and metoclopramide are also accepted as gastroprokinetic agents. Domperidone is a selective D₂ receptor antagonist and metoclopramide possesses 5-HT₄ receptor agonistic activity as well as D₂ receptor antagonistic activity. Although domperidone was reported to enhance the gastric motor activity but not to stimulate small intestinal and colonic motor activity in conscious dogs (Miyashita et al., 1991).

A potent anti-AChE inhibitor, neostigmine is well known to improve postoperative ileus, and it is worth while to notice that such old drug is recently prescribed in patients with acute colonic pseudo-obstruction. However, the use of neostigmine is limited because of its side effects. Basically, neostigmine dose dependently and significantly enhanced the antral and coline motor activity in dogs at 30 and 100 μg/kg i.v. but tended to increase blood pressure and to suppress respiration (Kishibayashi et al., 1994). Also, dogs often collapsed when neostigmine was intravenously administered at 1000 μg/kg (Iwanaga et al., 1990). Itopride did not cause any adverse effects such as salivation, snivel, vomiting, and diarrhea based on anti-AChE action throughout our experiments. The inhibitory action of itopride on AChE was 100 times stronger than that on butyrylcholinesterase, whereas the inhibitory action of neostigmine on AChE was 10 times stronger than that on butyrylcholinesterase (Iwanaga et al., 1994). The selectivity on AChE seems a cause of the differences of safety window. Several studies on proarrhythmic potential of itopride demonstrate that itopride is devoid of QT prolongation at least at the present dose levels (Kakiuchi et al., 1997). Furthermore, itopride was reported not to penetrate into the blood-brain barrier (Yamada et al., 1994).

In conclusion, it is anticipated that itopride could be of value as a safe and feasible alternative to other existing prokinetic agents for the treatment of functional bowel disorders such as functional constipation and constipation-dominant irritative bowel syndrome, without an excessive increase in dose.

### References


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**Fig. 7.** Effects of itopride (A), cisapride (B), and mosapride (C) on gastric emptying in rats. Gastric emptying was measured by the phenol red method. 0.05% phenol red was orally given at 30 min after oral drug administration. Gastric emptying was assayed at 15 min after the administration of phenol red. Each column represents the mean ± S.E.M. of 8 to 11 rats. *, p < 0.05; ***, p < 0.01 compared with control (vehicle).

### Notes

- **itopride**
  - In conclusion, it is anticipated that itopride could be of value as a safe and feasible alternative to other existing prokinetic agents for the treatment of functional bowel disorders such as functional constipation and constipation-dominant irritative bowel syndrome, without an excessive increase in dose.

- **References**


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