

Investigation into the Effects of Mosapride on Motility of Guinea Pig Stomach, Ileum, and Colon

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Mosapride citrate (Mosapride) is a new prokinetic agent that enhances the gastrointestinal (GI) motility by stimulation of 5-HT₄ receptors. This agent stimulates acetylcholine release from enteric cholinergic neurons in the GI wall. It was reported in several studies that mosapride selectively enhanced the upper, but not lower, GI motor activity. However, in these studies other 5-HT₄ receptor agonists exerted stimulating effects on the motility of the colon. Moreover, it is well known that the receptors of 5-HT₄ are also located in the colon. The purpose of this study was to estimate the effect of mosapride on the motility of the stomach, ileum and colon in the guinea pig and to investigate whether or not mosapride influenced the colonic motility. Mosapride significantly increased the amplitude of the contraction waves in the guinea pig stomach by electrical stimulation. In addition, it significantly increased the number of peaks, the area under the curve and the propagation velocity of the peristaltic contraction of the guinea pig ileum in a concentration dependent fashion. Mosapride also significantly shortened the transit time of the guinea pig colon. Accordingly, we concluded that mosapride exerted prokinetic effect on the entire GI tract of the guinea pig. Based on the possibility of similar results in humans, we suggest the potential use of mosapride for lower GI motor disorders such as constipation and upper GI motor disorders such as gastroesophageal reflux disease or gastroparesis.

Key Words: Mosapride, gastrointestinal motility, 5-hydroxytryptamine

Received September 12, 2002

Accepted May 28, 2003

This study was supported in part by a research fund from Daewoong Co.

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INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is an important brain neurotransmitter which is involved in depression, migraine, and other neuro-psychiatric illnesses.¹ But about 95% of 5-HT is found in the gastrointestinal (GI) tract, where it has been estimated that the total serotonin content is about 10 mg; 90% is in enterochromaffin cells and 10% in enteric neurons.¹ The remaining 5% of 5-HT is found in the brain.¹ Virtually all of 5-HT in the blood is derived from the GI tract.¹⁻³ In addition 5-HT receptors are present on enteric neurons, enterochromaffin cells, GI smooth muscles and possibly on enterocytes and immune tissues.^{1,4}

In the gut, 5-HT is released from enterochromaffin cells of the intestine by mechanical or vagal stimulation.^{1,5} It has diverse motor and sensory functions in the GI tract through submucosal and myenteric neurons that respond to 5-HT through a variety of receptors.¹ 5-HT initiates the responses as diverse as nausea, vomiting, intestinal secretion and peristalsis and it plays a role in bowel physiology as an enteric neurotransmitter.⁶

The multiple 5-HT receptor subtypes that have been cloned to date are the largest of all known neurotransmitter receptor families.⁷⁻¹⁰ The 5-HT₁, 5-HT₂, 5-HT₄, 5-HT₅, 5-HT₆, and 5-HT₇ receptor families are members of the super-family of G-protein-coupled receptors, while the 5-HT₃ receptor, on the other hand is a ligand-gated ion channel. The motility of the GI tract is either enhanced or inhibited via the multiple 5-HT receptor subtypes.⁸ 5-HT can stimulate the cholinergic neurons to release acetylcholine, which results in smooth muscle contraction, or it can stimulate the inhibitory nitrenergic neurons to release nitric oxide,

which results in smooth muscle relaxation.^{1,6,11} It is generally accepted that 5-HT₁ receptor is a relaxant in the smooth muscle of the GI tract, while 5-HT₂, 5-HT₃, and 5-HT₄ receptors are contractile.⁸ Among these 5 HT receptors, the 5-HT₄ receptor, in particular, located in the myenteric plexus may participate in the GI motility.⁸

Mosapride citrate (Mosapride), a substituted benzamide, is a relatively new selective 5-HT₄ receptor agonist although its metabolite (M1) has an affinity for 5-HT₃ receptor and has proved to be a 5-HT₃ antagonist.¹²⁻¹⁴ It is free of dopamine D₂ receptor antagonistic properties.¹² In several experimental studies, mosapride selectively stimulates the upper, but not lower GI motility.¹⁵⁻¹⁷ Mine Y, et al. reported that mosapride selectively stimulated the upper GI motility both *in vivo* and *in vitro*, compared with other 5-HT₄ receptor agonists.¹⁶ But we questioned why mosapride did not influence the colonic motility, because 5-HT₄ receptors were found in the colon as well as the stomach and ileum.⁸ Therefore, we studied whether or not mosapride exerted an effect on colonic motility, and on the motility of the stomach and ileum of the guinea pig.

MATERIALS AND METHODS

Animals

Male guinea pigs of Hartley strain (Nihon SLC INC., Shizuoka) weighing 250 g were individually housed at 22-24°C.

Drugs

The drugs used in this study were mosapride (Daewoong Pharm Co, Seoul, Korea), tetrodotoxin and atropine (both Sigma Chemical Co, St. Louis, MO, USA), and GR113808 (Tocris Cookson Inc, Ellisville, MO, USA).

Experimental apparatus

BIOPAC TSD 105 (BIOPAC systems Inc, Santa Barbara, CA, U.S.A.) was used to estimate the tension of the circular muscle, and BIOPAC MP 100 (BIOPAC systems Inc, Santa Barbara, CA,

U.S.A.) was used to analyze the data. A peristaltic pump (Masterflex 7523-30 with cartridge 3519-85, Cole-Palmer, Chicago, IL, U.S.A.) was used to induce the peristaltic contraction wave. The method designed by Tonini, et al.¹⁸ was used as the experimental bath for the estimation of the peristalsis (Fig. 1).

Electrically evoked contraction in the guinea pig antral muscle

Electrical stimulation was used to estimate the contractile activity of the circular muscle of the stomach. Guinea pigs were stunned by a blow to the head, and killed by cutting the carotid arteries. The stomach was extracted, and flushed clean with Krebs-Henseleit (K-H) solution (pH 7.4, NaCl 118 mM, KCl 4.8 mM, CaCl₂ 2.5 mM, KH₂PO₄ 1.2 mM, MgSO₄ 1.5 mM, NaHCO₃ 25 mM, glucose 11 mM). The antrum of the stomach was left and the remainder was removed. Then it was suspended immediately in organ bath containing K-H solution. The solution was maintained at 37°C and saturated continuously with 95% O₂ and 5% CO₂. The preparations were pinned on the mucosal side uppermost so that the mucosa and submucosa could be delicately removed to expose the underlying circular muscle layer. These muscle strips were 3 mm in width, and 10 mm in length. One side of the muscle strips was connected to electrode in a bath and the other side was connected to a transducer. A tension of 1 g was ap-

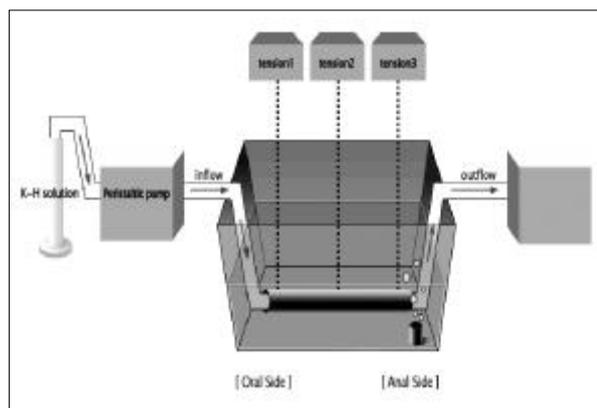


Fig. 1. The chamber for the peristaltic contraction study. The peristaltic chamber was used to evaluate the peristaltic activity that was induced by continuous intraluminal perfusion via a peristaltic pump.

plied, and the responses were recorded isometrically through a force displacement transducer (Fig. 2). Muscle strips of the antrum were left to reach equilibrium in K-H solution for approximately 60 min prior to the start of the experiment. Then the muscle strips were stimulated at 35 V with 0.2 Hz square-wave pulses (1 ms in duration) from an electrical stimulator via an electrode connected to one side of the muscle strips. Ten stimulations were given at 1 min intervals, and the mean amplitude of the last three stimulations was measured. Bathing solution was changed with new K-H solution, and thereafter mosapride was administered at increasing concentrations (10^{-9} - 10^{-6} M) without washing between concentrations. After observation of the response for 5 min following the administration of each concentration, ten stimulations were done at 1 min intervals. The mean amplitude of the last three stimulations, of the 10 stimulations, was measured.

Estimation of the peristaltic contraction in the guinea pig ileum

The abdominal cavity was opened and the distal ileum was excised 15 cm from the ileocecal junction. The mesenteric attachment was trimmed away, and flushed clean with K-H solution. Then

it was placed immediately into a bath containing K-H solution. The solution was maintained at 37 °C and saturated with 95% O₂ and 5% CO₂. The mechanical activity of the circular muscle in the fixed guinea pig ileum was monitored using three small clips arranged at intervals of 2.5 cm. These were attached via the serosal surface to the underlying circular muscle of the ileum, and were connected via thread to independent tension transducers. Initial tension was routinely set to 1g. Tissues were left to equilibrate in K-H solution for approximately 60 min prior to the start of the experiment. Then K-H solution was pumped (0.4 mL/min) into the lumen of the ileal segment for 20 min through the peristaltic pump, after which peristaltic activity could be induced. After setting the mosapride concentrations to 10^{-9} M, 10^{-8} M, 10^{-7} M, and 10^{-6} M, each concentration was added continuously and increasingly in the bath solution and in the ileum lumen for 20 min without washing between concentrations. The activity of the peristaltic contraction was determined by measurement of, 1) the number of peaks, 2) the area under curve, and 3) the propagation velocity of the peristaltic contraction. The number of peaks and the area under the curves of the last 10 min was calculated among the duration of total 20 min. The method for the estimation of the velocity of the peristaltic waves is described in Fig. 3.

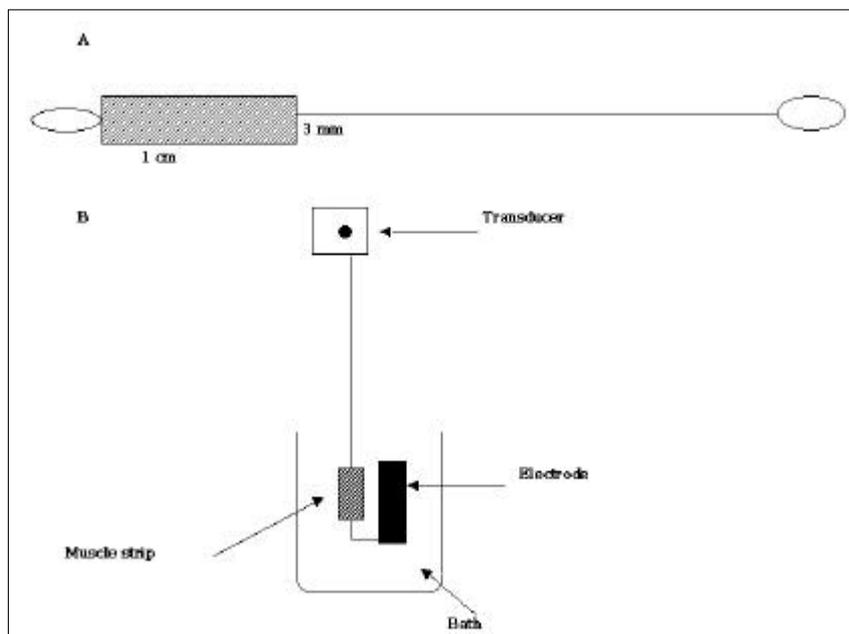


Fig. 2. Electrically evoked contractions in the antral muscle. (A) The antral muscle strips were 3 mm in width, and 10 mm in length. (B) One side of muscle strips was connected to electrode in bath after making ring via thread. The other side of muscle strips with long thread was connected to transducer. The muscle strips were stimulated at 35 V with 0.2 Hz square-wave pulses (1 ms in duration) from electrical stimulator via electrode connected on one side of the muscle strips.

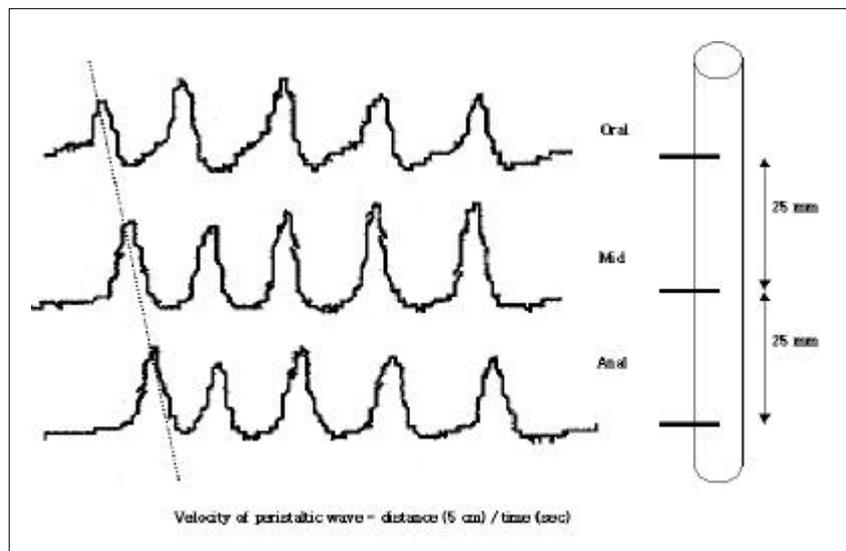


Fig. 3. The propagation velocity of the peristaltic contraction. The velocity was measured by dividing the distance from the oral to the anal side (5 cm) by the time (sec) that first peristaltic wave moved from the oral to anal side.

To evaluate the mechanism of mosapride's action, additional experiments were performed in the presence of tetrodotoxin (10^{-6} M), atropine (10^{-6} M), and GR113808 (10^{-7} M). After the control study, mosapride (10^{-8} M) was administered, and thereafter tetrodotoxin or atropine was added to both the perfusion fluid and the chamber fluid. GR113808 was administered 10 min before mosapride was administered.

Estimation of the transit time in the guinea pig colon

The method for the estimation of colonic transit time is described in Fig. 4. The distal colon (approximately 10 cm from the anus) was excised, flushed clean with K-H solution, and immediately placed into a bath containing K-H solution at 37 °C saturated with 95% O₂ and 5% CO₂. Both ends of the colon were connected to the two sides (oral and anal sides) in the chamber. After stabilization for about 60 min, artificial feces (length 10 mm, width 4 mm) were inserted into the oral side of the lumen, and K-H solution was pumped (0.4 mL/min) into the lumen of the colon. Artificial feces could then be moved from the oral to the anal side of the colon. Total 10 cm was observed and the time taken for moving about 2 cm was measured. Immediately after the control test, mosapride (10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} M) was applied into the bath and the lumen in the same way, by

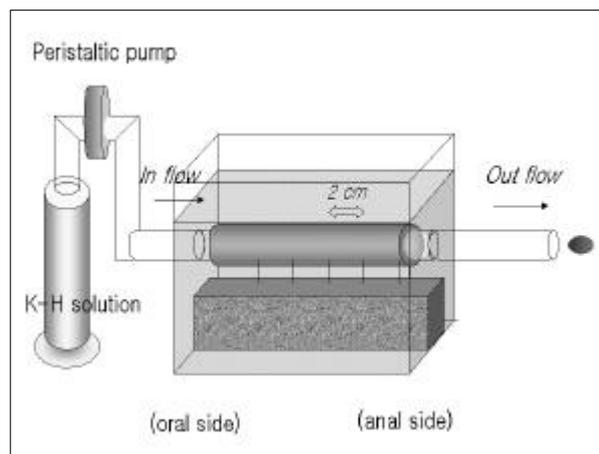


Fig. 4. The chamber for the colonic transit time study. Artificial feces were inserted into the oral side of lumen, and moved toward the anal side by intraluminal perfusion via a peristaltic pump.

increasing the concentrations without washing between concentrations. For each sample, the time taken for moving 2 cm was measured and the last four measurements were selected by value of the means.

Statistics

The result for each variable was represented as a percentage of the measurements before administration of mosapride (% control), and recorded as the mean with the standard error. Wilcoxon signed rank test was used for statistical analysis,

and the significance level was set at $p < 0.05$.

RESULTS

Effects of mosapride on the electrically evoked contraction of the guinea pig stomach

Mosapride (10^{-9} - 10^{-7} M) significantly increased the mean amplitude of the electrically evoked antral muscle compared to the controls ($n=6$, $p < 0.05$). At mosapride concentrations of 10^{-9} M, 10^{-8} , 10^{-7} M, and 10^{-6} M, the results were $130.93 \pm 11.47\%$, $126.3 \pm 8.1\%$, $115.8 \pm 5.5\%$, $102.2 \pm 3.8\%$, respectively (Fig. 5).

Effects of mosapride on the peristaltic contraction of the guinea pig ileum

The number of peaks of the peristaltic contraction

Peristaltic waves with multiple peaks were induced when K-H solution was administrated through the peristaltic pump. The perfusion times of K-H solution free of mosapride and containing mosapride were both 20 min. The mean values of the number of peaks over the last 10 min at the oral, mid and anal transducers were measured, and each value was added ($n=5$). Mosapride (10^{-9} - 10^{-6} M) significantly increased the number of peaks of the peristaltic contractions in a concentration dependent manner (Fig. 6) ($p < 0.05$). At

mosapride concentrations of 10^{-9} M, 10^{-8} M, 10^{-7} M, and 10^{-6} M, the results were $143.0 \pm 5.2\%$, $159.8 \pm 15.8\%$, $183.9 \pm 13.4\%$, $202.0 \pm 15.4\%$, respectively (Fig. 7).

The area under the curve of the peristaltic contraction

The area under the curve was measured at the oral, mid, and anal tension transducers to investigate the effect of mosapride on the motor

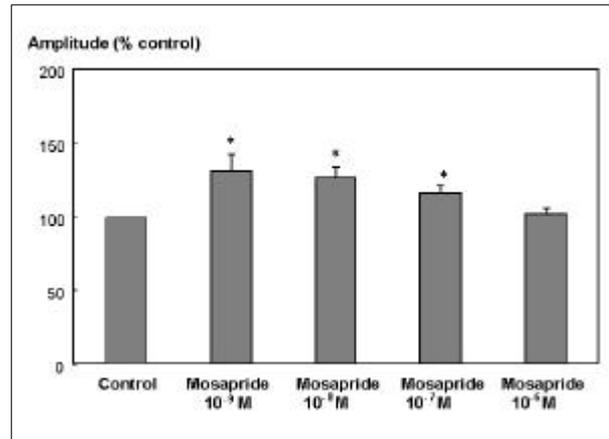


Fig. 5. The effect of mosapride on the contractility of the antral muscle by electrical stimulation. Mosapride (10^{-9} - 10^{-7} M) significantly increased the amplitude of the antral muscle than control after electrical stimulation. Data are expressed as % change from the amplitude of the antral muscle of control. Values are mean \pm standard error of 6 experiments. *Significant increase above control levels, $p < 0.05$.

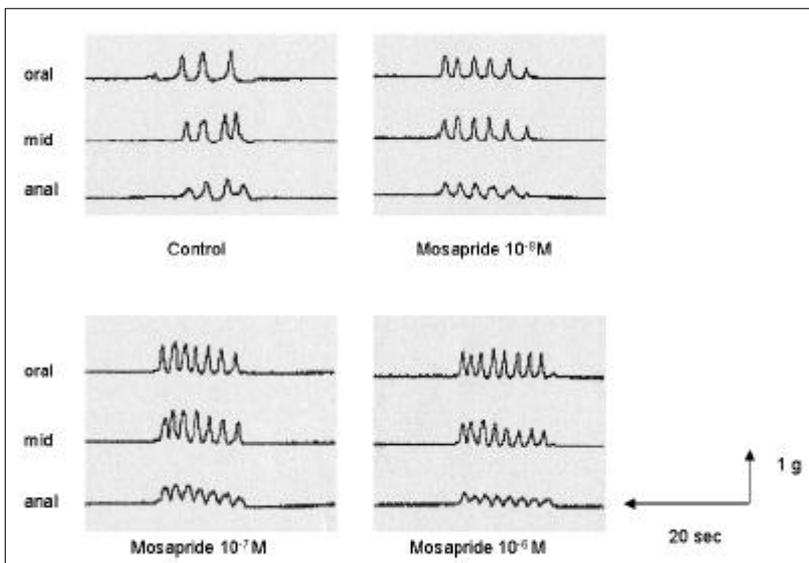


Fig. 6. The records of the peristaltic activity in the guinea pig ileum. The guinea pig ileum normally exhibited multi-peaked peristaltic contractions by continuous perfusion of K-H solution via a peristaltic pump. When mosapride was administrated to the perfusion fluid and the chamber fluid, it increased the number of peaks and the area under curve of the peristaltic contraction.

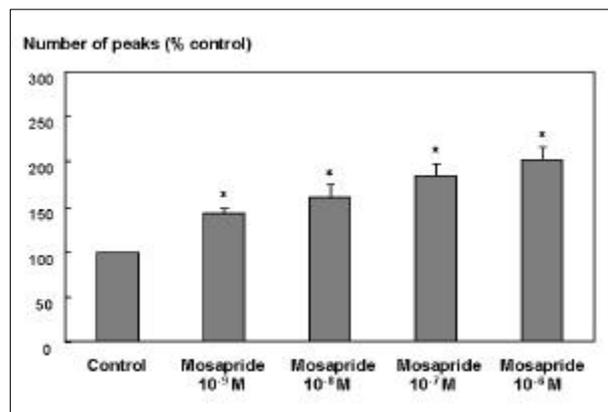


Fig. 7. The effect of mosapride on the number of peaks of the peristaltic contraction. Mosapride (10^{-9} - 10^{-6} M) significantly increased the number of peaks of the peristaltic contraction in concentration-dependent fashion. Data are expressed as % change from the number of peaks of control. Values are mean \pm standard error of 5 experiments. *Significant increase above control levels, $p < 0.05$.

activity of the circular muscle during induction of peristalsis ($n=5$). The mean values of the area under curve of the last 10 min were analyzed among the total duration of 20 min. Mosapride (10^{-8} - 10^{-6} M) significantly increased the area under the curve measured at the oral tension transducers compared to the controls ($p < 0.05$). At mosapride concentrations of 10^{-9} M, 10^{-8} M, 10^{-7} M, and 10^{-6} M, the results were $141.7 \pm 15.0\%$, $165.7 \pm 20.1\%$, $181.0 \pm 15.4\%$, $182.6 \pm 16.8\%$, respectively (Fig. 8). In addition, some concentrations of mosapride significantly increased the area under the curve measured at the mid and anal tension transducers, although the results were not dose-dependent. The magnitude of the increase of the area under the curve had a tendency to decrease as the measuring site became more distal. The mean amplitude of the contraction waves was not changed significantly ($p > 0.05$).

The velocity of the peristaltic contraction

The propagation velocity of the peristaltic contraction was increased in proportion to the mosapride concentrations. At mosapride concentrations of 10^{-8} M, 10^{-7} M, and 10^{-6} M, the results were $161.4 \pm 5.4\%$, $196.1 \pm 10.1\%$, and $259.1 \pm 22.4\%$, respectively (Fig. 9); all significantly increased compared with the controls ($n=6$, $p < 0.05$).

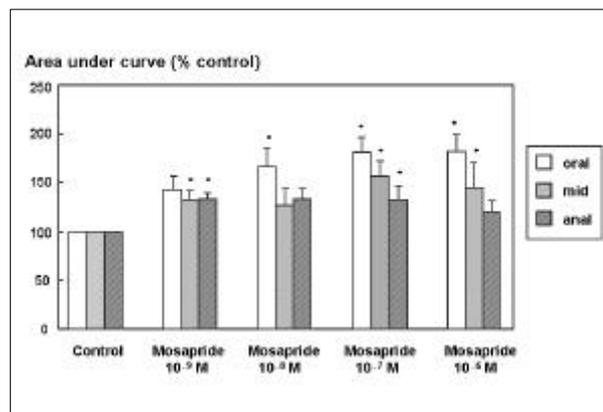


Fig. 8. The effect of mosapride on the area under the curve of the peristaltic contraction. Mosapride (10^{-9} - 10^{-6} M) significantly increased the area under the curve of the peristaltic contraction measured at oral tension transducers. Also, some concentrations of mosapride significantly increased the area under the curve measured at the mid and anal tension transducers, although the results were not dose-dependent. Data are expressed as % change from the area under the curve of the peristaltic contraction of control. Values are mean \pm standard error of 5 experiments. *Significant increase above control levels, $p < 0.05$.

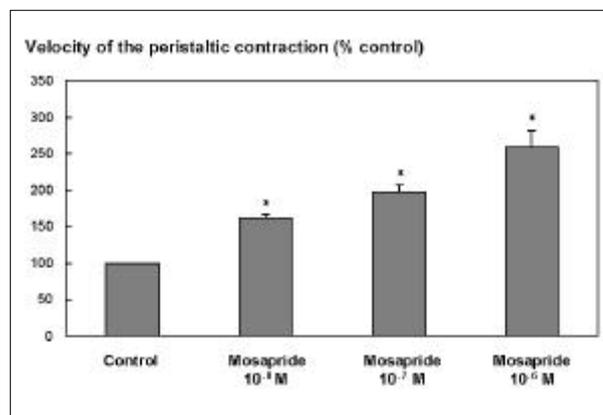


Fig. 9. The effect of mosapride on the propagation velocity of the peristaltic contraction. Mosapride (10^{-8} - 10^{-6} M) significantly increased the velocity in a concentration dependent manner. Data are expressed as % change from the velocity of the peristaltic contraction of control. Values are mean \pm standard error of 6 experiments. *Significant increase above control levels, $p < 0.05$.

Effects of tetrodotoxin, atropine, GR113808

Addition of tetrodotoxin (10^{-6} M) and atropine (10^{-6} M) in the perfusion fluid and the chamber fluid abolished and decreased the peristaltic contraction in mosapride-treated preparations, respec-

tively. Pretreatment with GR 113808 (10^{-7} M), a selective 5-HT₄ antagonist, did not affect the basal ileal peristaltic activity. However, the administration of mosapride did not increase the number of peaks or the area under the curve of the peristaltic contraction (Fig. 10).

Effects of mosapride on the motility of the guinea pig colon

At mosapride concentrations of 10^{-9} M, 10^{-8} M, 10^{-7} M, and 10^{-6} M, the colonic transit time were $83.0 \pm 2.6\%$, $65.61 \pm 5.2\%$, $58.7 \pm 8.6\%$, and $89.8 \pm 5.6\%$, respectively (Fig. 11); 10^{-9} - 10^{-7} M mosapride significantly decreased the colonic transit time compared with the controls ($n=5$, $p<0.05$).

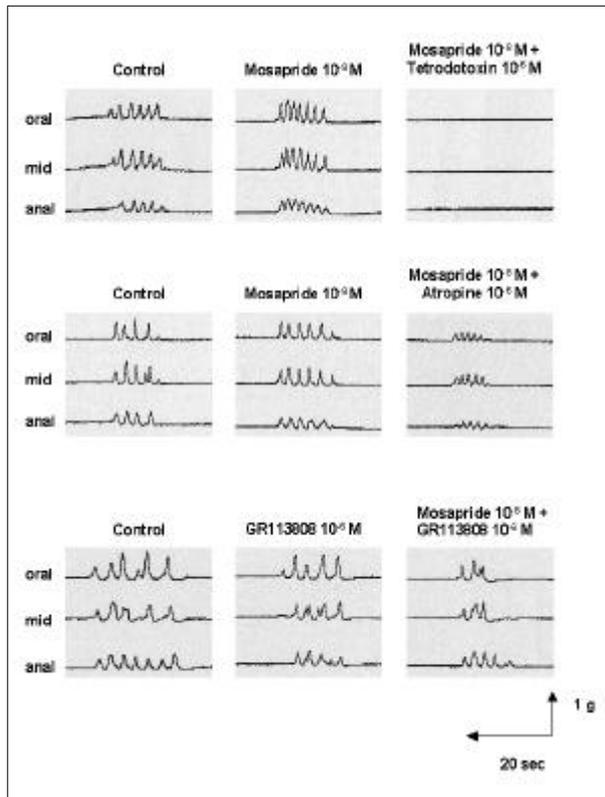


Fig. 10. The effect of tetrodotoxin, atropine, and GR113808 on the peristaltic activity in mosapride-treated preparation (guinea pig ileum). Addition of tetrodotoxin and atropine in the perfusion fluid and the chamber fluid abolished and decreased the peristaltic contraction, respectively. GR113808 did not affect the basal ileal peristaltic activity, and when mosapride was administered 10 min after addition of GR113808, mosapride did not increase the number of peaks and the area under the curve of the peristaltic contraction in the ileum.

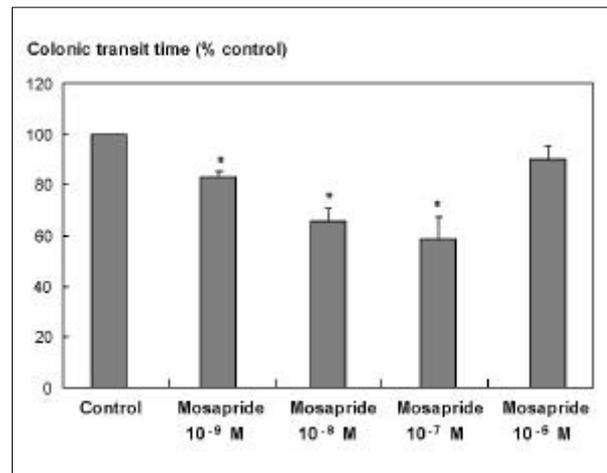


Fig. 11. The effect of mosapride on the colonic transit time. Mosapride (10^{-9} - 10^{-7} M) significantly decreased the time required that artificial feces moved from the oral to the anal side than control. Data are expressed as % change from the colonic transit time of control. Values are mean \pm standard error of 6 experiments. *Significant increase above control levels, $p<0.05$.

The higher concentration of mosapride (10^{-6} M) had no significant effect on the colonic transit time.

DISCUSSION

The 5-HT₄ receptors, first identified in fetal mouse collicular cell cultures and named by Dumuis, et al.¹⁹ are positively coupled to adenylyl cyclase in brain tissue²⁰ and smooth muscle.²¹ In 1990, Craig and Clarke pharmacologically identified the neuronal 5-HT₄ receptor in the guinea-pig ileum.²² The 5-HT₄ receptors are located on enterocytes, enterochromaffin cells, smooth muscle cells and neurons.²³ Neurons known to possess 5-HT₄ receptors include the intrinsic primary afferent neurons and the cholinergic interneurons that activate the excitatory and inhibitory neurons involved in peristalsis, and possibly visceral afferent neurons.²³

5-HT₄ receptor isoforms were first identified in the rat, in which two splice variants of the receptor, 5-HT_{4L} and 5-HT_{4S}, differ in the length and sequence of their C-termini,²⁴ which contain sites of phosphorylation by protein kinase. Recently, four variants of the human 5-HT₄ receptor; hs-

HT_{4(a)}, hs-HT_{4(b)}, hs-HT_{4(c)}, and hs-HT_{4(d)}, were identified.^{25,26} The 5-HT₄ receptors have been identified in a variety of central and peripheral tissues.²⁰ In the GI tract, stimulation of 5-HT₄ receptors potentiated the 'twitch' response in electrical field stimulation of the guinea pig ileum,^{22,27} and induced contraction of the guinea pig ileum²⁸ and distal colon.²⁹ When the gut is stimulated, the consequent release of 5-HT from mucosal enterochromaffin cells, stimulates the intrinsic primary afferent neurons via 5-HT₄ receptors and facilitates acetylcholine release from the neurons of the myenteric plexus.¹

Furthermore, the association of 5-HT₄ to the peristaltic reflex has been recognized. Grider JR, et al.³⁰ showed that the selective 5-HT₄ agonists added to the intestinal mucosa triggered the peristaltic reflex in human jejunal and rat and guinea pig colonic segments. They illustrated that the peristaltic reflex induced by mucosal stimuli was mediated by intrinsic sensory CGRP (calcitonin gene-related peptide) neurons activated by 5-HT released from enterochromaffin cells. They explained that 5-HT acted on 5-HT₄ receptors located on nerve terminals of intrinsic CGRP neurons, and that ultimately CGRP neurons relayed sensory stimuli to the same populations of interneurons coupled to the excitatory motor and inhibitory motor neurons.

The agonists of the 5-HT₄ receptors are currently known to include three structurally distinct chemical classes: indole-based molecules, substituted benzamides and benzimidazolones.¹⁶ Among them, substituted benzamides such as metoclopramide, zacopride, cisapride and renzapride, and benzimidazolones derivatives such as BIMU 1 and BIMU 8 are notable agonists at 5-HT₄ receptors in the GI tissues.¹⁶ Mosapride citrate, a substituted benzamide, is a new 5-HT₄ receptor agonist. In clinical studies, mosapride alleviates the dysfunctions of the GI motility such as non-ulcer dyspepsia, gastroparesis, gastric stasis and gastro-esophageal reflux disease.^{31,32}

It has been shown that 5-HT₄ mediated acetylcholine release from the post-ganglionic neurons of the myenteric plexus is an important mechanism of the prokinetic effects of mosapride.³¹ In a conscious dog study, the GI motor activity-stimulating effects of mosapride were antagonized by

the treatment with GR-113808, a selective 5-HT₄ receptor antagonist.¹⁶ In addition, mosapride did not stimulate the GI motor activity when treated with atropine, but did when treated with vagotomy.¹⁵ Furthermore, mosapride had no effect on the GI motor activity when stimulated by methacholine, a cholinomimetic that was hydrolyzed by cholinesterase.¹⁵ Accordingly, the results strongly suggest that mosapride activates 5-HT₄ receptors in the myenteric plexus to enhance acetylcholine release from the enteric neurons.

Receptor ligand binding studies demonstrated that mosapride showed no affinity for dopamine D₂, adrenergic α 1, adrenergic α 2, 5-HT₁ and 5-HT₂ receptors except a weak affinity for 5-HT₃, whereas other benzamides showed high affinity for several of the existing 5-HT and other neurotransmitter receptors.¹² In a study of the binding affinity of 5HT₄ receptor agonists in the guinea pig ileum using a selective 5-HT₄ receptor radioligand [3H]GR113808, 5-HT₄ agonists displayed the following order of inhibition potency: BIMU-8 > cisapride > mosapride > renzapride > 5-HT > zacopride > metoclopramide.³³ Mosapride had an affinity about threefold less than cisapride and about 2- to 12-fold more than zacopride, renzapride, metoclopramide.³³ In experimental studies of gastric emptying in the rat, of gastroduodenal motor activity in the conscious dog, and of electrically evoked ileal contraction in the guinea pig, the enhancing effects of mosapride were as potent as cisapride and more potent than metoclopramide.^{12,15}

However, the prokinetic effect of mosapride on the GI motor activity was somewhat different from that of cisapride or other 5-HT₄ receptor agonists. In several studies, mosapride selectively enhanced the motor activity in the upper GI tract.¹⁵⁻¹⁷ Mine Y, et al.¹⁶ reported that mosapride (0.3 - 3 mg/kg i.v.) stimulated the antral motility without affecting the colonic motility in the conscious dog with force transducers implanted, but that cisapride, zacopride and BIMU-8 (0.1 - 1 mg/kg i.v.) stimulated both the antral and the colonic motility. Mosapride, even at doses 10 times higher than those that enhanced the antral motility, failed to produce the colonic contraction. In addition, mosapride enhanced the electrically evoked contraction of the guinea pig ileum, but

did not evoke the contraction of the guinea pig distal colon. In contrast, other 5-HT₄ receptor agonists exhibited similar potencies in all preparations examined. The potency of mosapride in the guinea pig isolated colon was 15- to 40-fold lower than that in other GI tissues. Mine Y, et al.¹⁶ explained that these different effects of 5-HT₄ receptor agonists on the GI motility might be due to the heterogeneity of 5-HT₄ receptors in the GI tract.

Ponti, et al.³⁴ explained the absence of significant stimulatory effects on the colon by some 5-HT₄ receptor agonists might be because of the behavior of these compounds or their metabolites as 5-HT₃ receptor antagonists and therefore their potential to inhibit, rather than stimulate, the colonic motility. Furthermore, some articles reported that stimulation of the 5-HT₄ receptors could act as an inhibitory response on the colonic motility in humans.^{35,36} Unlike the contractile effect of the colonic motility in guinea pig preparations, stimulation of the 5-HT₄ receptor relaxed of the circular muscle in isolated human colonic preparations. Mclean PG, et al.³⁶ showed that the inhibitory responses appeared to be induced by stimulation of the 5-HT₄ receptors mainly located on the smooth muscle cells, while Briejer MR, et al.³⁷ did that the excitatory responses were mediated by stimulation of the receptor located on the myenteric cholinergic or tachykininergic neurons.

In-vitro receptor autoradiograms of the stomach and colon indicated that the density of 5-HT₄ receptors in the myenteric plexus of human tissues was lower than that in guinea pig tissues, although the distribution of 5-HT₄ receptors in the two tissues was similar, and accordingly that the receptors in the human colon predominated in the smooth muscle.⁸ Nevertheless, this article concluded that stimulation of the 5-HT₄ receptors might enhance the motility of the human colon due to the same pattern of localization of 5-HT receptors as that in the guinea pig.⁸

Therefore, in the present study we attempted to investigate whether or not mosapride has an effect on the colonic motility. Furthermore, we investigated the effects of mosapride on the motility of stomach and ileum. First, we examined the effect of mosapride on the stomach. Mosapride

(10⁻⁹-10⁻⁷ M) significantly increased the amplitude of the electrically evoked antral muscle, but not dose-dependently. The reason for the lack of a demonstrable dose-related effect of the drug on the antral motility is unclear. Possibly, the dose range used in this study was at the upper end of the dose-response curve. Because it is known that the distribution of 5-HT₄ receptors and the binding affinity of mosapride on the 5-HT₄ receptors differ in each regions of GI tract,^{16,38} it is assumed that the dose of mosapride having prokinetic effect varies according to the regions of the GI tract.

The effect of mosapride on the ileum was evaluated by measuring the number of peaks, the area under the curve, and the propagation velocity of the peristaltic contraction. In a dose-dependent manner, mosapride significantly increased the number of peaks, the area under the curve, and the propagation velocity of the peristaltic contraction. Interestingly, mosapride increased the area under the curve more at the oral side than at the mid or anal side. We assumed that perhaps mosapride influenced the motility of the more proximal part of the ileum than that of the distal part, and that this therefore helped to move the contents of the small intestine to the lower part more effectively. The area under the curve of the peristaltic contraction reflects the multiplication of the amplitude and the duration of the peristaltic waves. As the amplitude of the peristaltic waves was not changed significantly in our study, we assumed that mosapride lengthened the duration of the peristaltic contraction by increasing the number of peaks of the peristaltic waves and consequently increased the peristaltic motor activity; therefore, improving the motility of the small intestine. To determine the mechanism of mosapride's action, we examined what changes in the peristaltic movement occurred after tetrodotoxin, atropine, and GR113808 were added. The peristaltic waves did not occur in the presence of tetrodotoxin, suggesting that mosapride acted on the enteric neurons, rather than directly on the muscle. Atropine decreased the peristaltic contraction, suggesting that atropine had an antagonistic effect on the cholinergic receptor of acetylcholine that might be increased by mosapride. When GR

113808 was added, mosapride did not increase the total number of peaks or the area under the curve of the peristaltic waves, suggesting that the contractile response of mosapride might be mediated by 5-HT₄ receptor.

Finally, we investigated whether mosapride also had an effect on the motility of the colon. Mosapride (10⁻⁹-10⁻⁷ M) significantly shortened the time for artificial feces to move from the oral to the anal side. These results are clearly different from previously reported results showing that mosapride slightly affects colonic motility in conscious dogs implanted with force transducers.^{15,16}

This discrepancy can be explained by the difference in the methods employed. In the present study, we examined the effect of mosapride on colonic transit *in vitro*, and the antagonistic effect for 5-HT₃ receptor of its metabolite (M1) could be omitted. Another possibility is that there are differences in the effects of mosapride on colonic motility between guinea pigs and dogs. Recently, Kadowaki, et al.³⁹ reported that mosapride increases the developed intraluminal pressure *in vitro* and enhances the propulsion of the intraluminal contents in the rat distal colon. These results are in agreement with our results, although the species are different. We suggest that *in vivo* studies are needed to more exactly specify the effect of mosapride on the colonic transit of guinea pig. In our study, a higher concentration of mosapride (10⁻⁶ M) had no effect on the colonic transit of guinea pigs. Similarly, an *in vitro* study of Jin, et al.⁴⁰ showed that a higher concentration of tegaserod, another selective 5-HT₄ receptor agonist, did not elicit an increase in velocity of fecal pellet propulsion in the guinea pig colon. A possible explanation is the desensitization of 5-HT₄ receptors located on the sensory neurons in the presence of a higher concentration of 5-HT₄ receptors agonists. This hypothesis can be derived from the study of Grider, et al.⁴¹ They demonstrated that the ability of tegaserod to cause desensitization is dependent on the concentration of, and time of exposure to, tegaserod in the rat colon. Further studies are needed to investigate whether mosapride, like tegaserod, desensitizes 5-HT₄ receptors at higher concentrations in the guinea pig colon.

Therefore, we concluded that mosapride stimu-

lated the motility of the colon, as well as of the small intestine and stomach, *in vitro* and acted on the cholinergic motor neurons through the stimulation of 5-HT₄ receptors. From these findings and the similarity in distribution of 5-HT₄ receptors between the guinea pig colon and the human colon, we suggested that mosapride has potential benefit for the disorders of the lower GI motility such as constipation.

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