Effects of Mosapride Citrate on Human Plasma Levels of Motilin, Gastrin, Somatostatin, and Secretin

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The effect of mosapride citrate (mosapride) on plasma levels of gastrointestinal peptides (motilin, gastrin, somatostatin, and secretin) was studied in five healthy volunteers. After a single oral administration of mosapride (15 mg), the plasma mosapride level (85.0 ± 13.7 ng/ml) was highest in the 60-min sample after the administration and then the plasma mosapride fell. Peak plasma motilin levels (18.6 ± 1.7 pg/ml) were achieved 60 min after administration of mosapride (p < 0.01 vs. placebo), and returned to baseline levels within a further 120 min. Plasma gastrin levels (42.4 ± 3.6 pg/ml) increased 60 min after administration of mosapride (p < 0.01 vs. placebo). Plasma somatostatin and secretin levels did not change significantly. These results suggest that the pharmacological effects of mosapride on gastrointestinal functions are closely related to changes in motilin-immunoreactive substance levels in human plasma.

Key words mosapride; motilin; gastrin; somatostatin; secretin

Mosapride citrate (mosapride) (4-amino-5-chloro-2-ethoxy-N-[4-(4-fluorobenzyl)-2-morpholinylmethyl]benzamide citrate) is a novel gastroprokinetic agent that enhances gastrointestinal motility by stimulating the serotonin (5-HT₄) receptor. This agent stimulates acetylcholine release from cholinergic neurons in the gastrointestinal wall and may enhance upper gastrointestinal motor activity in the postprandial state in conscious dogs.

We previously confirmed that cisapride causes significant increases in the level of motilin, a powerful inducer of gastrointestinal motor activity, in human plasma. This result indicates that the actions of cisapride are closely related to changes in motilin in human plasma. Cisapride is a nonselective 5-HT₄ receptor agonist and mosapride is a selective 5-HT₄ receptor agonist. Furthermore, both agents have been used to improve gastrointestinal motility functions. Their effects on gastrointestinal motility are mainly regulated by hormonal and neuronal mechanisms. Therefore we examined the plasma levels of brain-gut peptides, which regulate gastrointestinal motility.

The gastrointestinal peptide motilin is found in specific endocrine cells of the upper small intestine of hogs. This peptide strongly stimulates fundic pouch activity and plays an important physiological role in intestinal contractility, particularly in the fundus and antral of pouch of the stomach. It is one of the most important factors controlling the regular occurrence of phase III interdigestive migrating contractions.

Gastrin was first detected in extracts of pyloric antral mucosa. The most potent actions of gastrin are stimulation of both gastric acid secretion and antral motility.

Somatostatin is present in high amounts in the stomach. This peptide inhibits the secretion of motilin, gastrin, and secretin, and it also inhibits gastric, duodenal, and biliary motility.

Secretin, first isolated from porcine small intestine, inhibits gastric acid secretion, gastrin release, and gastrointestinal motility.

The purpose of this study was to determine the effects of mosapride on plasma levels of motilin-, gastrin-, somatostatin- and secretin-immunoreactive substances (IS) in healthy individuals.

MATERIALS AND METHODS

Subjects Five healthy male volunteers, aged 25—31 (median 28) years, and weighing 54—70 (median 65.1) kg, participated in the study. Each subject received information about the study’s scientific purpose, which was approved by the Ethics Committee of Oita Medical University, and gave informed consent. No subject received any medication for two weeks preceding the test and no stimulator of gastrointestinal motility, except for mosapride, was administered to any subject during the study.

Study Schedules Mosapride (Gasmotin tablets, Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) was given orally as a single dose of 15 mg with water to the 5 volunteers. Two weeks later, placebo (lactose tablets) was given orally to the volunteers. Venous blood samples (10 ml) were taken from a forearm vein before, and at 30, 60, 120, 180, 240, and 300 min after administration. The study was carried out from 14:00 to 19:00 to avoid the effects of lunch. All subjects ate lunch at 12:00.

Determination of Mosapride Levels in Plasma The concentration of mosapride was determined by the modified method of Yokoyama et al. and Sakashita et al. Standard mosapride was supplied by Dainippon Pharmaceutical Co. Ltd. Plasma samples (1 ml) were diluted four-fold with water, loaded on Sep-Pak C18 (Millipore Corp., Milford, MA, U.S.A.), and washed with 3 ml of 30% acetonitrile. Mosapride was eluted with methanol 1 ml and methanol/10% acetic acid 3 ml (95/5, v/v). The eluate was evaporated to dryness and reconstituted in a 200-μl mobile phase, and an aliquot of 40 μl of the solution was injected onto the chromatograph. HPLC was carried out using a guard-Pak precolumn and a C18 column (Cosmosil 5C18-AR, Nacalai Tesque, Kyoto, Japan) at 40°C and UV detection was at 272 nm. Methanol/50 mmol/l citrate buffer adjusted to pH 3.0 with sodium hydroxide containing 50 mmol/sodium 1-heptanesulfonate (64/36, v/v) was used as the mobile phase at a flow rate of 0.8 ml/min. The concentration of mosapride was proportional to the peak area over the range of 10—1000 ng/ml.

Preparation of Plasma Extracts The blood samples...
were placed in chilled tubes containing aprotinin 500 kallikrein inhibitor units/ml (TrasyloL, Bayer Co., Ltd., Leverkusen, Germany) and EDTA 1.2 mg/ml (Wako Pure Chemical Industries, Ltd., Osaka, Japan). After centrifugation (1670×g, 4 °C, 20 min), plasma samples were diluted with 4% acetic acid, pH 4.0, and loaded onto Sep-Pak C18 cartridges (Millipore). After washing with 4% acetic acid, pH 4.0, each peptide in plasma was eluted with 70% acetonitrile in 0.5% acetic acid, pH 4.0. The eluates were concentrated by spin-vacuum evaporation, lyophilized, and stored (−40 °C) until highly sensitive enzyme immunoassay (EIA). The recovery of plasma motilin-, gastrin-, somatostatin-, and secretin-IS was >93% with this extracting procedure (data not shown).

EIAs for Motilin-, Gastrin-, Somatostatin-, and Secretin-IS EIA for motilin-IS was performed as previously described.17) Antiserum (Y121) was obtained from Peptide Institute, Inc. (Osaka, Japan). Motilin was labeled with β-d-galactosidase (Boehringer Mannheim Corp., Mannheim, Germany). EIA was performed by the delayed-addition method. Separation of bound and free materials was performed using an immunoplate (Nunc-Immuno Module Maxisorp F8, InterMed, Denmark) coated with anti-rabbit IgG (53641, ICN Pharmaceuticals, Inc., Ohio, U.S.A.). The EIAs for gastrin-IS,18) somatostatin-IS,19) and secretin-IS20) were essentially the same, except for using the antiserum for gastrin (A600/R1B; Biogenesis, Ltd., U.K.), somatostatin (RA-08-108; Cambridge Research Biochemicals, Cambridge, U.K.), and secretin (RA-08-105; Cambridge Research Biochemicals). The EIAs for motilin-, gastrin-, somatostatin-, and secretin-IS were specific and highly sensitive to detection limits of 0.80, 0.04, 0.10, and 1.7 fmol/ml, respectively.

Data Analysis All values are expressed as means±S.D. Comparison of mean values was made by analysis of variance and Dunnett’s test. A value of p<0.05 was regarded as significant.

RESULTS AND DISCUSSION

Mosapride improves gastrointestinal motility and reduces gastric stasis or gastroesophageal reflux.21-22) Mosapride also alleviates gastrointestinal dysfunction.23) In this study, four peptides (motilin, gastrin, somatostatin, and secretin) that regulate gastrointestinal motility were examined to study the effects of mosapride.

The profiles of average plasma mosapride concentrations against time after the oral administration of mosapride 15 mg are shown in Fig. 1. The plasma level (85.0±13.7 ng/ml) was highest in the 60 min sample. After oral administration of a lactose tablet (placebo), motilin, gastrin, somatostatin, and secretin levels were within baseline levels throughout the 300-min study period (Fig. 2).

The highest plasma motilin-IS levels (18.6±1.7 pg/ml) were recorded 60 min after oral administration of mosapride, and declined to baseline level by 180 min. Mosapride caused significant increases in motilin-IS at 30, 60, and 120 min compared with placebo (Fig. 2a). The release of motilin (AUC0→180 min) increased by 24.6% with mosapride (Table 1). Recently, we reported that cisapride raises plasma motilin levels and stimulates gastrointestinal motility. The release of motilin (AUC0→180 min) after oral administration of cisapride increased by 31.3% (vs. placebo).31) Mosapride is similar to cisapride in its effects on the release of motilin. In this study, motilin-IS levels after mosapride administration were enhanced by several pathways. Mosapride may stimulate upper gastrointestinal motor activity by stimulating a nonclassical serotonergic-like receptor or 5-HT₄ receptor that increases acetylcholine release from the enteric cholinergic nerve terminal of the gut. Fox et al.24) and Poitras et al.25) reported that muscarinic receptors are present on the membrane of motilin-secreting cells, and acetylcholine is a major regulator of motilin release. Our findings suggest that mosapride may stimulate motilin cells via muscarinic receptors. The elevated motilin level also appeared to indicate that mosapride might enhance motor activity in the small intestine, including phase III contractions of the migrating motor complex.7,8) Furthermore, the time to peak plasma mosapride levels (Tmax) after administration of mosapride was 60 min. The time for the increase in motilin levels to occur after mosapride administration corresponded approximately to the Tmax values of mosapride.

After oral administration of mosapride, the plasma levels of gastrin-IS was the highest at 42.4±3.6 pg/ml in the 60-min sample. The release of gastrin (AUC0→180 min) after placebo and mosapride was similar (Table 1). Gastrin is associated with gastrointestinal motility involving cholinergic nerves. Mosapride did not significantly alter gastrin-IS levels between 120—300 min compared with placebo, but a temporary elevation in the plasma gastrin-IS level was seen 60 min after administration of mosapride (Fig. 2b). Gastrin secretion after mosapride administration might be caused by stimulation of the gastric mucosal G cells.26) The levels of somatostatin-IS and secretin-IS were unchanged after administration of mosapride (Figs. 2c, d). Somatostatin, which is widely distributed in the gastrointestinal tract, participates in the control of gut motility by exerting both inhibitory and stimulating influences.27-28) Mosapride does not appear to affect gastric acid secretion.29) The prokinetic effect of mosapride on gastrointestinal motor activity was somewhat different from that of cisapride. Specifically, mosapride selectively enhances the motor activity of the upper gastrointestinal tract, such as of the stomach and duodenum, whereas cisapride stimulates the motor activity in all sites of the gastrointestinal tract from the stomach to the colon in conscious dogs.21) The selectivity of mosapride in
the digestive period may offer an advantage over cisapride. The motility-enhancing effects of cisapride on the lower gastrointestinal tract have been reported to cause adverse effects such as diarrhea, abdominal cramps, and abdominal pain. Accordingly, mosapride, which selectively enhances upper gastrointestinal motility, is expected to have few adverse effects in humans.

Based on the results of this study, we conclude that mosapride may improve gastrointestinal motility by significantly increasing motilin-IS levels in plasma. The results indicate that the action of mosapride is closely related to changes in motilin-IS levels in plasma.

REFERENCES