Omeprazole Raises Somatostatin and Motilin in Human Plasma

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Omeprazole, a proton pump inhibitor (PPI), is widely used in treatment of peptic ulcer, gastro esophageal reflux disease and eradication of Helicobacter pylori. PPIs inhibit final gastric acid secretion stage by blocking H+K+-ATPase. But the mechanism except for gastric antisecretory effect has not understood clearly. So, we examined the effects of omeprazole on the levels of gastrointestinal peptides (somatostatin, motilin, gastrin, vasoactive intestinal peptide (VIP), substance P (SP) and calcitonin gene-related peptide (CGRP)) in plasma from healthy subjects. After a single oral administration of omeprazole, the plasma omeprazole concentration was maintained at 120 min. Omeprazole caused a significant increase of plasma somatostatin-immunoreactive substance (IS) levels at 60—240 min and plasma motilin-IS levels at 120—180 min, compared with a placebo group, respectively. The physiological release of plasma gastrin-IS was reduced by the administration of omeprazole at 60 min, but the medicine did not alter the levels of VIP-, CGRP- and SP-IS. These results suggested that the pharmacological effects of omeprazole on regulation of gastrointestinal function are closely related to changes of somatostatin-, motilin- and gastrin-IS levels in human plasma.

Key words omeprazole; somatostatin; motilin; proton pump inhibitor

Proton pump inhibitors (PPIs) inhibit gastric acid secretion by blocking H+K+-ATPase at the terminal steps of gastric acid secretion process. So, PPIs are assumed that their potency of gastric antisecretory effect is stronger than that of the histamine H2-receptor antagonists.

Omeprazole, one of the PPIs, was discovered by Fellenius et al.1) and is widely used in treatment of peptic ulcer, gastro esophageal reflux disease and eradication of Helicobacter pylori together with amoxicillin and clarithromycin. Furthermore, it is known that PPIs not only inhibit gastric acid secretion, but also protect gastric mucosa.2,3) But the mechanism except for gastric antisecretory effect is not known well.

In recent years, there are some reports that the drugs to treat gastrointestinal diseases have been elucidated pharmacologically from the viewpoints of gastrointestinal peptide (somatostatin, motilin, gastrin, calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and substance P (SP)) levels.4,5) Somatostatin inhibits secretion of motilin, gastrin and secretion, and participates in regulating gastrointestinal motility.6—8) Motilin has powerful fundic pouch motor-stimulating activity, and is one of the most important factors controlling the regular occurrence of phase-3 contractions of the migrating motor complex (MMC).9) VIP has vasodilating effect, and is an important neurotransmitter for the enteric nervous system.10) CGRP is known to inhibit acid secretion and to stimulate mucosal blood flow.11) SP co-exists with CGRP in the sensory afferent neurons of the gastrointestinal mucosa.12)

The purpose of this study is to examine the effects of omeprazole on plasma levels of gastrointestinal peptides (somatostatin-, gastrin-, motilin-, VIP-, CGRP- and SP-immunoreactive substance (IS)).

MATERIALS AND METHODS

Materials Omeprazole (Omeprazon tablets; Mitsubishi Pharma Corporation, Osaka, Japan) were used. Lactose (Merck Hoei Co., Ltd., Osaka, Japan) was used as placebo. Synthetic human gastrin I, somatostatin, motilin, VIP, CGRP and its fragment (8—37), and SP were purchased from Peptide Institute, Inc. (Osaka, Japan). Fragment gastrin I (2—17) was purchased from Sigma (St Louis, MO, U.S.A.). VIP fragment peptide was supplied by Professor H. Yajima (Kyoto University, Kyoto, Japan). Antiserum to gastrin (A600/R1B), VIP (A604/R1B) were purchased from Biogenes (Poole, U.K.), CGRP (CA1132) was purchased from Affinity Research Products Ltd. (Nottingham, U.K.), somatostatin (RA-08-108) and SP (RA-08-095) from Cambridge Research Biochemicals (Cambridge, U.K.) and motilin (Y121) from Yanaihara Institute (Shizuoka, Japan). All other reagents were analytical reagent grade from commercial sources.

Subjects Five healthy male volunteers, aged 25—30 years (median 27 years), weighing 55—68 kg (median 63 kg), participated in this study. Each subject received information about this study’s scientific purpose, which was approved by the Ethics Committee at Oita Medical University, and subsequently gave informed consent. No subject received any medication for a month preceding the test and no stimulator or inhibitor of gastrointestinal motility was administered to any subject for 3 h before the study commenced and during the experiments.

Study Schedules Omeprazole at a dose of 20 mg, or placebo was administered orally with 100 ml water. The dose of omeprazole in this study was the maximum daily dose used in clinical therapy. Venous blood samples (10 ml) were taken from a forearm vein before and at 30, 60, 90, 120, 180, 240 and 360 min after administration of the drug. The study was carried out from 11:30 to 17:30 h.

Determination of Omeprazole Levels in Plasma The plasma concentration of omeprazole was determined by the modified method of Lagerstrom et al.13) Standard omeprazole was supplied by Mitsubishi Pharma Corporation (Osaka, Japan). A 1 ml of the plasma sample is transferred to a centrifuge tube, mixed with 100 μl of 1 N sodium dihydrogen phosphate, and was then eluted with 8 ml dichloromethane. The elute was evaporated to dryness under reduced pressure. The residue was dissolved in 650 μl of mobile phase and...
200 μl of the solution was subjected to HPLC. HPLC was carried out using a C18 column (Cosmosil 5C18-AR; Nacalai Tesque, Kyoto, Japan) with UV detection at 302 nm, and 0.05 M phosphate buffer (pH 7.5)—acetonitrile (7:3, v/v) was used as a mobile phase at a flow rate of 1.0 ml/min.

Preparation of Plasma Extracts The blood samples were placed in chilled tubes containing 500 kallikrein inhibitor units/ml of aprotinin and 1.2 mg/ml of EDTA. After centrifugation, plasma samples were diluted with 4% acetic acid buffer (pH 4.0), and loaded onto a C18 reversed-phase cartridge (Sep-Pak C18; Millipore Corp., Milford, MA, U.S.A.). After washing with 4% acetic acid buffer plasma peptides were eluted with 70% acetonitrile in 0.5% acetic acid buffer (pH 4.0). Eluates were concentrated by spin-vacuum evaporation, lyophilized and stored at −40°C until used. The recovery of plasma somatostatin-, motilin-, gastrin-, VIP-, CGRP- and SP-IS was >90% with this extracting procedure (data not shown).

Enzyme Immunoassays for Somatostatin-, Motilin-, Gastrin-, VIP-, CGRP- and SP-IS Peptide levels in plasma were measured using a highly sensitive enzyme immunoassay for somatostatin, gastrin, motilin, VIP, CGRP and SP as previously described. The assay was performed by a delayed addition method. Separation of bound and free antigen was performed on an anti-rabbit IgG (55641, ICN Pharmaceuticals, Inc., Ohio, U.S.A.) coated immunoplate (Nunc-Immuno Module Maxisorp F8, InterMed, Denmark). Human somatostatin, fragment gastrin I (2—17), motilin, fragment VIP (11–28), SP, and human CGRP (8—37) were conjugated with N-(maleimidocaproyloxy)-succimide according to the methods of Kitagawa et al. The enzyme immunoassays for somatostatin-, motilin-, gastrin-, VIP-, CGRP- and SP-IS were specific and highly sensitive to detection limits of 0.10, 0.04, 0.80, 1.00, 0.40 and 0.08 fmol/well, respectively.

Statistical Analysis All values are expressed as means ± standard derivation (S.D.). Comparison of mean values was made by Mann Whiney U test. A p-value less than 0.05 indicated statistical significance.

RESULTS

The profiles of average plasma omeprazole concentrations against time after oral administration 20 mg of the drug are shown in Fig. 1. The plasma level was highest in the 120 min (2445 ± 857.83 ng/ml).

Omeprazole caused significantly increase in plasma somatostatin-IS levels at 60—240 min (19.9 ± 7.7 pg/ml at 60 min, 20.0 ± 10.1 pg/ml at 90 min, 20.5 ± 5.0 pg/ml at 120 min, 18.4 ± 8.7 pg/ml at 180 min, 12.7 ± 2.5 pg/ml at 240 min) compared with placebo (9.5 ± 4.1 pg/ml at 60 min, 8.7 ± 2.4 pg/ml at 90 min, 8.5 ± 3.4 pg/ml at 120 min, 9.1 ± 1.9 pg/ml at 180 min, 9.5 ± 1.3 pg/ml at 240 min), respectively (Fig. 2a). Omeprazole caused significant increase in plasma motilin-IS levels at 120—180 min (28.7 ± 3.7 pg/ml at 120 min, 24.6 ± 3.6 pg/ml at 180 min) compared with placebo (25.2 ± 2.8 pg/ml at 120 min, 17.2 ± 2.6 pg/ml at 180 min), respectively (Fig. 2b). A temporary elevation in plasma gastrin-IS levels of placebo at 60 min (80.0 ± 8.0 pg/ml) was significantly reduced by administration of omeprazole (33.3 ± 23.1 pg/ml) (Fig. 2c).

DISCUSSION

Somatostatin, a 14-amino acid residue polypeptide, is broadly distributed in the pancreatic islet, the gastrointestinal tract and the central nervous system (CNS). In the stomach and the pancreatic islet, somatostatin is present in endocrine-type D cells, whereas in the intestine and the CNS, it is present in the neurons. In the gastrointestinal tract, somatostatin is released by stimulating of nutrient (ex. proteins and amino acids) and gastrin. Generally, somatostatin acts as an inhibitor of other hormones (insulin, gastrin, motilin and secretin) release. In this study, omeprazole raised plasma somatostatin-IS levels. This increase might contribute to change the gastrointestinal functions through the effect of somatostatin; inhibition of the secretion of other hormones. Actually, in our study, plasma gastrin-IS levels were inhibited at 60 min. Furthermore, somatostatin participates in regulating gastrointestinal motility. In the stomach, somatostatin induces phase-3 activities in the interdigestive state, whereas it inhibits motilin-induced gastrointestinal MMC in the digestive state. In the intestine, somatostatin stimulates peristalsis. In the peristaltic reflex, somatostatin caused descending caudal relaxation via VIP and γ-aminobutyric acid release. Octreotide, one of the somatostatin analogues, has a longer half-life than somatostatin and stimulates MMC-like activity in the small intestine. Omeprazole might affect the gastrointestinal motility through the somatostatin and expect to apply the drug to the peristalsis disorders.

Motilin, a 22-amino acid residue polypeptide, is a powerful inducer of gastrointestinal motor activity in the fundus and the antral pouch of the stomach. It plays an important physiological role in intestinal contractility, and is one of the most important factors controlling the regular occurrence of phase-3 contractions of migrating motor complex. Allen et al. suggested that oral administration of 40 mg omeprazole was no significant effect on basal or postprandial levels of plasma motilin levels. These differences might occur that Allen et al. assessed plasma concentrations at two points (before or 6 h after administration), but we assessed eight points’ plasma concentrations. Six hours after administration, our results were also no significant effect between omeprazole group and placebo group. In this study, because plasma motilin-IS levels significantly increased after single administration of omeprazole, the medicine might regulate gastrointestinal motility by accelerating gastric emptying.

Gastrin, a 17-amino acid residue polypeptide, stimulates
acid secretion. Although gastrin release is mediated by various mechanisms, generally gastrin is secreted as a result of stimulation of the gastric mucosal G cells. In Fig. 2c, gastrin-IS level of placebo was increased at 60 min. This temporary elevation of gastrin-IS levels might be caused by a direct stimulation of gastric mucosa G cells. In this study, placebo stimulate gastrin-release was reduced by omeprazole. We thought the changes of somatostatin-IS might be closely related to inhibition of gastrin-IS.

In our study, omeprazole did not change CGRP and SP. In gastrointestinal system, CGRP and SP play important role in mucosal protective mechanism. We considered that cytoprotective effects of the drug might be due to other mechanisms without relationship with CGRP and SP.

In conclusion, ingestion of omeprazole caused changes in the plasma somatostatin-, motilin- and gastrin-IS levels. We hypothesized that pharmacological effects of omeprazole might be closely related to changes of plasma somatostatin-, motilin- and gastrin-IS levels, which are related to regulation of gastrointestinal function.

REFERENCES

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