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Effects of histamine H_2-receptor antagonists on human plasma levels of calcitonin gene-related peptide, substance P and vasoactive intestinal peptide

Hiroki Itoh, Takafumi Naito and Masaharu Takeyama

Abstract

The effects of the histamine H_2-receptor antagonists (H_2-antagonists), ranitidine, nizatidine, cimetidine and famotidine, on plasma levels of gastrointestinal peptides, calcitonin gene-related peptide (CGRP), substance P (SP), and vasoactive intestinal peptide (VIP) was investigated with respect to regulation of gastric mucosal blood flow, in healthy volunteers. H_2-Antagonists or placebo was orally administered to five healthy male volunteers. Venous blood samples were taken before and after drug administration. The levels of plasma gastrointestinal peptides were determined by enzyme immunoassay. The administration of ranitidine and nizatidine caused significant increases in plasma CGRP and SP levels at 30 to 120 min compared with the placebo group. Peak plasma CGRP levels (39.8 ± 3.1 and 40.6 ± 3.6 pg mL^{-1}) were achieved 60 min after administration of ranitidine and nizatidine, respectively. Maximum plasma SP levels (21.3 ± 5.2 and 22.8 ± 4.2 pg mL^{-1}) were reached 60 min after administration of ranitidine and nizatidine, respectively. However, all H_2-antagonists did not alter the levels of VIP. The released CGRP and SP by ranitidine and nizatidine administration may produce a gastroprotective effect, increase mucosal blood flow, and inhibit acid secretion in the gastrointestinal tract.

Introduction

Histamine H_2-receptor antagonists (H_2-antagonists) have proved effective in patients with gastric ulcers, gastroesophageal reflux disease and gastritis, and their most probable mechanism of action is the inhibition of gastric acid secretion (Deakin & Williams 1992). Konturek et al (1981) have demonstrated that ranitidine prevents the gastric mucosal injury induced by aspirin plus hydrochloric acid, independently of its antisecretory property. Thus, it is possible that ranitidine prevents the gastric mucosal injury not only by inhibiting gastric acid secretion, but also by inhibiting neutrophil activation. To our knowledge, there are no reports of the effects of other H_2-antagonists in humans. Their effects on gastrointestinal function are mainly regulated by hormonal and neuronal mechanisms; however, the mechanisms are not clear. We therefore examined the plasma levels of gut peptides that regulate gastrointestinal function.

Calcitonin gene-related peptide (CGRP) is a 37-amino acid peptide that is produced by a tissue-specific alternative RNA processing of the calcitonin gene (Amara et al 1982). The actions of CGRP in the stomach include inhibition of acid secretion (Tache et al 1984), inhibition of motility (Katsoulis & Conlon 1989) and stimulation of blood flow (Bauerfeind et al 1989). Substance P (SP), an undecapeptide, was first detected by von Euler & Gaddum (1931) in extracts from equine intestine and brain. It is widely distributed in the central and peripheral nerve endings (Hokfelt et al 1977), and is implicated as a sensory neurotransmitter (Otsuka et al 1982) and an excitatory transmitter to intestinal muscles (Costa et al 1985; Grider 1989). This peptide participates in the regulation of gastric mucosal blood flow by activation of mast cells (Gronbech & Lacy 1994). Vasoactive intestinal peptide (VIP), a 28-amino acid residue neuropeptide, is widely distributed in the central and peripheral nervous systems (Fahrenkrug 1993). This peptide has a vasodilating effect and is an important neurotransmitter for the enteric nervous system (Goyal et al 1980; Grider et al 1985).

Department of Clinical Pharmacy, Oita Medical University, Hasama-machi, Oita 879-5593, Japan

Hiroki Itoh, Takafumi Naito, Masaharu Takeyama

Correspondence: H. Itoh, Department of Clinical Pharmacy, Oita Medical University, Hasama-machi, Oita 879-5593, Japan.
E-mail: itoh@oita-med.ac.jp

The purpose of this study was to determine the effects of H_2-antagonists (ranitidine, nizatidine, cimetidine and famotidine) on the plasma levels of the gastrointestinal peptides, CGRP, SP and VIP immunoreactive substances (IS), in healthy subjects.

Materials and Methods

Materials

Lactose (Merck Hoei Co. Ltd, Osaka, Japan) was used as placebo. CGRP, SP and synthetic VIP were purchased from Peptide Institute Inc. (Osaka, Japan). Antisera to CGRP and VIP (A604/R1B) were purchased from Biogenesis, Ltd (Poole, UK). Antiserum to SP (RA-08-095) was purchased from Genosys Biotechnologies Ltd (London, UK). All other reagents were reagent grade and are commercially available.

Subjects

Five healthy male volunteers, aged 23–28 years (medians 26 years), 53–68 kg (63.2 kg), participated in the study. Each subject received information about the scientific purpose of the study, which was approved by the Ethics Committee of Oita Medical University, and gave informed consent. No subject received any medication for 2 weeks preceding the test and no stimulator of gastrointestinal motility was administered to any subjects during the study.

Study schedules

Each subject was administered H_2-antagonists and placebo at an interval of 3 weeks and the result for each subject was the mean of two measurements. Therapeutic equivalent doses of ranitidine (300 mg, Zantac tablets; Sankyo Co. Ltd, Tokyo, Japan), nizatidine (300 mg, Acinon capsules; Zeria Pharmaceutical Co. Ltd, Saitama, Japan), cimetidine (800 mg, Tagamet tablets; Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan), famotidine (40 mg, Gaster tablets; Yamanouchi Pharmaceutical Co. Ltd, Tokyo, Japan), or placebo were administered orally with 100 mL water. Venous blood samples (15 mL) were taken from a forearm vein before, and at 30, 60, 120, 180, 240 and 300 min after administration of the H_2-antagonists. The study was carried out from 1400 to 2000 h to avoid the effects of lunch (1200 h).

Preparation of plasma extracts

Blood samples were placed in chilled tubes (4°C) containing aprotinin (500 kallikrein inhibitor units mL^{-1}) and EDTA (1.2 mg mL^{-1}). After centrifugation (1670 *g*, 4°C, 20 min), plasma samples were diluted fivefold with 4% acetic acid (pH 4) and loaded onto C18 reversed-phase cartridges (Sep-Pak C18; Millipore Corp., Milford, MA, USA). After washing with 4% acetic acid, gastrointestinal peptides in plasma were eluted with 70% acetonitrile in 0.5% acetic acid (pH 4). Eluates were concentrated by spin-vacuum evaporation, lyophilized and stored until use. The recovery of plasma CGRP-IS, SP-IS and VIP-IS was >93% with this extracting procedure (data not shown).

Enzyme immunoassay for CGRP-IS, SP-IS and VIP-IS

Peptide levels in plasma were measured using highly sensitive enzyme immunoassays for CGRP (Nagano et al 1998), SP (Takeyama et al 1990a), and VIP (Takeyama et al 1990b), 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., OH, USA) coated immunoplate (Nunc-immuno Module Maxisorp F8, InterMed, Denmark). CGRP, SP and VIP were conjugated with β-D-galactosidase (Boehringer Mannheim Corp., Mannheim, Germany) by *N*-(ϵ-maleimidocaproyloxy)-succinimide according to the methods of Kitagawa et al (1981). Tubes containing antiserum for each peptide and extracted samples (or standard) were incubated at 4°C for 24 h, and then enzyme-linked antigen was added. After incubation for another 24 h, each antigen/antibody solution was added to secondary-antibody coated immunoplates and the plates were incubated overnight. Then, after washing with phosphate buffer, 4-methylumbelliferyl-β-D-galactopyranoside was added to each well. The plates were incubated again at 37°C for 3 h and the fluorescence intensity of each well was measured with an MTP-100F microplate reader (Corona Electric, Ibaraki, Japan). The enzyme immunoassays for CGRP-IS, SP-IS and VIP-IS were specific and highly sensitive to detection limits of 0.08, 0.4, and 1.0 fmol/well, respectively.

Data analysis

The area under the plasma concentration–time curve (AUC) was calculated using the trapezoidal method. All values are expressed as means ± s.d. Comparison of mean values was made by two-way analysis of variance and $P < 0.05$ was considered statistically significant.

Results

After oral administration of a lactose tablet (placebo), CGRP, SP and VIP levels were within baseline levels throughout the 300-min study period.

Effect of H_2-antagonists on plasma levels of CGRP-IS

There was no significant difference between mean plasma levels of CGRP-IS before administration of ranitidine, nizatidine, cimetidine, famotidine, or placebo. Maximum

plasma CGRP-IS levels (40.6±3.6 and 39.8±3.1 pg mL^{-1}) were reached 60 min after oral administration of nizatidine and ranitidine, respectively, and declined to baseline within a further 60–120 min. Ranitidine and nizatidine caused significant increases in plasma CGRP levels at 30 to 120 min compared with the placebo group, whereas plasma CGRP levels were not significantly changed after administration of famotidine or cimetidine. Administration of ranitidine and nizatidine caused a significant increase in the release of CGRP (7824±214.7 and 7977±268.2 pg min^{-1} mL^{-1}) compared with the placebo group (6876±451.6 pg min^{-1} mL^{-1}), respectively.

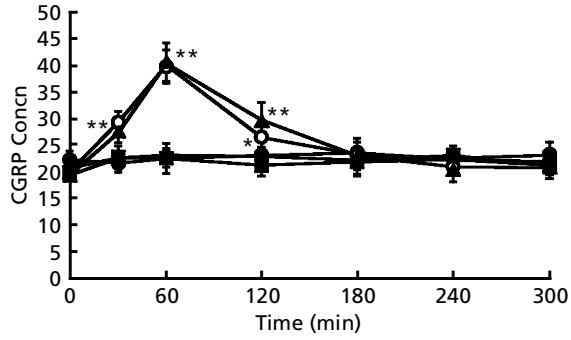

Figure 1 Plasma calcitonin gene-related peptide (CGRP) concentrations in healthy male subjects after oral administration of ranitidine (○), nizatidine (▲), cimetidine (△), famotidine (■), or placebo (●). Each value represents the mean±s.d., n = 5. $*P < 0.05$ and $**P < 0.01$ compared with placebo.

Effect of H_2-antagonists on plasma levels of SP-IS

There was no significant difference between mean plasma levels of SP-IS before administration of ranitidine, nizatidine, cimetidine, famotidine, or placebo. Maximum plasma SP-IS levels (22.8±4.2 and 21.3±5.2 pg mL^{-1}) were reached 60 min after oral administration of nizatidine and ranitidine, respectively, and declined to baseline within a further 60–120 min. Ranitidine and nizatidine caused significant increases in plasma SP levels at 30 to 120 min compared with the placebo group, whereas plasma SP levels were not significantly changed after administration of famotidine or cimetidine. Administration of ranitidine and nizatidine caused a significant increase in the release of SP (3905.7±321.5 and 3975.9±418.0 pg min^{-1} mL^{-1}) compared with the placebo group (2991.3±182.8 pg min^{-1} mL^{-1}), respectively.

Effect of H_2-antagonists on plasma levels of VIP-IS

There was no significant difference between mean plasma levels of VIP-IS before administration of ranitidine, nizatidine, cimetidine, famotidine, or placebo. Plasma VIP levels were not significantly changed after administration of the H_2-antagonists.

Discussion

The H_2-antagonists, ranitidine, nizatidine, cimetidine and famotidine are very different chemically. Cimetidine has the imidazole ring of histamine. Ranitidine has an alkyl furan ring, whereas famotidine and nizatidine have thiazole rings. In this study, gut-regulated peptide (CGRP, SP and VIP) levels, which regulate gastrointestinal function, were examined to study their relationship with H_2-antagonists.

The action of CGRP in the stomach includes stimulation of gastric blood flow (Bauerfeind et al 1989), inhibition of motility (Katsoulis & Conlon 1989), and inhibition of gastric acid secretion (Tache et al 1984). The time course of the CGRP-IS plasma level after administration of H_2-antagonists is shown in Figure 1. Ranitidine and nizatidine caused a significant increase in CGRP-IS at 30–120 min compared with placebo. The release of CGRP (AUC) increased by 14.1% with ranitidine, and by 16.4% with nizatidine (compared with placebo) (Table 1). The CGRP released by ranitidine and nizatidine administration may produce a gastroprotective effect, increase mucosal blood flow, and inhibit acid secretion in the gastrointestinal tract by stimulating CGRP-containing nerves. In addition, ranitidine and nizatidine have anti-acetylcholinesterase (AChE) activity not found with cimetidine and famotidine (Mizumoto et al 1990; Ueki et al 1993). Trasforini et al (1994) reported that pyridostigmine, an AChE inhibitor, induced a significant rise in basal plasma CGRP and that the cholinergic system might be an important regulator of CGRP release under physiological conditions. Ranitidine and nizatidine inhibit AChE activity, and might raise CGRP levels.

SP is co-released with CGRP from sensory neurons (Rydning et al 1999), but the role of this peptide in gastric blood flow regulation is largely unknown. The time course of the SP-IS plasma level after administration of H_2-antagonists is shown in Figure 2. Ranitidine and nizatidine caused a significant increase in SP-IS at 30–120 min compared with placebo. The release of SP (AUC) increased by 31.2% with ranitidine, and by 33.3% with nizatidine (compared with placebo) (Table 1). SP may have a distinct role in the regulation of gastric mucosal blood flow (Gronbech & Lacy 1994). SP is co-localized with CGRP in sensory neurons and released together with CGRP (Kwok & Mclntosh 1990). In particular, neuropeptides, such as SP and CGRP, are important regulators of mucosal blood flow.

VIP has a vasodilating effect and functions as a neurotransmitter for the enteric nervous system (Fahrenkrug 1993). H_2-Antagonists had no effect on plasma VIP-IS levels compared with the placebo group. Furthermore, plasma VIP-IS levels remained constant before and after administration (approx. 8 pg mL^{-1}).

Our studies of gut hormone levels offer another viewpoint on the mechanism of H_2-antagonists. Ingestion of

Table 1 Total amount of peptides released after administration of H_2-antagonists to healthy male subjects.

	Calcitonin gene-related peptide	Substance P	Vasoactive intestinal peptide
Placebo	6876±451.6	2991.3±182.8	2542.5±236.4
Ranitidine	7824±214.7**	3905.7±321.5**	2780.7±36.6
Nizatidine	7977±268.2**	3975.9±418.0**	2814±107.2
Cimetidine	6736.5±286.6	3084.6±122.1	2729.1±176.6
Famotidine	6625.5±472.7	3109.2±122.9	2792.1±176.0

Each value represents the mean±s.d., n = 5. **$P < 0.01$ compared with placebo.

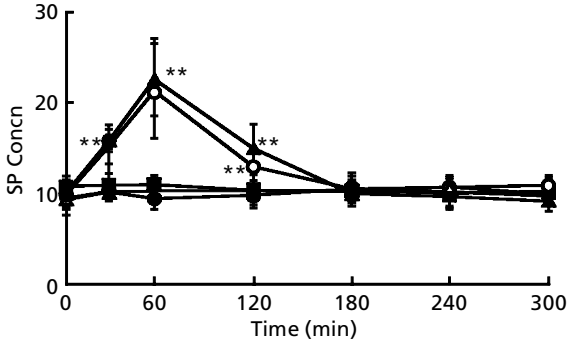

Figure 2 Plasma substance P (SP) concentrations in healthy male subjects after oral administration of ranitidine (○), nizatidine (▲), cimetidine (△), famotidine (■), or placebo (●). Each value represents the mean±s.d., n = 5. **$P < 0.01$ compared with placebo.

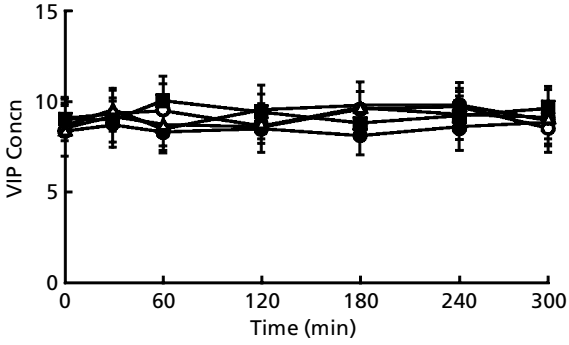

Figure 3 Plasma vasoactive intestinal peptide (VIP) concentrations in healthy male subjects after oral administration of ranitidine (○), nizatidine (▲), cimetidine (△), famotidine (■), or placebo (●). Each value represents the mean±s.d., n = 5.

ranitidine and nizatidine causes changes in the plasma levels of CGRP-IS and SP-IS. We hypothesize that their gastrointestinal effects might be closely related to changes in CGRP and SP plasma levels, which are related to the regulation of gastric mucosal blood flow and gastroprotective activity. Thus, the administration of ranitidine and nizatidine may contribute to an increase in the gastric mucosal blood flow.

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