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Communication

# Cetraxate raises levels of calcitonin gene-related peptide and substance P in human plasma

Fumihiko Katagiri, Yuhki Sato, Hiroki Itoh and Masaharu Takeyama

## Abstract

Cetraxate hydrochloride (cetraxate), an anti-ulcer drug, produces a dose-related increase in mucosal blood flow. Recently, it was found that capsaicin-sensitive afferent nerves play an important role in gastric mucosal defence. Capsaicin stimulates afferent nerves and enhances the release of calcitonin gene-related peptide (CGRP) and substance P in the stomach. We studied the effect of cetraxate on human plasma CGRP and substance P in healthy subjects. Cetraxate (800 mg) or placebo were orally administered to five healthy males. Blood samples were taken before, and at 20, 40, 60, 90, 120, 180 and 240 min after administration, followed by the extracting procedure, and submitted to a highly sensitive enzyme immunoassay system for CGRP and substance P. Single administration of cetraxate caused significant increases in plasma CGRP concentration at 60–120 min compared with placebo. Cetraxate significantly increased plasma substance P levels at 40–90 min compared with placebo. In this study, we hypothesized that cetraxate might indirectly stimulate capsaicin-sensitive afferent nerves and increase mucosal blood flow, and that this may be a key mechanism underlying its gastroprotective action.

## Introduction

Gastric acid secretion and cytoprotective factors play an important role in the genesis of gastric ulcer, apart from the significant contribution of *Helicobcter pylori*. The local release of vasodilator mediators in the gastric mucosal microcirculation is of paramount importance in the maintenance of mucosal integrity and defence.

Cetraxate was introduced in 1976 as an anti-ulcer drug with a mucosal protective effect (Suzuki et al 1976). Kuribayasi et al (1988) demonstrated that cetraxate is a potent cytoprotective agent that can effectively prevent gastric mucosal necrosis induced by HCl–ethanol in rats. Its efficacy is ascribed to the promotion of an increase in gastric mucosal blood flow. Animal experiments have shown that cetraxate significantly reverses the reduction of gastric mucosal blood flow induced by smoking (Shibata et al 1993). Murakami et al (1991) devised a non-invasive technique for the continuous measurement of human gastric mucosal blood flow by laser method and studied the effect of cetraxate on human gastric mucosal microcirculation. They reported that cetraxate produced a dose-related increase in gastric mucosal blood flow.

On the gastroprotective function as a neural emergency system, sensory afferent neurons in the gastrointestinal mucosa regulate levels of neuropeptides (calcitonin gene-related peptide (CGRP), and tachykinins (substance P, etc.)) and play various physiological roles (Holzer 1998). Renzi et al (1991) and Whittle (1991) suggested that CGRP and substance P might play an important local role in gastroduodenal ulcer genesis.

CGRP possesses several potent biological actions including vasodilatation, being the most powerful vasoactive substance described to date, and it increases mucosal blood flow (Bauerfeind et al 1989; Katsoulis & Conlon 1989). CGRP is known to co-exist with tachykinins in the population of sensory neurons in man (Ekström et al 1998). Substance P is widely distributed in the central and peripheral divisions of the nervous system and in the entero-endocrine cells of the gut (Pernow 1983), and it participates in the regulation of gastrointestinal motility and secretion (Schmidt & Holst 2000).

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

Fumihiko Katagiri, Yuhki Sato, Hiroki Itoh, Masaharu Takeyama

**Correspondence:** F. Katagiri, Department of Clinical Pharmacy, Oita University Hospital, Hasama-machi, Oita 879-5593, Japan.
E-mail: FKATA@med.oita-u.ac.jp

The purpose of this study was to determine the effects of cetraxate on plasma levels of CGRP-like immunoreactive substance (IS) and substance P-IS in healthy subjects.

## Materials and Methods

### Materials

Cetraxate (Neuer capsule; Daiichi Seiyaku Co. Ltd, Tokyo, Japan) were used. Lactose (Merck hoei Co. Ltd, Osaka, Japan) was used as placebo.

Synthetic human gastrin I (G17), somatostatin, porcine motilin, VIP, human CGRP and its fragment (8–37) and substance P were purchased from the Peptide Institute (Osaka, Japan). Fragment mini gastrin I was purchased from Sigma Chemical (St Louis, MO). Motilin and VIP fragment peptide were supplied by Professor H. Yajima (Kyoto University, Kyoto, Japan). Antisera to gastrin (A600/R1B), VIP (A604/R1B) and CGRP were purchased from Biogenesis (Poole, UK), somatostatin (RA-08-108) and substance P (RA-08-095) from Cambridge Research Biochemicals (Cambridge, UK) and motilin (Y121) from Yanaihara Institute (Shizuoka, Japan). All other reagents were analytical reagent grade from commercial sources.

### Subjects

Five healthy male subjects, aged 24–29 years (median 28 years), 55–68 kg (median 62 kg), participated in the study. Each subject received information on the scientific purpose of the study and gave written consent. The study was approved by the ethical committee of Oita Medical University. The subjects did not receive any medication for one week before the study, and fasted for 2 h before the study commenced and during the experiments.

### Study schedule

Cetraxate (800 mg) or placebo were administered orally with 100 mL water. Each subject was administered these drugs with an interval of four weeks. The dose of cetraxate 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 20, 40, 60, 90, 120, 180 and 240 min after, administration. The study was carried out from 1400 to 1600 h.

### Enzyme immunoassay (EIA) of gastrin, somatostatin, motilin, VIP, CGRP and substance P

The blood samples were placed in chilled tubes containing 500-kallikrein inhibitor units/mL of aprotinin and 1.2 mg $mL^{-1}$ of EDTA. After centrifugation, plasma samples were diluted five fold with 4% acetic acid (pH 4.0) and loaded onto C18 reversed-phase cartridge (Sep-Pak C18; Millipore Corp., Milford, MA). After washing with 4% acetic acid, plasma peptides were eluted with 70% acetonitrile in 0.5% acetic acid (pH 4.0). Eluates were concentrated by spin-vacuum evaporation, lyophilized and stored at −40 °C until assayed. The recovery of plasma gastrin-, somatostatin-, motilin-, VIP-, CGRP- and substance P-IS was > 90% with this extracting procedure (data not shown).

EIAs for gastrin (Takeyama et al 1993), somatostatin (Takeyama et al 1990a), motilin (Naito et al 2002), VIP (Takeyama et al 1990b), CGRP (Nagano et al 1998) and substance P (Takeyama et al 1990c) were performed as previously described. Each 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) coated immunoplate (Nunc-Immuno Module Maxisorp F8; InterMed, Denmark). The fluorescent product 4-methylumbelliferon was measured with an MTP-100F microplate reader (Corona Electric, Ibaraki, Japan). Mini gastrin I, human somatostatin, porcine motilin, fragment VIP (11–28), human CGRP (8–37) and substance P were conjugated with $\beta$-D-galactosidase (Boehringer Mannheim, Mannheim, Germany) by *N*-(ε-maleimidocaproyloxy)-succimide, according to the method of Kitagawa et al (1981). The EIAs for gastrin, somatostatin, motilin, VIP, CGRP and substance P were specific and highly sensitive to detection limits of 0.04, 0.10, 0.80, 1.00, 0.08 and 0.4 fmol/well, respectively.

### Statistical analysis

Results are expressed as mean ± s.d. Comparison of mean values was made by the Mann–Whitney U-test and $P < 0.05$ was considered statistically significant.

## Results and Discussion

The plasma CGRP-IS level–time profile after administration of cetraxate to healthy subjects is shown in Figure 1. Cetraxate significantly increased CGRP-IS at 60–120 min and 240 min ($20.7 \pm 6.0$ pg $mL^{-1}$ at 60 min, $11.1 \pm 10.8$ pg $mL^{-1}$ at 90 min, $7.6 \pm 1.8$ pg $mL^{-1}$ at 120 min, $11.8 \pm 0.7$ pg $mL^{-1}$ at 240 min) compared with the response of the placebo group ($5.2 \pm 1.2$ pg $mL^{-1}$ at 60 min, $5.7 \pm 1.1$ pg $mL^{-1}$ at 90 min, $5.4 \pm 0.4$ pg $mL^{-1}$ at 120 min, $3.8 \pm 1.2$ pg $mL^{-1}$ at 240 min). Figure 2 shows plasma substance P-IS levels after administration of cetraxate to healthy subjects. Cetraxate significantly increased substance P-IS at 40–90 min ($29.6 \pm 2.5$ pg $mL^{-1}$ at 40 min, $34.4 \pm 2.5$ pg $mL^{-1}$ at 60 min, $34.0 \pm 1.3$ pg $mL^{-1}$ at 90 min) compared with the response of the placebo ($20.4 \pm 6.5$ pg $mL^{-1}$ at 40 min, $21.5 \pm 2.5$ pg $mL^{-1}$ at 60 min, $23.3 \pm 3.2$ pg $mL^{-1}$ at 90 min). The plasma somatostatin-IS levels were significantly increased at 20 min ($28.1 \pm 11.6$ pg $mL^{-1}$) compared with placebo ($12.8 \pm 1.1$ pg $mL^{-1}$) (Figure 3). At 240 min, the plasma gastrin-IS levels ($12.3 \pm 4.7$ pg $mL^{-1}$) were significantly suppressed by cetraxate compared with placebo ($20.8 \pm 6.4$ pg $mL^{-1}$) (Figure 4). Cetraxate did not, however, alter levels of motilin and VIP (Figures 5 and 6).

CGRP is a powerful vasoactive substance, which is released from the sensory afferent nerve endings against gastric mucosal injury in the stomach (Holzer 1998).

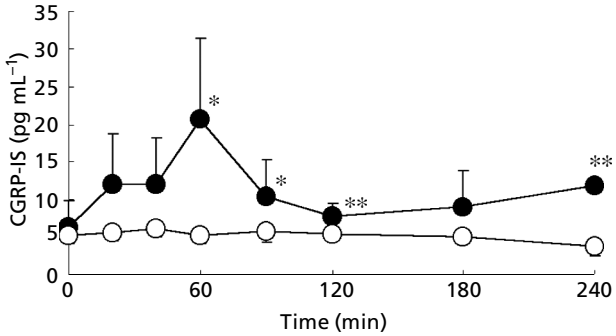


**Figure 1** Effect of oral cetraxate 800 mg (●) or placebo (○) on plasma CGRP levels in healthy subjects. Each value represents the mean ± s.d., n = 5. *$P < 0.05$ and **$P < 0.01$, compared with placebo.

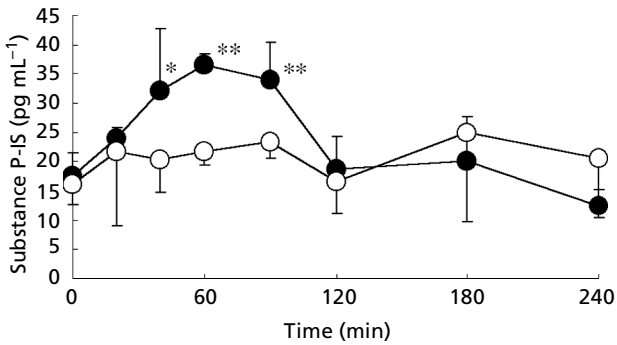


**Figure 2** Effect of oral cetraxate 800 mg (●) or placebo (○) on plasma substance P levels in healthy subjects. Each value represents the mean ± s.d., n = 5. *$P < 0.05$ and **$P < 0.01$, compared with placebo.

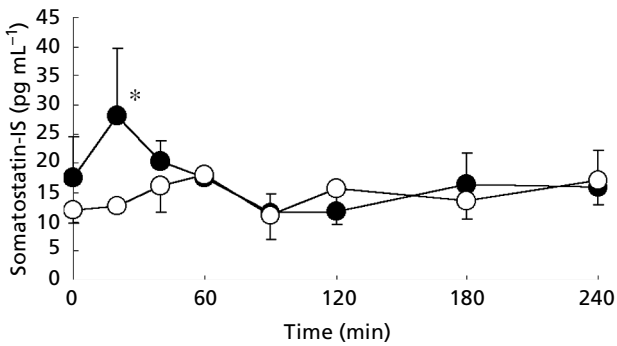


**Figure 3** Effect of oral cetraxate 800 mg (●) or placebo (○) on plasma somatostatin levels in healthy subjects. Each value represents the mean ± s.d., n = 5. *$P < 0.05$ compared with placebo.

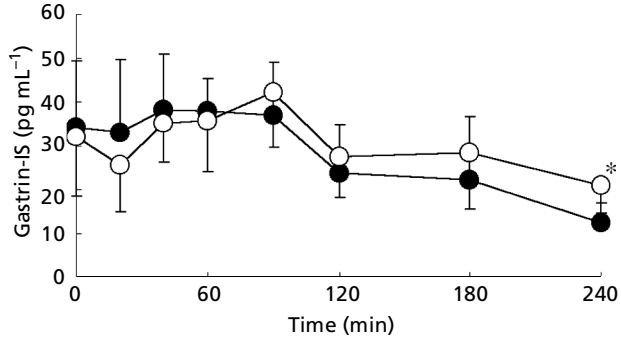


**Figure 4** Effect of oral cetraxate 800 mg (●) or placebo (○) on plasma gastrin levels in healthy subjects. Each value represents the mean ± s.d., n = 5. *$P < 0.05$ compared with placebo.

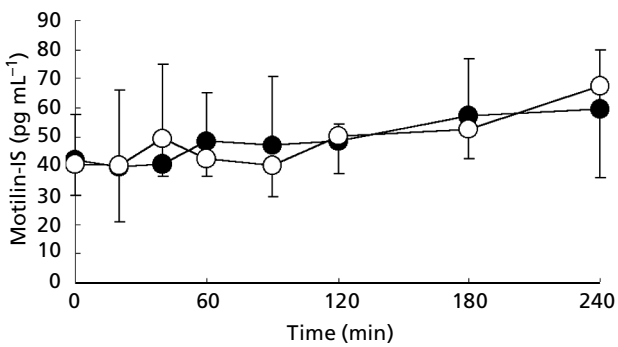


**Figure 5** Effect of oral cetraxate 800 mg (●) or placebo (○) on plasma motilin levels in healthy subjects. Each value represents the mean ± s.d., n = 5.

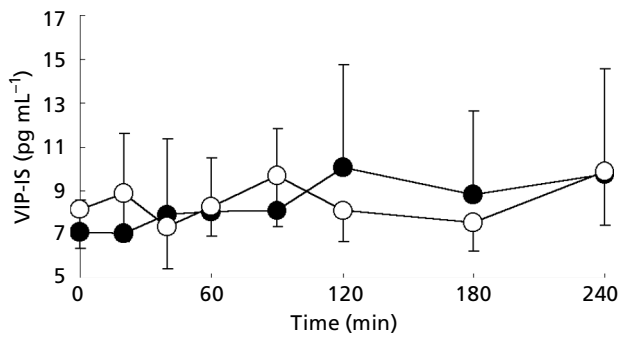


**Figure 6** Effect of oral cetraxate 800 mg (●) or placebo (○) on plasma VIP levels in healthy subjects. Each value represents the mean ± s.d., n = 5.

CGRP increases gastric mucosal flow as a gastroprotective factor (Holzer et al 1991). Substance P, a tachykinin, co-exists with CGRP in the sensory afferent neurons of the gastrointestinal mucosa, and is released with acetylcholine in response to depolarizing stimuli in the enteric nerve system (Hellström et al 1991).

Peptic ulcer results from a pathological mismatch between injurious influences and protective mechanism in the gastroduodenal mucosa. Accordingly, ulcer therapy has long relied on the suppression of aggressive factors such as acid and pepsin. Although a deficiency of cytoprotective prostaglandin is known to be responsible for the mucosal

lesions caused by non-steroidal anti-inflammatory drugs, the possibility that chemosensitive afferent neurons monitor noxious tissue challenge and, in turn, activate mechanisms of protection was not considered until recently. Renzi et al (1991) investigated, using duodenal ulcergens, the endogenous levels of duodenal CGRP-IS and substance P-IS, and reported a decrease of duodenal CGRP and substance P. Whittle (1991) investigated the role of CGRP and substance P in modulating gastric mucosal integrity. Capsaicin, the pungent ingredient in red pepper, such as paprika or chilli, stimulates capsaicin-sensitive afferent neurons, which release CGRP and substance P from their nerve endings (Chen et al 1992). The results of several neurochemical and pharmacological studies support the hypothesis that capsaicin-sensitive afferents afford gastric mucosal protection via release of CGRP and substance P from their peripheral nerve endings. The anti-lesion action of the primary transmitters CGRP and substance P involves secondary messengers such as nitric oxide (NO) (Lambrecht et al 1993; Ströff et al 1996). NO is thus an important mediator of the gastroprotective pathways stimulated by capsaicin-evoked release of CGRP and substance P from extrinsic afferent nerve fibres. The source of NO involved in the anti-lesion actions of capsaicin, CGRP and substance P has not been identified so that both a neural and endothelial origin is conceivable.

In this study, cetraxate raised plasma CGRP- and substance P-IS levels. It is known that cetraxate increases mucosal prostaglandin (PG) $E_2$ and 6-keto-$PGF_{1\alpha}$ in rat stomach and promotes biosynthesis of $PGE_2$ and $PGI_2$. $PGE_2$ and $PGI_2$ exert their cytoprotective effect via capsaicin-sensitive afferent nerves (Arai 2003; Takeuchi 2003). Cetraxate might also exert its cytoprotective action through this pathway. It is also known that cetraxate suppresses gastric acid secretion against kallikrein. Kawashima et al (2002) reported CGRP increased somatostatin secretion and decreased gastric acid secretion via somatostatin-induced reduction of gastrin and histamine. In this study, plasma somatostatin-IS levels were significantly increased, but earlier than plasma CGRP-IS levels. The result was different from Kawashima's report. Furthermore, plasma gastrin-IS levels did not decrease, compared with placebo, until 240 min. There are long time lags between induction of somatostatin and reduction of gastrin. We think the effect of cetraxate on suppression of gastric acid secretion needs more investigation.

In conclusion, a single administration of cetraxate caused significant increases of plasma CGRP and substance P concentration compared with placebo in healthy subjects. Considering that cetraxate produces a dose-related increase in mucosal blood flow, we hypothesized that cetraxate might indirectly stimulate capsaicin-sensitive afferent nerves and increase mucosal blood flow, resulting in a gastroduodenal protective action.

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