Comparison of the Effects of Cetraxate and its Major Metabolite on Human Plasma Gastrin, Somatostatin, Calcitonine Gene-Related Peptide and Substance P in Human Plasma

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Cetraxate hydrochloride (cetraxate), an antiulcer drug, produces a dose related increase in mucosal blood flow. We have reported that cetraxate increases plasma calcitonin gene-related peptide (CGRP) and substance P in healthy human subjects (*J. Pharm. Pharmacol.*, **56**, p. 557, 2004). Cetraxate is rapidly metabolized to tranexamic acid in plasma. We investigated the effect of tranexamic acid on human plasma CGRP, substance P, gastrin and somatostatin. Tranexamic acid at a dose of 500 mg or placebo was orally administered in five healthy male volunteers. The blood samples were taken before and at 20, 40, 60, 90, 120, 180 and 240 min after administrations, followed by the extracting procedure, and submitted to the high sensitive enzyme immunoassay system for CGRP and substance P as previously developed. Single administration of tranexamic acid caused significant increases of plasma CGRP concentration at 60–90 min compared with placebo, but the level-time profile was a little different from that of cetraxate. Tranexamic acid had no significant effect on plasma gastrin, somatostatin and substance P levels compared with placebo. In this study, we thought that the gastroprotective effect of cetraxate might not be due to plasma metabolite, tranexamic acid, but direct stimulation of gastric mucosa.

Key words —— cetraxate, tranexamic acid, mucosal blood flow, neuropeptide

INTRODUCTION

In genesis of gastroduodenal ulcer, gastric acid secretion and cytoprotective factors play an important role, apart from the significant contribution of

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Helicobacter pylori. The local release of vasodilating mediators in the gastric mucosal microcirculation is of paramount importance in the maintenance of mucosal integrity and defense.

Cetraxate was introduced in 1976 as an anti-ulcer drug with a mucosal protective effect.[1] Murakami *et al.*[2] devised a non-invasive technique to measure continuously human gastric mucosal blood flow by laser method and studied the effect of cetraxate on human gastric mucosal microcirculation. They reported that cetraxate produce a dose dependent increase in gastric mucosal blood flow. Recently, cetraxate has been used in the treatment of eradication of *Helicobacter pylori* with proton pump inhibitor.[3,4] In the treatment of eradication of *Helicobacter pylori*, cetraxate play a role that the resistance of gastric mucosa is improved.

On the gastroprotective function as a neural emergency system, sensory afferent neurons in the gastrointestinal mucosa regulate neuropeptides [calcitonin gene-related peptide (CGRP) and tachykinins (substance P *etc.*)] levels and play various physiological roles.[5] Renzi *et al.*[6] and Whittle[7] reported that the lack of CGRP and substance P might cause gastroduodenal ulcer.

CGRP possesses several potent biological activities, including vasodilatation, being the most powerful vasoactive substance described to date, and it increases mucosal blood flow.[8,9] CGRP is known to coexist with tachykinins, in the population of sensory neurons in human.[10] Substance P is widely distributed in the central and peripheral divisions of the nervous system and in the enteroendocrine cells of gut,[11] and it participates in the regulation of gastrointestinal motility and secretion.[12]

We reported that single administration of cetraxate caused significant increases in plasma CGRP and substance P levels.[13] Cetraxate is absorbed from stomach and intestine, and by esterase,
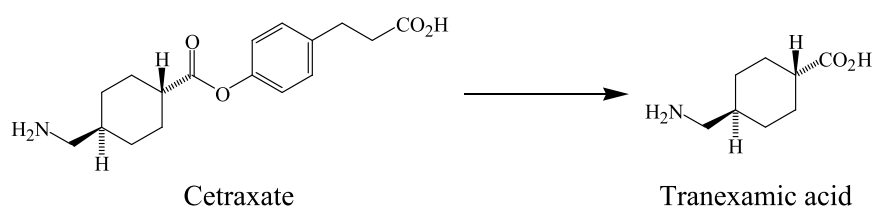
Fig. 1. The Structure of Cetraxate and Tranexamic Acid

rapidly metabolized to tranexamic acid and 3-(*p*-hydroxyphenyl)propionic acid in plasma (Fig. 1). The rate of metabolism is too fast to detect plasma concentration of cetraxate. Therefore the cytoprotective effect of cetraxate assumed to be due to direct local effect on gastric mucosa. But administration of high dose tranexamic acid has effects of curing serotonin-induced ulcer (not published). The another metabolite, 3-(*p*-hydroxyphenyl)propionic acid also has the effect of curing a kind of gastric ulcer, but the drug is not used clinically.

The purpose of this study was to determine the effects of tranexamic acid on plasma levels of CGRP-like immunoreactive substance (IS), substance P-IS and other gastrointestinal peptides in healthy subjects.

MATERIALS AND METHODS

Materials —— Cetraxate (Neuer capsule; Daiichi Seiyaku Co., Ltd., Tokyo, Japan), tranexamic acid (Transamin 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, human CGRP and its fragment (8-37) and substance P were purchased from the Peptide Institute (Osaka, Japan). Fragment gastrin I (2-17) was purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Antiserum to gastrin (A600/R1B) and CGRP were purchased from Biogenesis (Poole, U.K.), somatostatin (RA-08-108) and substance P (RA-08-095) from Cambridge Research Biochemicals (Cambridge, U.K.). All other reagents were analytical reagent grade from commercial sources.

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

Study Schedule —— Cetraxate (800 mg), tranexamic acid (500 mg) or placebo was administered orally with 100 ml water. Each subject was administered with these drugs at an interval of four weeks. The dose of tranexamic acid in this study was the corresponding dose that 800 mg cetraxate, which is maximum dose as a daily dose in clinical therapy, was all metabolized to tranexamic acid. 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.

Determination of Tranexamic Acid Levels in Plasma —— The plasma concentration of tranexamic acid was determined by the modified method of Matsubayashi *et al.*[14] Standard tranexamic acid (lot TSAAJ04) was supplied by Daiichi Seiyaku Co., Ltd., A 0.5 ml of plasma samples were added to 1.4 ml of ethanol and the contents was mixted on the vortex mixer. After centrifugation (15000 g, 5 min), the supernatant was added to 1 ml of 10 mM borax solution (pH 9.2) and 13 μl of phenyl isothiocyanate and kept at 40°C for 30 min. The resulting mixture was extracted with 5 ml xylene and centrifuged (2500 g, 10 min, 0°C). The organic layer was discarded and aquerious layer was acidified with 1 ml of 35% (w/w) hydrochloric acid. The solution was heated at 80°C for 10 min, then evaporated to dryness under reduced pressure. The residue was dissolved in 1 ml of 0.1 M borax solution and extracted 3 ml of benzene. The organic extracts were evaporated to dryness under reduced pressure. The residue was redissolved in 1 ml of mobile phase, and a 20-μl portion was injected into the HPLC. HPLC was carried out using a C18 column (Cosmosil 5C18-AR; Nacalai Tesque, Kyoto, Japan) with UV detection at 254 nm, and 20 mM phosphate buffer (pH 7.0)-ethanol (9 : 1) was used as a mobile phase at a flow rate of 1.7 ml/min.

Enzyme Immunoassay (EIA) of Gastrin, Somatostatin, 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 of EDTA. After centrifugation, plasma samples were diluted fivefold with 4% acetic acid (pH 4.0) and loaded onto C18 reversed-phase cartridge (Sep-Pak C18; Millipore Corp., Milford, MA, U.S.A.). 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-, CGRP- and substance P-IS was > 90% with this extracting procedure (data not shown).

EIA for gastrin,[15] somatostatin,[16] CGRP,[17] and substance P[18] were performed 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). The fluorescent product 4-methylumbelliferon was measured with an MTP-100F microplate reader (Corona Electric, Ibaraki, Japan). Gastrin I (2-17), human somatostatin, human CGRP (8-37) and substance P was conjugated with β-D-galactosidase (Boehringer Mannheim, Mannheim, Germany) by N-(ε-maleimidocaproyloxy)-succimide according to the method of Kitagawa *et al*.[19] The EIA for gastrin, somatostatin, CGRP and substance P was specific and highly sensitive to detection limits of 0.04, 0.10, 0.08 and 0.4 fmol/well, respectively.

Statistical Analysis —— Values obtained at a given time points were compared between groups by Mann-Whitney U test and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Figure 2 showed plasma tranexamic acid levels after its oral administration. The drug concentration was peaked at 180 min (4.4 ± 0.8 mg/ml). The relative recovery of plasma tranexamic aci was 98% at the concnentration 4.4 mg/ml.

The plasma CGRP-IS level-time profile after administration of cetraxate and tranexamic acid is shown in Fig. 3a. Cetraxate caused significant in-
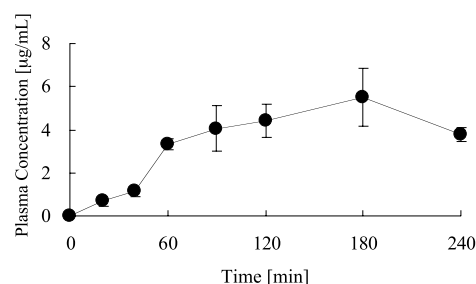

Fig. 2. Plasma Tranexamic Acid Levels after Oral Administration of 500 mg of the Drug

Each value represents the mean ± S.D., $n = 5$.

crease in CGRP-IS between 60–120 min and 240 min (20.7 ± 6.0 pg/ml at 60 min, 11.1 ± 10.8 pg/ml at 90 min, 7.6 ± 1.8 pg/ml at 120 min and 11.8 ± 0.7 pg/ml at 240 min), compared with the response of the placebo group (5.2 ± 1.2 pg/ml at 60 min, 5.7 ± 1.1 pg/ml at 90 min, 5.4 ± 0.4 pg/ml at 120 min and 3.8 ± 1.2 pg/ml at 240 min). Tranexamic acid caused significant increase in CGRP-IS between 60–90 min and 180–240 min (10.8 ± 5.7 pg/ml at 60 min, 11.7 ± 3.7 pg/ml at 90 min, 6.5 ± 2.0 pg/ml at 180 min and 7.7 ± 2.3 pg/ml at 240 min), compared with the response of the placebo group (5.0 ± 0.2 pg/ml at 180 min). Figure 3b showed plasma substance P-IS levels after administration of cetraxate and tranexamic acid. Cetraxate significantly increased substance P-IS levels between 40–90 min (29.6 ± 2.5 pg/ml at 40 min, 34.4 ± 2.5 pg/ml at 60 min and 34.0 ± 1.3 pg/ml at 90 min) compared with the response of the placebo (20.4 ± 6.5 pg/ml at 40 min, 21.5 ± 2.5 pg/ml at 60 min and 23.3 ± 3.2 pg/ml at 90 min). The plasma somatostatine-IS levels were significantly increased at 20 min (28.1 ± 11.6 pg/ml) compared with placebo (12.8 ± 1.1 pg/ml) after administration of cetraxate (Fig. 3c). At 240 min, the plasma gastrin-IS levels (12.3 ± 4.7 pg/ml) were significantly suppressed compared with placebo (20.8 ± 6.4 pg/ml) after administration of cetraxate (Fig. 3d). But tranexamic acid did not alter levels of substance P, somatostatin and gastrin-IS (Fis. 3b–3d).

Tranexamic acid binds lysine-binding site strongly, which is fibrin affinity site of plasmin and plasminogen and inhibits that plasmin or plasminogen binds fibrin.[20,21] Plasmin is activated from plasminogen by plasminogen activator and resolves fibrin, which is pillar of blood coagulation. Therefore, it is considered that tranexamic acid inhibits fibrin resolution and stops bleeding. Furthermore
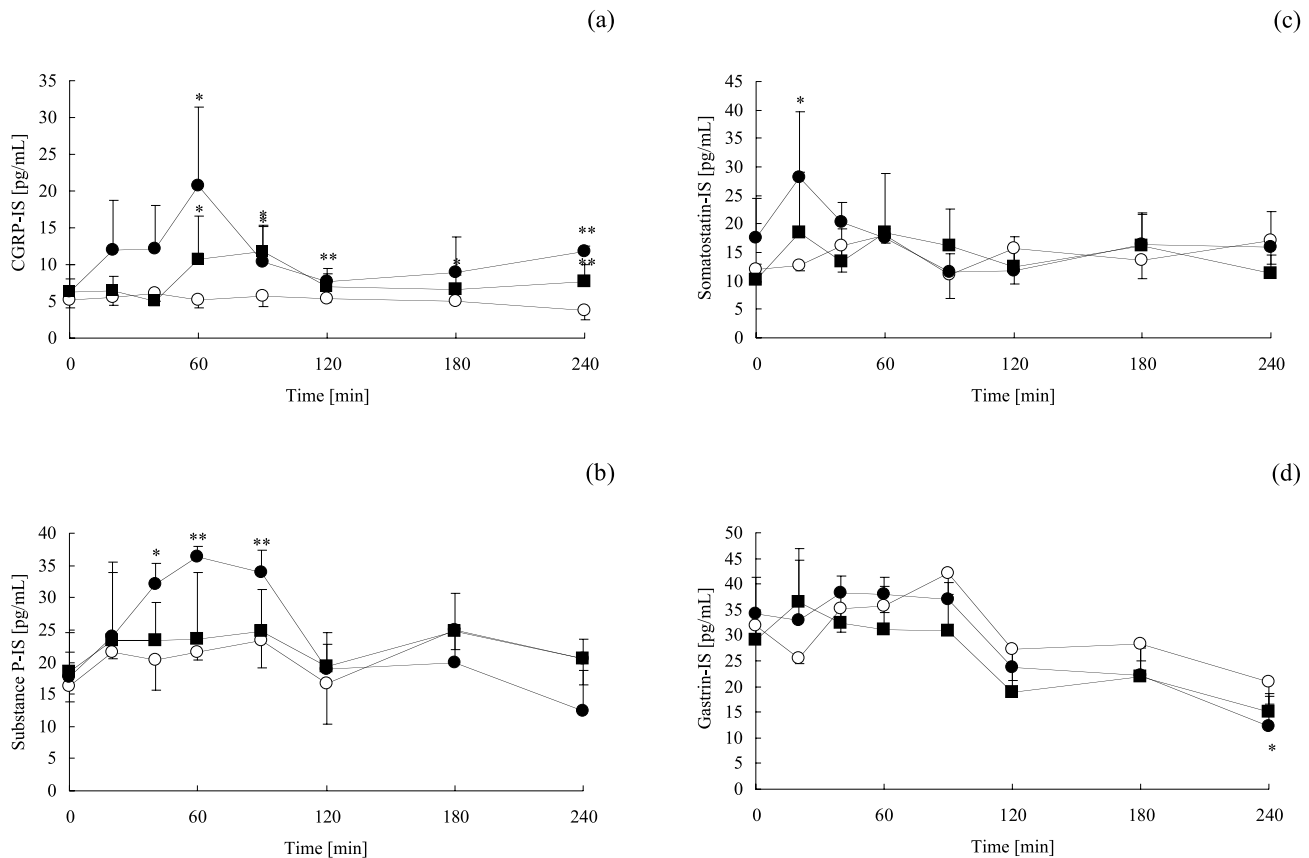
(a)

(c)

(b)

(d)

Fig. 3. Effect of Cetraxate (●), Tranexamic Acid (■) or Placebo (○) on Plasma CGRP- (a), Substance P- (b), Somatostatin- (c) and Gastrin-IS (d) Levels
Each value represents the mean ± S.D., $n = 5$. *$p < 0.05$ and **$p < 0.01$, significantly different compared with placebo.

tranexamic acid suppressed genesis of kinin and other bioactive peptides, which caused promotion of capillary permeability, allergy or inflammation diseases.[22] Clinically, it is used to stop bleeding and to treatment with allergy or inflammation diseases in dermatology and otolaryngology fields.[23] Although the medicine is not used to treat gastrointestinal disease, in this study, we investigated the effect of tranaxamic acid as a metabolite of cetraxate on gastrointestinal peptide.

CGRP is a powerful vasoactive substance, which is released from the sensory afferent nerve endings against gastric mucosal injury in the stomach. CGRP increases gastric mucosal flow as a gastroprotective factor.[5] Substance P, tachykinins, coexists 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.[24]

In this study, CGRP-IS levels were raised significantly but substance P was not altered. The Tmax of tranexamic acid was from 120 to 180 min, in this study, Tmax was almost 180 min (Fig. 2). Considering CGRP-IS levels were significantly increased from 60 to 90 min, the increase might not be related between the plasma concentration changes of tranexamic acid and that of CGRP-IS levels. Because the changes of CGRP-IS levels after tranexamic acid administration was similar to the changes after cetraxate administration, tranexamic acid also might have direct effects to gastric mucosa as cetraxate stimulates directly gastric mucosa.

The anti-lesion action of the primary transmitters CGRP involves secondary messengers such as nitric oxide (NO).[25,26] NO, an endothelium derived relaxing factor, stimulates vascular smooth muscle, resulted in relaxing vascular smooth muscle and vasodilation.[27] The pharmacological effect of cetraxate is considered that it increases gastric mucosal blood flow *via* vasodilation of gastric microcirculation and cetraxate shows gastroprotective effects. On tranexamic acid, there were no information to vasodilate, but its effects on cochlear blood flow was reported.[28] Very high dose administration

of tranexamic acid might cause increase of blood flow, but daily dose administration (up to 2000 mg) might not cause to improve blood flow and show gastric protective effects. Furthermire, we hypothesized that the gastroprotective effect of cetraxate was caused by stimulation of capsaicin-sensitive afferent nerves, which released CGRP and substance P.) Considering that tranexamic acid did not alter substance P-IS levels, we thought that the increase of CGRP-IS after administration of tranexamic acid might not be by stimulating capsaicin-sensitive afferent nerves, but there was the other mechanism.

In conclusion, single administration of tranexamic acid caused significant increases of plasma CGRP concentration compared with placebo group. Although it is not denied that tranexamic acid increases blood flow *via* directly or indirectly stimulation of afferent nerve, the contributions for pharmacological effects of cetraxate might be much little.

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