

The Effect of Nichin-to on the Plasma Gut-Regulatory Peptide Level in Healthy Human Subjects

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(Received November 1, 2004; Accepted December 9, 2004)

Nichin-to, a traditional Chinese herbal (Kampo) medicine, has been used to treat nausea and vomiting. Most of traditional herbal medicines are prepared from several different herbs. For example, Nichin-to is a combination of five herbs: *Pinelliae Tuber*, *Zingiberis Rhizoma*, *Poria*, *Glycyrrhizae Radix* and *Aurantii Nobilis Pericarpium*. Thus, to determine the exact pharmacological mechanisms of Chinese herbal medicines is very difficult. However, the pharmacological effects of some Chinese herbal medicines can be elucidated from the changes in plasma levels of neuropeptides. In this study, we investigated the effects of Nichin-to on the plasma levels of gut-regulated peptides [gastrin, somatostatin, motilin, vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and substance P] in healthy human subjects. A single oral administration of Nichin-to caused significant ($p < 0.05$) increases in plasma levels of gastrin-, somatostatin-, VIP-, motilin-, CGRP- and substance P-immunoreactive substance (IS), compared with placebo group. In conclusion, these results might indicate that the pharmacological action of Nichin-to is closely related to changes in these peptide levels in human plasma, and we hypothesize that the pharmacological effect of Nichin-to might be due to improvement of digestion, and accelerating the rate of gastric emptying and intestinal propulsion.

Key words — Nichin-to, gut-regulated peptide, Kampo, nausea, vomiting

INTRODUCTION

In many cases, traditional Chinese herbal (Kampo) medicines have been used in the empirical treatment of chronic hypofunction. In recent years, some Kampo medicines used to treat those gastrointestinal diseases have been elucidated pharmacologically by studying gut-regulated hormone levels.^{1–4)}

Nichin-to, a Kampo medicine, is prepared from five herbs: *Pinelliae Tuber*, *Zingiberis Rhizoma*, *Poria*, *Glycyrrhizae Radix* and *Aurantii Nobilis Pericarpium*. The medicine has been used for thousands of years for the treatment of nausea, vomiting, chronic gastritis and gastroduodenal ulcer.

Emesis is a complex process coordinated by the vomiting center in the lateral reticular formation of the medulla. This center receives input from the chemoreceptor trigger zone (CTZ) in the area

postrema. The blood-brain barrier is poorly developed in the area postrema, such that the CTZ is readily accessible to emetic substances in the circulation. Some peripheral signals bypass the CTZ, reaching the emetic center via the solitary tract nucleus (*e.g.* from the stomach and small intestine). Moreover, emesis is enhanced by conditions that slow gastric emptying.

Serotonin (5-hydroxytryptamine, 5-HT), acting at 5-HT₃ receptors, is an important emetic signal and transmitter in the afferent pathways from the stomach and small intestine, in both the CTZ and in the solitary tract nucleus. Dopaminergic D₂ receptors are implicated in emetic signaling through the CTZ and in the solitary tract nucleus, as is acetylcholine, which acts via muscarinic receptors in the solitary tract nucleus. Antagonism of transmission through these pathways contributes to the antiemetic effects of D₂ and 5-HT₃ antagonists. Moreover, dopamine receptors in the stomach appear to mediate the inhibition of gastric motility that occurs during nausea and vomiting, and these receptors may provide a site of action for antiemetic dopamine receptor antagonists. These dopamine receptors also participate in

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reflexes that result in relaxation of the upper portion of the stomach and delayed gastric emptying in response to gastric distension by food.

It has been assumed that one of the gastrointestinal motility regulatory factors related with empirical effects of Kampo medicine is the induction of changes in the levels of peptides [gastrin, somatostatin, motilin, vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and substance P] in plasma. Itoh *et al.* reported that cisapride, a dopamine D₂ receptor antagonist and nonselective serotonin 5-HT_{1, 3, 4} receptor agonist, induced a rise in plasma levels of motilin, gastrin and somatostatin,⁵ while mosapride, a selective serotonin 5-HT₄ agonist, raised motilin and gastrin⁶ plasma levels. We have investigated the effects of Sho-hange-ka-bukuryo-to, which is a Kampo medicine closely related to Nichin-to, on plasma gut-regulatory peptide levels.⁴

On the basis of traditional Kampo theory, the Kampo medicine has been used for improvement of abnormal condition of the subjects. But the levels of plasma gut-regulatory peptides are easily influenced by meals, stress or diseases. Therefore, at first, we investigated the effects on healthy subjects, whose environmental condition were easily controlled.

The purpose of this study was to determine the effects of Nichin-to on the plasma levels of the gut-regulate peptides gastrin-, somatostatin-, motilin-, VIP-, CGRP-, substance P-immunoreactive substance (IS), in healthy subjects.

MATERIALS AND METHODS

Materials — Nichin-to (lot 21010041), prepared as a 3.0 g dried powder extract of *Pinelliae Tuber* (5.0 g), *Glycyrrhizae Radix* (1.0 g), *Poria* (5.0 g), *Zingiberis Rhizoma* (1.0 g) and *Aurantii Nobilis Pericarpium* (4.0 g) were kindly supplied by Tsumura Co., Ltd. (Tokyo, Japan). The placebo was maltose, the vehicle for the above formulations, (Sunmalt; Hayashibara Co., Ltd., Okayama, Japan).

Synthetic human gastrin I (G17), somatostatin, motilin, VIP, substance P, and CGRP and its fragment (8-37), were purchased from the Peptide Institute (Osaka, Japan). Fragment gastrin I (2-17) was purchased from Sigma Chemicals (St. Louis, MO, U.S.A.) and the VIP fragment peptide was supplied by Professor H. Yajima (Kyoto University, Kyoto, Japan). Antisera to gastrin (A600/R1B), VIP (A604/

R1B) and CGRP were purchased from Biogenesis (Poole, U.K.). Antisera to somatostatin (RA-08-108) and substance P (RA-08-095) were purchased from Cambridge Research Biochemicals (Cambridge, U.K.) and antiserum to motilin (Y121) was purchased from Yanaihara Institute (Shizuoka, Japan). All other reagents were analytical grade from commercial sources.

Subjects — Five healthy male volunteers (non smokers), aged 24–29 years (median 28 years), weighing 55–68 kg (median weight 62 kg), participated in the study. Each subject received information on the 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 at least one month before the study, and fasted for 2 hr before drug administration and during the experiments.

Study Schedule — Nichin-to or placebo was administered orally at a dose of 7.5 g with 100 ml water. Each subject was given both the placebo and Nichin-to, a month apart. The dose of Nichin-to used in this study was the maximum daily dose used in clinical therapy. Venous blood samples (10 ml) were taken from a forearm vein just before the drug was administered, and at 20, 40, 60, 90, 120, 180 and 240 min after administration (eight blood samples were taken during each experiment). All subjects ate lunch at 11:45–12:00, and the study was carried out from 14:00 until 18:00.

Enzyme Immunoassay (EIA) of Gastrin, Somatostatin, Motilin, VIP, CGRP, Substance P — The blood samples were placed in chilled tubes containing 500 units/ml of the kallikrein inhibitor 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 a 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). Elutes 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).

Enzyme immunoassay (EIA) for gastrin,⁷ somatostatin,⁸ motilin,² VIP,⁹ CGRP¹⁰ and substance P¹¹ was performed as previously described, 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). Fragment gastrin I (2-17), human somatostatin, porcine motilin, fragment VIP (11-28), human CGRP (8-37) and substance P was conjugated with β -D-galactosidase (Boehringer Mannheim, Mannheim, Germany) by *N*-(ϵ -maleimidocaproyloxy)-succinide according to the method of Kitagawa *et al.*¹² The EIA for gastrin, somatostatin, motilin, VIP, CGRP and substance P was 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 \pm S.D. (pg/ml). Comparison of mean values was made by the Mann Whitney U test and $p < 0.05$ was considered statistically significant.

RESULTS

The plasma gastrin-IS level-time profile after the administration of Nichin-to or placebo is shown in Fig. 1a. Nichin-to caused significant increases in plasma gastrin-IS levels at 60 and 120 min (47.8 ± 5.4 pg/ml at 60 min, 38.9 ± 7.8 pg/ml at 120 min) compared with the placebo group (31.3 ± 3.6 pg/ml at 60 min, 29.5 ± 4.6 pg/ml at 120 min).

The plasma somatostatin-IS level-time profile after the administration of Nichin-to or placebo is shown in Fig. 1b. Nichin-to caused significant increases in somatostatin-IS at 20 and 60 min (15.4 ± 3.7 pg/ml at 20 min, 14.4 ± 1.1 pg/ml at 60 min) compared with the placebo group (11.7 ± 1.9 pg/ml at 20 min, 10.0 ± 1.2 pg/ml at 60 min).

The plasma motilin-IS level-time profile after the administration of Nichin-to or placebo is shown in Fig. 1c. Nichin-to caused significant increases in motilin-IS at 90 min (80.9 ± 10.0 pg/ml) compared with the placebo group (52.6 ± 10.9 pg/ml).

The plasma VIP-IS level-time profile after the administration of Nichin-to or placebo is shown in Fig. 1d. Nichin-to caused significant increases in VIP-IS at 40 min (10.0 ± 1.8 pg/ml) compared with the placebo group (7.4 ± 1.9 pg/ml).

The plasma CGRP-IS level-time profile after the administration of Nichin-to or placebo is shown in Fig. 1e. Nichin-to caused significant increases in CGRP-IS at 60 min (33.5 ± 8.4 pg/ml) compared

with the placebo group (20.8 ± 4.5 pg/ml).

The plasma substance P-IS level-time profile after the administration of Nichin-to or placebo is shown in Fig. 1f. Nichin-to caused significant increases in substance P-IS at 90 min (51.6 ± 10.2 pg/ml) compared with the placebo group (36.6 ± 6.0 pg/ml).

DISCUSSION

Gastrin stimulates gastric acid secretion involving G cells, and it is associated with a mechanism of gastrointestinal motility involving the cholinergic nervous system.¹³ In Fig. 1, the temporary elevation of gastrin-IS levels of the Nichin-to and placebo groups (20 min) might be caused by stimulation of gastric mucosa G cell. Nichin-to significantly increased gastrin-IS levels at 60 and 120 min compared with the response of the placebo group. Therefore, we propose that the Nichin-to group showed a two-phase (20 min: non-specific, 60–120 min: Nichin-to specific) increase in this study. The plasma gastrin-IS level-time profile after the administration of Nichin-to was similar to that of Rikkunshi-to.¹ The gastrin-IS release by Nichin-to might be related to its pharmacological effect; normalization of gastric function.

Somatostatin inhibits the secretion of other hormones, including gastrin, insulin, and motilin.¹⁴ In the gastrointestinal tract, gastric acid and pepsin secretion, and gastric emptying are inhibited by somatostatin.¹⁵ In the interdigestive state somatostatin induces phase-3 activities,¹⁶ and in the digestive state it inhibits gastric emptying¹⁷ and slows gastrointestinal transit.¹⁸ Somatostatin inhibits the secretion of gastrin, but in this study, while somatostatin-IS levels were significantly increased, gastrin-IS levels were not inhibited. This implies that the intercellular communication between somatostatin and gastrin is paracrine and somatostatin might not inhibit all pathways of gastrin release. A previous report on Ninjin-to also showed the same phenomena.¹⁹ Further studies are needed to elucidate the mechanisms involved.

Motilin has a powerful fundic pouch motor-stimulating activity.²⁰ 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 the migrating motor complex (MMC).²¹ Motilin participates in regulating gastrointestinal motility with somatostatin, and stimulates gastric emptying and postpran-

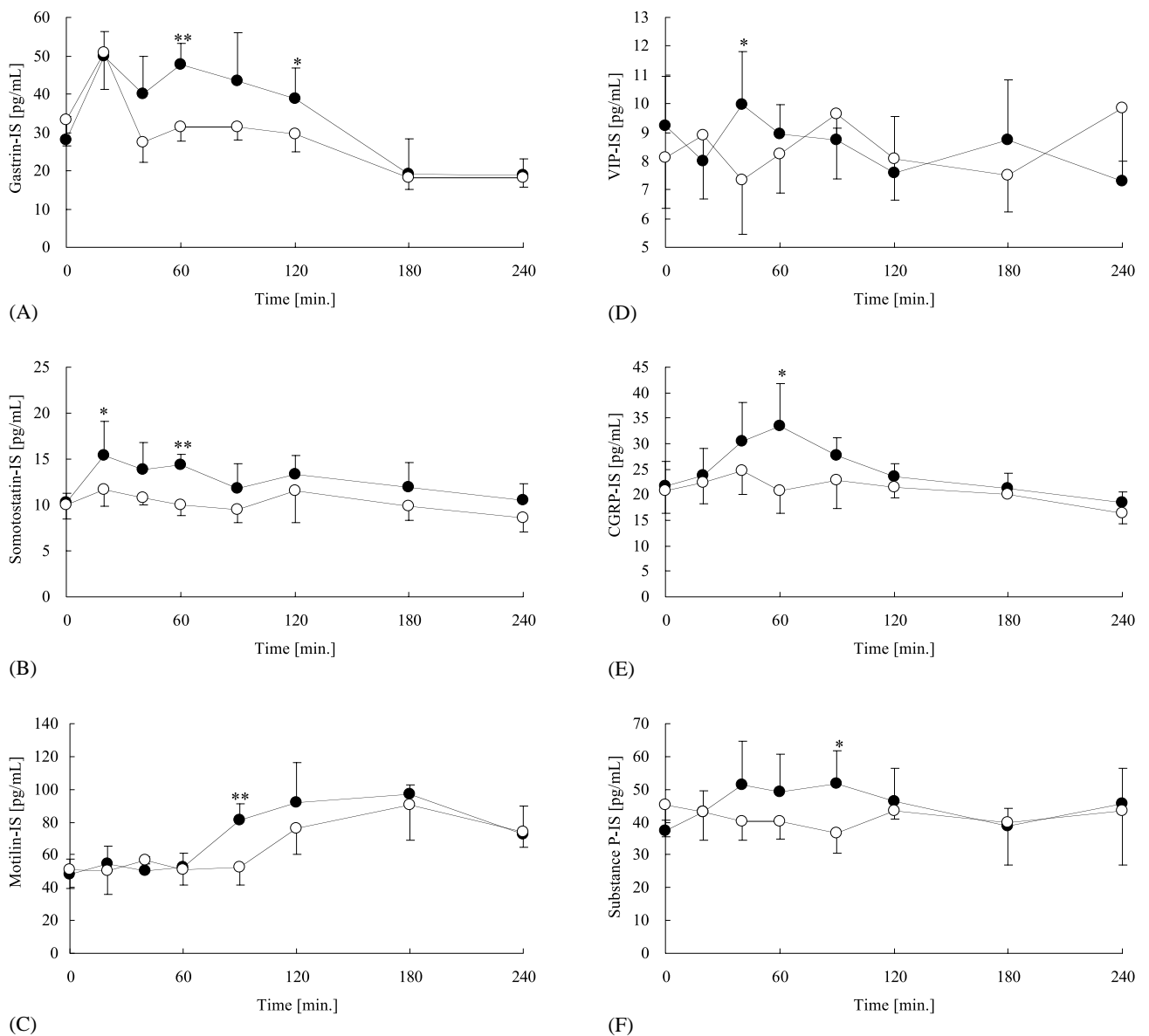


Fig. 1. Effect of Nichin-to (●) or Placebo (○) on Plasma Gastrin- (a), Somatostatin- (b), Motilin- (c), VIP- (d), CGRP- (e) and Substance P-IS (f) Levels

Each value represents the mean \pm S.D., $n = 5$. * $p < 0.05$ and ** $p < 0.01$, significantly different from placebo.

dial gastric contraction. Its actions result from its interaction with a specific receptor for the peptide in the antrum and duodenum. The antibiotic erythromycin also acts as an agonist at these receptors. This interaction may account for the prokinetic effects of erythromycin and other macrolide antibiotics.²²⁾ In this study, following increases of somatostatin-IS levels at 40–60 min, motilin-IS levels were increased at 90 min. This supports the theory that Nichin-to promotes gastric emptying.

CGRP is a powerful vasoactive substance, which is released from the sensory afferent nerve endings

after gastric mucosal injury (acid and the other noxious chemicals such as capsaicin, ethanol, *etc.*) in the stomach. CGRP increases gastric mucosal blood flow as a gastroprotective factor.²³⁾ In this study, because Nichin-to raised plasma CGRP-IS levels, Nichin-to might directly stimulate CGRP-containing nerves, or indirectly secrete CGRP accompanied by the stimulation of other secretion cells.

VIP is widely distributed in the central and peripheral nervous system.²⁴⁾ This peptide has a vasodilating effect and is an important neurotransmitter for the enteric nervous system.²⁵⁾

Substance P, a tachykinin neuropeptide, 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 nervous system.²⁶⁾ Substance P causes direct contractions of circular muscle in most regions of the mammalian intestine, stimulates small intestine motility and controls human small intestinal motility.²⁵⁾ Furthermore, this peptide influences the rate of gastric emptying.²⁷⁾

The intestine of humans is capable of organized propulsion of intraluminal contents. Propulsion of chyme or feces along the gut depends on the enteric nervous system, which coordinates slow wave activity and on associated contractions and relaxations of the muscle layers. This coordinated propulsive activity is called peristalsis. Somatostatin and VIP are considered to regulate relaxations, substance P is considered to regulate contractions, and CGRP is considered to regulate both of them.²⁵⁾ In this study, somatostatin-, VIP-, CGRP- and substance P-IS levels were increased after Nichin-to administration. This could indicate that Nichin-to might promote the peristaltic reflex.

Nichin-to is widely used in treatment of nausea and vomiting. We investigated the changes of gastrointestinal peptides in healthy human plasma after Nichin-to administration, but the relationship between changes of gastrointestinal peptides and emesis is not known. The pharmacological mechanism is also not known and there are no reports on the pharmacological effects in humans. Therefore, further studies such as clinical trials in patients suffering nausea and vomiting are needed to elucidate these pharmacological mechanisms and effects.

In conclusion, Nichin-to, a Kampo medicine that is used to treat nausea and vomiting, raised plasma gastrin-, somatostatin-, motilin-, VIP-, CGRP- and substance P-IS levels. We hypothesized that the pharmacological effects of the drug might be closely related with these peptides, normalize gastric digestion, and accelerate the rate of gastric emptying and intestinal propulsion.

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