Effects of Ninjin-to on Levels of Brain-Gut Peptides (Motilin, Vasoactive Intestinal Peptide, Gastrin, and Somatostatin) in Human Plasma

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Received July 21, 2000; accepted October 24, 2000

We examined the effects of Ninjin-to, a traditional Chinese (Kampo) medicine, on the levels of brain-gut peptides (motilin, vasoactive intestinal peptide (VIP), gastrin, and somatostatin) in plasma from healthy subjects. A single oral administration of Ninjin-to, at a dose of 6.0 g, caused significant increases in plasma motilin levels at 40 to 90 min and somatostatin levels at 20 to 90 min, compared with a placebo treated group. Transient elevations of gastrin levels in the placebo group were inhibited by administration of Ninjin-to, but the medicine did not alter the levels of VIP. In conclusion, these results suggest that pharmacological effects of Ninjin-to on gastrointestinal functions closely relate to changes of motilin, gastrin, and somatostatin-immunoreactive substance levels in human plasma.

Key words Ninjin-to; motilin; somatostatin; vasoactive intestinal peptide; gastrin; Kampo

Ninjin-to, a traditional Chinese (Kampo) herbal medicine, is prepared from four crude herbs: Ginseng Radix, Glycyrrhizae Radix, Atractylodis Rhizoma, and Zingiberis Siccatum Rhizoma. Ninjin-to has been used for thousands of years for the treatment of gastroenteritis, esogastritis, gastric atony, gastrectasia, vomiting, and anorexia experientially.

In recent years, some Chinese herbal medicines of experimental gastrointestinal effects have been elucidated pharmacologically. One of them, Dai-kenchu-to, increases gastrointestinal motility and improves bowel obstruction. These effects were reported to cause significant increases in the levels of motilin and vasoactive intestinal peptide (VIP) in plasma. Ninjin-to contains two of the same herbs as Dai-kenchu-to: Ginseng Radix and Zingiberis Siccatum Rhizoma. Furthermore, both herbal medicines have been used to improve gastrointestinal functions. Those effects of gastrointestinal motility are mainly regulated by hormonal and neuronal mechanism. Therefore, we examined the plasma levels of brain-gut peptides, which regulate gastrointestinal motility.

The brain-gut peptide, motilin, is a 22-amino acid residue polypeptide and has a powerful fundic pouch motor-stimulating activity. It plays an important physiological role in intestinal contractility, and was one of the most important factors controlling the regular occurrence of phase-3 contractions of migrating motor complex (MMC).

VIP, a 28-amino acid residue neuropeptide, widely distributes in the central and peripheral nervous system. This peptide has a vasodilating effect, and is an important neurotransmitter for the enteric nervous system.

Gastrin, a 17-amino acid residue polypeptide, stimulates gastric acid secretion. This peptide is associated with a mechanism of gastrointestinal motility involving the cholinergic nervous system.

Somatostatin, a 14-amino acid residue polypeptide, inhibits the secretion of other polypeptides, including gastrin, insulin, and motilin. In the gastrointestinal tract, gastric acid and pepsin secretion and gastric emptying are inhibited by somatostatin.

The purpose of this study was to determine the effects of Ninjin-to on the plasma levels of motilin-, VIP-, gastrin-, and somatostatin-immunoreactive substance (IS) in healthy subjects.

MATERIALS AND METHODS

Materials Ninjin-to (EK-32, lot. 01BJ), prepared as a 3.0 g dried powder extract in the following proportions: Ginseng Radix (3.0 g), Glycyrrhizae Radix (3.0 g), Atractylodis Rhizoma (3.0 g), and Zingiberis Siccatum Rhizoma (3.0 g), was kindly supplied by Kaneko Co., Ltd. (Tokyo, Japan). A mixture of sucrose and cellulose, consisting of EK-32 as additive, was used as a placebo. All other reagents were reagent grade and commercially available. Synthetic porcine motilin, that has a sequence identical to human motilin, synthetic vasoactive intestinal peptide (VIP), synthetic human gastrin I (G17), and synthetic somatostatin were purchased from Peptide Institute Inc. (Osaka, Japan). Antiserum to motilin (A600/R1B), VIP (A604/R1B), and gastrin (A600/R1B) were kindly supplied by Kanebo Co., Ltd. (Tokyo, Japan). A mixture of sucrose and cellulose, consisting of EK-32 as additive, was used as a placebo. All other reagents were reagent grade and commercially available.

Subject Five healthy male volunteers (non-smokers) participated in this study. Each subject received information about the study’s scientific purpose, which was approved by the Ethics Committee at Oita Medical University, and gave informed consent. No subject received any medication for two weeks preceding the test and no stimulator of gastrointestinal motility, except Ninjin-to, was administered to any subject during the study.

Study Schedule Ninjin-to and placebo were packaged identically. Ninjin-to was given orally as a single dose of 6.0 g with water to the 5 volunteers. Two weeks later, the same dose of a placebo was given orally to the volunteers. Venous blood samples (10 ml) from a forearm vein were taken to measure the levels of each peptide in plasma by enzyme immunoassay (EIA). Samples were taken before and at 20, 40, 60, 90, 120, 180, and 240 min after administration of the drug. All subjects ate lunch at 11:45—12:00 and the...
study was carried out from 14:00 (2 h after lunch) until 18:00.

**Preparation of Plasma Extracts** The blood samples were placed in chilled tubes containing 500 kallikrein inhibitor units/ml of aprotinin (Trasylol®, Bayer Co., Ltd., Leverkusen, Germany) and 1.2 mg/ml of EDTA (Wako Pure Chemical Industries, Ltd., Osaka, Japan). After centrifugation (1670×g, 4 °C, 20 min), plasma samples were diluted with 4 ml of 4% acetic acid, pH 4.0, and loaded onto Sep-Pak® C18 cartridges (Millipore Co., Massachusetts, U.S.A.). After washing with 4% acetic acid, pH 4.0, each peptide in plasma was eluted with 70% acetonitrile in 0.5% acetic acid, pH 4.0. The eluates were concentrated by spin-vacuum evaporation, lyophilized, and stored (−20 °C) until high sensitive enzyme immunoassay (EIA). The recovery of plasma motilin-, VIP-, gastrin-, and somatostatin-IS was >93% with this extracting procedure (data not shown).

**EIA for Motilin-, VIP-, Gastrin, and Somatostatin-IS** Peptide levels in plasma were measured using EIA for motilin-15) VIP-16) gastrin-17) and somatostatin-IS18) as previously described. The assay was performed by a delayed addition method. Separation of bound and free antigen (peptides) was performed on an anti-rabbit IgG coated immunoplate (Nunc-Immuno Module Maxisorp F8, InterMed, Denmark). Human motilin, fragment of VIP (positions 11—28), mini gastrin I, and human somatostatin were conjugated with β-galactosidase by N-(ε-maleimidocaproyloxy)-succinimide according to the methods of Kitagawa et al.19) 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 put in secondary-antibody coated immunoplates and the plates were incubated overnight. Then, after washing with phosphate buffer, MUG was added to each well. The plates were incubated again at 37 °C for 3 h and the fluorescence intensity (λ_{Ex} 360 nm, λ_{Em} 450 nm) of each well was measured with a MTP-100F microplate reader (Corona Electric, Ibaraki, Japan). The detection unit of motilin-, VIP-, gastrin-, and somatostatin-IS was 0.80, 1.00, 0.04, and 0.10 fmol/well, respectively.

**Data Analysis** All values are expressed as mean±S.D. Comparisons of mean values were made by analysis of variance and Dunnett’s test. A value of p<0.05 was regarded as significant.

**RESULTS AND DISCUSSION**

Ninjin-to has frequently used for thousands of years to improve gastrointestinal functions. In this study, four types of brain-gut peptide (motilin, VIP, gastrin, and somatostatin), which regulates gastrointestinal motility, were examined to study the effects of Ninjin-to.

Motilin is a powerful inducer of gastrointestinal motor activity in the fundus and the antral pouch of the stomach.4) The plasma motilin-IS level-time profile after a single oral administration of Ninjin-to or placebo is shown in Fig. 1(A). Ninjin-to caused significant increases in motilin-IS at 40 min (41.1±11.7 pg/ml), 60 min (50.5±11.4 pg/ml), and 90 min (61.1±12.1 pg/ml) compared with the response of the placebo treated group (about 25 pg/ml), whereas in the placebo group, the basal line of plasma motilin-IS levels might be the effect of circadian rhythms. Accordingly, those changes of motilin-IS levels in the Ninjin-to treated group suggest that this drug might affect motilin-IS levels in human plasma through some pathways.

VIP has a vasodilating effect and functions as a neurotransmitter for the enteric nervous system.7) Ninjin-to had no effect on plasma VIP-IS levels compared with the placebo treated group (Fig. 1(B)). Furthermore, plasma VIP-IS levels of both groups remained constant before and after adminis-
transit (about 8 pg/ml).

Gastrin is associated with a gastrointestinal motility involving cholinergic nerves. Somatostatin is the first of the gut regulatory peptides to have a significant therapeutic use. To date most clinical experience has been with one analogue, octreotide. This peptide pharmaceutical may be useful for treatment of intestinal dysmotility. Some of these effects of peptides might correspond with experiential gastrointestinal actions of Ninjin-to.

Motilin participates in regulating gastrointestinal motility with somatostatin. In this study, motilin-IS levels by Ninjin-to administration were enhanced by several pathways. That elevation also seemed to indicate that Ninjin-to might enhance motor activity in a small intestine, including phase-3 contractions of MMC. We hypothesize that Ninjin-to might improve the gastrointestinal functions by increasing motilin- and somatostatin-IS levels in plasma. Although further studies are needed to elucidate the mechanism, our findings indicated the experiential gastrointestinal effects of Ninjin-to are closely related to changes in brain-gut peptide levels in plasma.

REFERENCES