Rikkunshi-to Raises Levels of Somatostatin and Gastrin in Human Plasma

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Received January 12, 2001; accepted April 2, 2001

Rikkunshi-to, a traditional Chinese (Kampo) medicine, has been used to treat chronic hypofunctions of the gastrointestinal tract. The effects of Rikkunshi-to on the plasma levels of gut-regulated peptide (somatostatin, motilin, gastrin, and vasoactive intestinal peptide (VIP)) levels were studied in healthy subjects. A single oral administration of Rikkunshi-to caused significant increases in plasma somatostatin and gastrin levels at 60 to 240 min compared with a placebo group. On the other hand, this medicine showed no effects on motilin and VIP levels. In conclusion, these results might indicate that the pharmacological action of Rikkunshi-to is closely related to changes in somatostatin- and gastrin-immunoreactive substance levels.

Key words Rikkunshi-to; somatostatin; gastrin; Kampo

Rikkunshi-to, a traditional Chinese (Kampo) herbal medicine, is prepared from eight crude herbs: Ginseng Radix, Atractylodis Rhizoma, Hoelen, Pinelliae Tuber, Aurantii Nobilis Pericarpium, Zizyphi Fructus, Glycyrrhizae Radix, and Zingiberis Rhizoma. This medicine was evaluated for its clinical usefulness in the treatment of chronic hypofunctions of gastrointestinal tract including gastric flatulence, anorexia, nausea, and vomiting. Recently, those effects of Rikkunshi-to were proved to be based on increased blood flow to the stomach, accelerated gastric emptying, and improved gastric mucosal damage.^{1–3)}

In recent reports, some Chinese herbal medicines used to treat those experiential gastrointestinal effects have been elucidated from the viewpoint of gut-regulated hormone levels. Among the medicines, Ninjin-to and Dai-kenchu-to regulated gastrointestinal motility. One of the factors of those effects was assumed to be due to causing increases in the levels of somatostatin, motilin, gastrin, and VIP (vasoactive intestinal peptide) in plasma.^{4–7)} Furthermore, the abnormality of gastrointestinal motility of non-ulcer dyspepsia as an indication of Rikkunshi-to was presumed to be caused by the obstruction of the automatic nervous system and by abnormal hormone levels.⁸⁾ Therefore, we examined the plasma levels of gut-regulated peptides (somatostatin, motilin, gastrin, and VIP).

Somatostatin acts as an inhibitor of hormone release. It participates in regulating gastrointestinal motility with motilin.^{9–14)} Motilin has powerful fundic pouch motor-stimulating activity,¹⁵⁾ and is one of the most important factors controlling the regular occurrence of phase-3 contractions of the migrating motor complex (MMC).^{16,17)} Gastrin stimulates acid secretion and gastrin release is mediated by various mechanisms.¹⁸⁾ VIP is widely distributed in the central and peripheral nervous system.¹⁹⁾ This peptide has a vasodilating effect and is an important neurotransmitter for the enteric nervous system.^{20–22)}

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

MATERIALS AND METHODS

Materials Rikkunshi-to (EK-43, lot. 26L99), prepared as a 4.1 g dried powder extract in the following proportions: Ginseng Radix (4.0 g), Atractylodis Rhizoma (4.0 g), Hoelen (4.0 g), Pinelliae Tuber (4.0 g), Aurantii Nobilis Pericarpium (2.0 g), Zizyphi Fructus (2.0 g), Glycyrrhizae Radix (1.0 g), and Zingiberis Rhizoma (0.5 g), was kindly supplied by Kanebo Co., Ltd. (Tokyo, Japan). An additive, consisting of EK-43, was used as a placebo. Synthetic somatostatin, porcine motilin, human gastrin I (G17), and VIP were purchased from Peptide Institute, Inc. (Osaka, Japan). An antiserum to somatostatin was purchased from Cambridge Res. Biochem. (Cambridge, U.K.). Antisera to motilin (A602/R1B), gastrin (A600/R1B), and VIP (A604/R1B) were purchased from Biogenesis, Ltd. (Poole, U.K.). All other reagents were reagent grade and commercially available.

Subject Five healthy male volunteers (non-smokers), aged 23–39 (median 28 years), 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 subsequently gave informed consent. No subject received any medication for a month preceding the test and no stimulator or inhibitor of gastrointestinal motility was administered to any subject during the study.

Study Schedule Rikkunshi-to at a dose of 6.0 g was orally administered. The same dose of a placebo was given to the volunteers. Venous blood samples from a forearm vein were taken before and at 20–240 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 and 1.2 mg/ml of EDTA. After centrifugation, plasma samples were diluted with 4% acetic acid, pH 4.0, loaded onto Sep-Pak[®] C18 cartridges (Millipore Co., Massachusetts, U.S.A.), and washed with 4% acetic acid. The peptides in plasma were eluted with 70% acetonitrile in 0.5% acetic acid, pH 4.0, lyophilized, and stored (–40 °C) until used. The recovery of plasma somatostatin-, motilin-, gastrin-, and VIP-IS was >93% with this extraction procedure (data not shown).

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Enzyme Immunoassay for Somatostatin-, Motilin-, Gastrin-, and VIP-IS Peptide levels in plasma were measured using a highly sensitive enzyme immunoassay for somatostatin-,²³⁾ motilin-,²⁴⁾ gastrin-,²⁵⁾ and VIP-IS²⁶⁾ 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). Human somatostatin, porcine motilin, mini gastrin I, and a fragment of VIP (positions 11–28) were conjugated with β -galactosidase by *N*-(ϵ -maleimidocaproyloxy)-succinimide according to the methods of Kitagawa *et al.*²⁷⁾ The detection unit of somatostatin-, motilin-, gastrin-, and VIP-IS was 0.10, 0.80, 0.04, and 1.00 fmol/well, respectively.

Data Analysis Somatostatin-, motilin-, gastrin-, and VIP-IS levels in plasma are expressed as the concentration of mean $\pm$ S.D. (pg/ml). 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

Rikkunshi-to has been frequently used to improve chronic hypofunctions of the gastrointestinal tract such as non-ulcer dyspepsia. The abnormality of gastrointestinal motility was presumed to be caused by an obstruction of the automatic nervous system and abnormal hormone levels. In this study, gut-regulated peptide (somatostatin, motilin, gastrin, and VIP) levels, which regulate gastrointestinal motility, were examined to study their relation with Rikkunshi-to.

Somatostatin acts as an inhibitor of hormone release and is a gastrointestinal motor regulator. The plasma somatostatin-IS level-time profile of Rikkunshi-to is shown in Fig. 1(A). Rikkunshi-to caused significant increases in somatostatin-IS at 60–240 min compared with the response of the placebo group. In this study, some pathways enhanced somatostatin-IS levels by Rikkunshi-to. Somatostatin participates in regulating gastrointestinal motility with motilin.^{9–14)} Somatostatin is the first of the gut regulatory peptides (to stimulate MMC-like activity) to have significant therapeutic use of its analogue.²⁸⁾ Increases in somatostatin might correspond with gastrointestinal motor regulation including the acceleration of gastric emptying by this medicine.

Motilin is a powerful inducer of gastrointestinal motor activity in the fundus and the antral pouch of the stomach. The plasma motilin-IS levels after an administration of Rikkunshi-to are shown in Fig. 1(B). Rikkunshi-to had no significant effect on plasma motilin-IS level. But, participating volunteers had different degrees of motilin plasma levels. Some of them caused about 1.3 times increases in motilin-IS at 60–90 min compared with the response of the placebo. The changes in plasma motilin-IS levels of both groups might be the effect of periodic rhythms.

Gastrin is associated with acid secretion in the stomach involving G cells. The plasma gastrin-IS level-time profile is shown in Fig. 1(C). Rikkunshi-to significantly increased gastrin-IS levels between 60–240 min compared with the response of the placebo group. Direct stimulation of gastric mucosal G cells might cause an elevation of both groups at 20 min. Therefore, we guessed that the Rikkunshi-to group

Fig. 1. Effect of Plasma Somatostatin (A)-, Motilin (B)-, Gastrin (C)-, and VIP (D)-Immunoreactive Substance (IS) Levels after the Oral Administration of Rikkunshi-to (●) or Placebo (■)

showed two-phase (20 min: non-specific effect, 60 min<: specific effect of Rikkunshi-to) increases in this study.

In general, somatostatin inhibited the secretion of motilin and gastrin. But in our study, Rikkunshi-to caused significant

increases in gastrin-IS levels. This implies that the intercellular communication between somatostatin and gastrin is paracrine, and somatostatin might not inhibit all pathways of gastrin release. Further studies are needed to elucidate the mechanism involved. The manifestation of dyspepsia is closely related to abnormalities of gastrointestinal motility and hypoacidity. Gastrin stimulates acid secretion and gastrin release is mediated by various mechanisms such as direct stimulation of G cells, cholinergic nerve mediation, stimulation of gastrin releasing peptide, pH of the stomach and so forth.¹⁸⁾ In this study, gastrin release by Rikkunshi-to might be related to normalization of gastric functions.

VIP has a vasodilating effect as a neurotransmitter for the enteric nervous system. Rikkunshi-to had no effect on plasma VIP-IS levels (Fig. 1(D)). Plasma levels of VIP-IS remained within constant ranges before and after administration. Probably, Rikkunshi-to had no effect of vasodilation or increase in blood flow in the gastrointestinal tract by stimulating VIP-containing nerves.

Rikkunshi-to has been reported to have a prokinetic action on gastric emptying and was useful in treating chronic dyspepsia. Using an acetaminophen absorption method and direct double sampling method proved those experiential effects of the medicine.^{1,29,30)} However, the mechanism accelerating gastric emptying by Rikkunshi-to is not known. From the viewpoint of herbs, Rikkunshi-to consists of eight herbs. Two of them, Ginseng Radix and Pinelliae Tuber, accelerate gastrointestinal motility and cause increases in intestinal propulsion.^{31,32)} Rikkunshi-to has a cytoprotection effect and totally treats gastrointestinal functions by regulating gastrointestinal motility.^{33–35)} Our studies of gut hormone levels might add a viewpoint to elucidate the mechanism of Rikkunshi-to.

Ninjin-to and Dai-kenchu-to, which have the effects of accelerating gastrointestinal motility and improving of gastrointestinal dysfunction, caused increases in plasma motilin levels.^{4–6)} In this study, Rikkunshi-to had no effects on motilin levels. That might be because of differences in indication. Rikkunshi-to has been used to regulate gastrointestinal motility to treat gastric flatulence, anorexia, vomiting, and nausea, including effects in the stomach. Rikkunshi-to caused increases both in plasma somatostatin and gastrin levels, which are related to the regulation of gastrointestinal motility. Ninjin-to caused increases in plasma somatostatin levels, too. We guess that the differences in changes of motilin and gastrin levels each might explain differences in indications between Rikkunshi-to and Ninjin-to.

In conclusion, taking Rikkunshi-to causes increases in the plasma levels of somatostatin- and gastrin-IS. We hypothesize that the gastrointestinal effects of Rikkunshi-to might be closely related to increases in somatostatin- and gastrin-IS levels in plasma.

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