Effect of Dai-kenchu-to on Levels of 3 Brain-gut Peptides (Motilin, Gastrin and Somatostatin) in Human Plasma

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We examined the effect of Dai-kenchu-to (DKCT), a traditional Chinese (Kampo) medicine, on the levels of 3 brain-gut peptides (motilin, gastrin and somatostatin) in plasma from 24 healthy subjects. A single oral administration of DKCT, at a dose of 7.5 g, caused significant increases in plasma motilin levels (about 12 pg/ml) at 60 to 90 min, compared with a placebo-treated group. Transient elevations of gastrin levels were noted after administration of both DKCT (25.9±1.4 pg/ml) and placebo (23.5±1.3 pg/ml). DKCT did not alter the levels (about 5.7 pg/ml) of somatostatin. In conclusion, these results indicate that the action of DKCT closely relates to changes in motilin-immunoreactive substance levels in human plasma.

Key words motilin; Dai-kenchu-to; Kampo; gastrin; somatostatin; gastrointestinal motility

Motilin, a powerful inducer of motor activity in the fundus and the antral pouch of the stomach, was first isolated from the small intestinal mucosa of hogs in 1971 by Brown.1 While most gastrointestinal peptides are released in response to the ingestion of a meal, motilin has the specific characteristic that it is released at about 100-min intervals during interdigestive periods2 when no nutrient is present, at least in the duodenum and the upper jejunum. Moreover, in man3 the release of the peptide, motilin, is inhibited by feeding. The increase in plasma motilin is abolished during passage through the ileus.4

In 1905, gastrin was first detected by Edkins5 in extracts from pyloric antral mucosa and shown to stimulate acid secretion. This peptide is associated with a mechanism of gastrointestinal motility involving the cholinergic nerves. Basson et al. reported that gastrin levels did not change in obstructed dogs6 and, furthermore, Dauchel et al. reported that pentagastrin did not reduce the time spent in the ileus.7

Somatostatin is a tetradecapeptide that was isolated from the ovine hypothalamus by Brazeau et al.,8 and identified on the basis of its action as an inhibitor of growth hormone (GH) release. Poitras et al. reported that intravenous somatostatin inhibited motilin release9 and Kraenzlin et al. also demonstrated a reduction in plasma motilin during infusion with a somatostatin analogue.10 In patients with intestinal pseudo-obstruction, the postprandial somatostatin response as markedly impaired.10

Dai-kenchu-to (DKCT), a traditional Chinese (Kampo) herbal medicine, is known to increase gastrointestinal motility and improve ileal function. DKCT is prepared from three herbs: ninjin (Ginseng radix), sanshou (Zanthoxyli fructus) and kankyou (Zingiberis sicaatum rhizoma). Sugiyama11 has reported that DKCT significantly increased plasma motilin levels in the treatment of diseases of the ileus. In that study, however, there was very little data. Furthermore, most reports hardly mention any changes in other brain-gut peptides in human plasma. However, we believe that motilin may play an important role in the action of DKCT.

The purpose of this study is to determine the effect of DKCT on the plasma levels of 3 brain-gut peptides [motilin-, gastrin- and somatostatin-immunoreactive substance (IS)] in healthy subjects.

MATERIALS AND METHODS

Materials DKCT (TJ-100), prepared as a dried powder extract, as purchased from Tsumura Co. Ltd. (Tokyo, Japan). A mixture of glucose and maltose (Summalto®, Hayashibara Co. Ltd., Okayama, Japan) was used as a placebo. Other reagents were of extra-pure grade and were from commercial sources. Synthetic porcine motilin, which has a sequence identical to human motilin, synthetic human gastrin I (G17) and synthetic somatostatin were purchased from Peptide Institute Inc. (Osaka, Japan). Antiserum to motilin (i602) and G17 (i600/001) were purchased from UCB Bioproducts SA (Allard, Belgium). An antiserum to somatostatin (RA08108) and the fragment of G17, [G17 (2—17)] was purchased from Cambridge Res. Biochem. (Cambridge, England). Goat affinity purified antibody to rabbit IgG (whole molecule) (55641) was purchased from ICN Pharmaceuticals, Inc. (Ohio, U.S.A.). 4-Methylumbelliferyl-β-D-galactopyranoside (MUG) was purchased from Sigma Chemical Co. (St. Louis, U.S.A.).

Subjects Twenty-four healthy male volunteers, aged 23—29 years (median 25 years), weighing 56—65 kg (median 61 kg), participated in the study. Each subject received information about the scientific purpose of the study, which was approved by our Ethics Committee at Oita Medical University, and gave informed consent. No subjects received any medication on the 7d preceding the test, and no stimulator of gastrointestinal motility, except DKCT, was administered to any subject during this study.

Study Schedule DKCT or placebo was orally administrated as a single dose of 7.5 g with water. DKCT and the placebo were packaged identically. Venous blood samples (10 ml) from a forearm vein were taken for EIA of the levels of the three brain-gut peptides in plasma. Samples were taken at 15, 30, 45, 60, 90, 120 and 180 min after administration of the drugs. All subjects took breakfast at 6:00 and the study was carried out from 8:00 (2 h after breakfast) to 11:00 h.

Preparation of Plasma Extracts The blood samples were placed in chilled (4°C) tubes containing 500 kallikrein inhibitor units/ml of aprotinin (Trasylo®, Bayer Co., Ltd., Leverkusen, Germany) and 1.2 mg/ml EDTA (Wako Pure

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Chemical Industries, Ltd., Osaka, Japan). After centrifugation (1670×g, 4°C, 20 min), plasma samples were diluted fivefold with 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, the 3 brain-gut peptides in plasma were individually eluted with 60% acetonitrile in 0.5% acetic acid, pH 4.0. Eluates were concentrated by spin vacuum evaporation, lyophilized and stored (~20°C) until use. The recovery of plasma motilin-, gastrin- and somatostatin-IS was >93% with this extracting procedure (data not shown).

**EIA for Motilin-, Gastrin- and Somatostatin-IS**

Brain-gut peptide levels in plasma were measured using high sensitivity enzyme immunoassay (EIA) for motilin, gastrin and somatostatin-IS as previously described. EIA was performed by a delayed addition method. Separation of bound and free materials was performed on an anti-rabbit IgG coated immunoplate (Nunc-Immuno Module Maxisorp F8, InterMed, Denmark). Human motilin, G17 (2–17), and somatostatin were conjugated with β-gal by N-[(ε-maleimidocaproyloxy)-succinimide according to the methods of Kitagawa. Test-tubes containing antisera for each peptide and sample (or standard) were incubated and enzyme-linked antigen was then added. After the test-tubes were incubated, the antigen-antibody solution for each sample was added to the secondary antibody-coated immunoplates and the plates were incubated and washed with a buffer. Then MUG was added to each well. The plates were then incubated and the fluorescence intensity (λex, 360 nm, λem, 450 nm) of each well was measured with an MTP-100F microplate reader (Corona Electric, Ibaraki, Japan). The detection limit of motilin-, gastrin- and somatostatin-IS was 0.80, 0.04 and 1.00 fmol/well, respectively.

**Data Analysis** All values are expressed as means±S.D. Comparisons of mean values were made by analysis of variance and Dunnett's test. *p*<0.05 was considered as indicative of statistical significance.

**RESULTS AND DISCUSSION**

**Effect of DKCT on Motilin-, Gastrin- and Somatostatin-IS Levels in Plasma**

Plasma levels of motilin in man change during the first 30 min following a meal. Therefore, to avoid the effects of a meal, this study was carried out 2 h after breakfast. The profiles of average plasma motilin-, gastrin- and somatostatin-IS levels against time, in 24 subjects, after a single oral administration of DKCT or placebo, are shown in Fig. 1. DKCT caused significant increases (about 12 pg/ml) in plasma motilin-IS levels at 60 to 90 min, compared with the placebo group. At this point, the motilin-IS levels had risen to about 1.8 times the levels at 0 min. In the control group, plasma motilin-IS levels did not change significantly. Our findings suggest that DKCT may affect motilin-IS levels in human plasma through stimulation of motilin cells.

Temporary elevations in plasma gastrin-IS levels were seen after administration of both DKCT (25.9±1.4 pg/ml) and placebo (23.5±1.3 pg/ml) at 15 min. After 30 min, the DKCT and placebo groups showed similar changes in gastrin-IS levels. Probably gastrin-IS was secreted as a result of stimulation of DKCT or placebo on the gastric mucosal G

![Fig. 1. Plasma Motilin-, Gastrin- and Somatostatin-immunoreactive Substance (IS) Levels after Oral Administration of DKCT and Placebo.](image)

Mean±S.D. levels in 24 male volunteers are given. ●, motilin-IS levels in DKCT group; ○, motilin-IS levels in placebo group; ■, gastrin-IS levels in DKCT group; □, gastrin-IS levels in placebo group; ▲, somatostatin-IS levels in DKCT group; △, somatostatin-IS levels in placebo group. *p*<0.05 compared with placebo group.

cells. Therefore, the changes in gastrin-IS levels are not due to the specific action of DKCT.

After 60 min, plasma somatostatin-IS levels in the DKCT group were higher than those in the placebo group. However, this difference was not significant. DKCT did not significantly alter the level of plasma somatostatin-IS between 0—180 min. Generally, somatostatin inhibits the secretion of gastrin and motilin and the gastrointestinal tract, gastric acid secretion, gastric emptying and duodenal motility are inhibited by somatostatin. As DKCT did not significantly alter the level (about 5.7 pg/ml) of plasma somatostatin-IS, this suggests that DKCT does not have an effect on somatostatin-IS in plasma.

DKCT has been used in the treatment of ileal disease and in improving gastrointestinal motility. The 3 ingredients of DKCT are n ninjin, sanshou, and kankyou. Kase et al. reported that sanshou and ninjin caused jejunal contracture with an elevation of tone in the rabbit, whereas kankyou suppressed contraction. They also reported that the intestinal contraction by DKCT was associated with the cholinergic nerves. Recently, Itoh found that muscarinic receptors are responsible for motilin release from motilin cells. In other
words, motilin cells are innervated by local or intramural cholinergic neurons. Therefore, DKCT may stimulate release of motilin through muscarinic receptors on motilin cells in the enteric nervous system. The released motilin may control motor activity in the small intestine, inducing phase III contractions of the migrating motor complex. As a consequence, the increased motilin in plasma may have a beneficial effect on the ileus by promoting gastrointestinal motility and transport of intestinal contents. Although further studies are needed, these pharmacological actions may normalize intestinal motility, improve edema of the intestinal wall and promote food absorption.

In this study, we conclude that DKCT may improve the action of the ileus and gastrointestinal motility by significantly increasing motilin-IS levels in plasma. These results indicate that the action of DKCT is closely related to changes in motilin-IS levels in plasma.

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