Pharmacological Differences between Liu-Jun-Zi-Tang, a Traditional Chinese Herbal Medicine, and Domperidone on Isolated Guinea-Pig Ileum

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The different characteristics of Liu-Jun-Zi-Tang, a spray-dried powdered extract from 8 Chinese herbs, and domperidone, a D2 antagonist, both of which are used clinically for the treatment of gastritis, were validated in isolated guinea pig ileal longitudinal muscle. Liu-Jun-Zi-Tang at 0.05 mg/ml did not affect the twitch response by electrical field stimulation, but it concentration-dependently inhibited the twitch response at concentrations of 0.1 and 1.0 mg/ml, and maximum inhibition occurred 5 min after application of Liu-Jun-Zi-Tang. In the presence of Liu-Jun-Zi-Tang, the concentration–response curve for ACh-contraction showed inhibition in a non-competitive manner, which is the same as domperidone. In the presence of Liu-Jun-Zi-Tang (1.0 mg/ml), ACh (10−7 M), histamine (5×10−7 M) and barium chloride (10−4 M)-induced basal contractions were inhibited by 24, 19 and 54 %, respectively. On the other hand, in the presence of domperidone (2×10−5 M), ACh- and barium chloride-induced basal contractions were inhibited by 28 and 63 %, respectively, similar to that in the presence of Liu-Jun-Zi-Tang. However, the inhibition of histamine-induced contraction in the presence of domperidone was significantly increased (81 %) compared with that in the presence of Liu-Jun-Zi-Tang. These findings suggest that Liu-Jun-Zi-Tang has milder effects on histamine-induced disorders than domperidone. In other words, domperidone has a more potent effect on histamine-induced disorders.

Key words Liu-Jun-Zi-Tang; electrical field stimulation; domperidone; histamine; guinea-pig ileum

Liu-Jun-Zi-Tang, a gastroprotective herbal medicine,1) was effective symptomatic relief in patients with dysmotility-like functional dyspepsia, and is widely used in the treatment of gastritis.2) Gastric motility may be regulated by multiple neural systems. Studies on the mechanisms involving the neuronal systems suggest the involvement of cholinergic3) and noradrenergic systems.4) Additionally, various studies have suggested the existence of dopamine neurons and receptors in these tracts.3) 5–30 Domperidone, a dopamine D2-receptor antagonist,10) is also clinically effective in treating functional gastrointestinal disorders such as gastroesophageal reflux, gastritis, gastric atony, gastroparesis, dyspepsia, anorexia, nausea and vomiting.11) However, the pharmacological differences between Liu-Jun-Zi-Tang and domperidone are unclear.

This study was undertaken to validate the different characteristics of Liu-Jun-Zi-Tang, a dried decoctum of 8 Chinese herbs, and domperidone, a dopamine D2-receptor antagonist,10) on isolated guinea pig ileum; both substances are clinically and widely used in the treatment of gastritis.2) 11

MATERIALS AND METHODS

Drugs The sources of the drugs were: Liu-Jun-Zi-Tang (TJ-43, Lot No. 80043002PI, Tsumura Co., Ltd., Tokyo, Japan), domperidone (Kyowa Hakkou Co., Ltd., Tokyo, Japan), acetylcholine chloride (Ovisot, Daichi Pharm. Inc., Osaka, Japan); histamine dihydrochloride (Wako Pure Chemical Industries Inc., Osaka, Japan); barium chloride (Wako Pure Chemical Inc., Osaka, Japan). All other drugs were commercially obtained. Liu-Jun-Zi-Tang (1 mg/ml), in the form of a spray-dried powder of the extract, was suspended with Krebs-bicarbonate solution at 37 °C and bubbled with 95% O2/5% CO2 for 60 min. Domperidone was dissolved in a small volume of 0.1 N acetic acid and diluted with distilled water. All other drugs were dissolved in distilled water.

Animals All experiments were performed on isolated ileum obtained from randomly-bred male guinea-pigs weighing between 350—450 g housed individually in hanging wire cages under a constant light cycle (light on 7:00 to 19:00) at 23±1 °C. Food and water were available ad libitum.

Guinea-Pig Myenteric Plexus-Longitudinal Muscle Preparation The animals were stunned and decapitated, and each ileum (ca. 3 cm) was quickly isolated about 10 cm away from the ileo-caecal junction. The myenteric plexus-longitudinal muscle (MPLM) was prepared by a method described previously.12-15) A glass rod was inserted into the lumen of an intestinal segment, and the MPLM was removed by rubbing the segment with a cotton swab soaked in Krebs’ solution. The preparations (2—2.5 cm in length) were suspended at a resting tension of 300—500 mg in a 5 ml organ bath between platinum ring electrodes (3.5 cm apart), placed at the top and the bottom of the bath. The bath contained Krebs-bicarbonate solution (mm: NaCl 118; KCl 4.7; CaCl2 2.5; KH2PO4 1.2; MgSO4 1.2; NaHCO3 25; Glucose 10), at 37 °C, and was bubbled with 95% O2/5% CO2.

Twitch Response by Electrical Field Stimulation Experiments were performed according to a method described previously.12-15) Rectangular electrical pulse field stimulations were applied at 0.1 Hz, 0.5 ms pulse width and maximum intensity using a DPS-160B stimulator (NEC-Sanei.
co., Tokyo, Japan) with a DPS-122 isolator (NEC-Sanei Co., Tokyo, Japan), and the responses were recorded isometrically on an SP-H5P recorder (Riken Densi Co., Tokyo, Japan) using an SD-1T force displacement transducer (Nihon Kohden Co., Tokyo, Japan).

**Effects of Liu-Jun-Zi-Tang and Domperidone on Twitch Response** The application of Liu-Jun-Zi-Tang was performed as follows: the medium in the bath was replaced with 0.25, 0.5 or 5 ml of Krebs-bicarbonate solution to final concentrations of 0.05, 0.1 and 1 mg/ml, respectively, with 1 mg/ml Liu-Jun-Zi-Tang, which was suspended in Krebs-bicarbonate solution for ca. 60 min at 37 °C, then bubbled with 95% O2/5% CO2, and subsequently equilibrated with continuous stimulation until a stable twitch response was obtained. The replacement with Liu-Jun-Zi-Tang was done between 40 and 50 min after setting the preparation. The responses were determined every 5 min for 20 min. The effects of Liu-Jun-Zi-Tang alone or on twitch response were assessed by percentage inhibition of the twitch contraction before Liu-Jun-Zi-Tang.

Effects of domperidone on the twitch response was determined according to the method previously described by us.\textsuperscript{16} Various concentrations of domperidone were added singly to a bath with a volume of 5 μl of each concentration, then equilibrated with continuous stimulation (stimulated for about 40 min) until a stable twitch response was obtained. The application of domperidone was performed between 40 and 50 min after setting the preparation. The responses were determined every 5 min for 30 min. The effect of domperidone on twitch response was assessed by the percentage inhibition of the twitch contraction preceding domperidone.

**Effects of Liu-Jun-Zi-Tang and Domperidone on Concentration–Response Curve for ACh-Evoked Contraction of Basal Tension** Concentration–response curves for ACh on basal tension were constructed. Each concentration of ACh was cumulatively applied in the presence and absence of Liu-Jun-Zi-Tang (0.05, 0.1, 1 mg/ml), which had been previously replaced, or domperidone (1, 10, 20, 100 μM), and added at a volume of 5 μl before 10 min, as we previously reported.\textsuperscript{16}

**Effects of Liu-Jun-Zi-Tang and Domperidone on ACh-, Histamine- and Barium Chloride-Induced Basal Contraction** Electrical field stimulation was stopped and ACh (10⁻⁷ M), histamine (5×10⁻⁷ M) and barium chloride (10⁻⁵ M), at which concentrations they induce submaximal contraction of the muscle, were added to the bath, respectively. Domperidone was added to the bath at the volume of 10 μl, and ACh, histamine and barium chloride were added in volumes of 5, 5 and 50 μl, respectively. After removal of these contractile drugs by washing the preparation, electrical field stimulation was re-started for continuous application. After a constant twitch response was obtained, Liu-Jun-Zi-Tang (1 mg/ml) or domperidone (2×10⁻⁵ M) was added to the bath by the method described above. After a constant twitch response was obtained in the presence of Liu-Jun-Zi-Tang or domperidone (35 min later), electrical field stimulation was stopped and the contractile drugs were again added in the presence of Liu-Jun-Zi-Tang or domperidone. The effects of domperidone or Liu-Jun-Zi-Tang on twitch response were quantified as a percentage of twitch height before and after Liu-Jun-Zi-Tang or domperidone. The effects of ACh-, histamine- and barium chloride-induced contraction were quantified as a percentage of contraction height before and after Liu-Jun-Zi-Tang or domperidone. The inhibitory effects were evaluated by comparison of each paired twitch inhibition, since the degree of twitch inhibition varies between preparations.

**Statistics** Values are expressed as means and standard error of the means (S.E.M.), with the number of preparations in parentheses. The significance of differences was evaluated by one-way analysis of variance with the Student’s t-test for each concentration of contractile drug. Differences were considered significant at p<0.05.

**RESULTS**

**Effects of Liu-Jun-Zi-Tang on Twitch Response** Typical tracings of the effects of Liu-Jun-Zi-Tang on twitch response are shown in Fig. 1. The responses were determined every 5 min for 30 min. Liu-Jun-Zi-Tang 0.05 mg/ml did not produce any effect on the 0.1 Hz-evoked twitch response, but it dose-dependently inhibited the twitch response between concentrations of 0.1 and 1 mg/ml at respective times, and the inhibition was maximal and continued after 5 min at the highest concentration used (1 mg/ml) (Fig. 2).

**Effects of Domperidone on Twitch Response** The effects of domperidone at concentrations from 1 to 20 μM were tested on 0.1 Hz-elicited twitch responses every 5 min during a 30-min period. Domperidone had no effect at a concentration of 1 μM. When the drug was applied at higher concentrations, 2—20 μM, a concentration-dependent inhibition of the twitch responses was observed, as previously shown by us.\textsuperscript{16}

**Effects of Liu-Jun-Zi-Tang and Domperidone on Concentration–Response Curve for ACh-Evoked Contraction of Basal Tension** In the presence of Liu-Jun-Zi-Tang, at 0.05 and 0.1 mg/ml, the concentration–response curve for
ACh-evoked contraction was not changed, but a higher concentration (1 mg/ml) of Liu-Jun-Zi-Tang inhibited the basal-contraction caused by ACh in a non-competitive manner (Fig. 3). Domperidone, 1 μM, did not change the concentration–response curve for ACh-evoked contraction, but at higher concentrations of 10, 20, 100 μM, domperidone inhibited the basal-contraction to ACh in a non-competitive manner, as previously shown by us.16)

Effects of Liu-Jun-Zi-Tang and Domperidone on ACh-, Histamine- and Barium Chloride-Induced Contraction

Typical tracings of the effects of Liu-Jun-Zi-Tang and domperidone on twitch contraction and on ACh-, histamine- and barium chloride-induced contraction are shown in Figs. 4 and 5, respectively. The twitch inhibition of Liu-Jun-Zi-Tang was not significantly different from that of domperidone in each group, ACh, histamine and barium chloride (Fig. 6 black column). Acetylcholine (10⁻⁷ M)-, histamine (5×10⁻⁷ M)- and barium chloride (10⁻⁴ M)-induced contraction were inhibited in the presence of Liu-Jun-Zi-Tang 1 mg/ml or domperidone 2×10⁻⁵ M. In the presence of Liu-Jun-Zi-Tang, submaximal basal contractions induced by ACh (10⁻⁷ M), histamine (5×10⁻⁷ M) and barium chloride (10⁻⁴ M), were inhibited by 24, 19 and 54%, respectively (Fig. 6 white column). On the other hand, in the presence of domperidone at 2×10⁻⁵ M, ACh- and barium chloride-induced basal contractions were inhibited by 28 and 63%, respectively, which was not significantly different from that by Liu-Jun-Zi-Tang (Fig. 6 left (ACh-group) and right (barium chloride-group)). However, histamine-induced contractions in the presence of domperidone were inhibited by 81%, which was significantly higher (p<0.01) than that in the presence of Liu-Jun-Zi-Tang (Fig. 6 middle (histamine-group)).
DISCUSSION

The presence of dopamine neurons in the alimentary tract is still under discussion. However, it has been demonstrated that the inhibitory effect of dopamine on gastrointestinal motility is reversed by dopamine antagonists.\textsuperscript{7,17,18} Many findings also suggest that there are dopaminergic nerves and specific dopamine receptors in this tract.\textsuperscript{6,8,9,14,19} This study was undertaken to characterize the different effects of \textit{Liu-Jun-Zi-Tang}, a Chinese herbal medicine, and domperidone on isolated ileum, since both are clinically effective in treating functional gastrointestinal disorders such as gastro-esophageal reflux, gastritis, gastric atony, gastroptosis, dyspepsia, anorexia, nausea and vomiting.\textsuperscript{2,11}

As shown in Figs. 1 and 2, the 0.1 Hz-induced twitch response was also inhibited by 1 and 0.1 mg/ml, but not by 0.05 mg/ml, of \textit{Liu-Jun-Zi-Tang}. As it is well known that the twitch response is due to acetylcholine output from coaxially stimulated myenteric neurons,\textsuperscript{20} this finding suggests the possibility that \textit{Liu-Jun-Zi-Tang} inhibits the release of ACh. Therefore, the effects of \textit{Liu-Jun-Zi-Tang} on ACh-induced contraction of basal tension, an indicator of direct action on the ACh receptor, was investigated. Additionally, it has been reported that domperidone similarly showed inhibition of the 0.1 Hz-evoked twitch response,\textsuperscript{16} and that the ACh-induced contraction was inhibited with non-competitive manner in the presence of 1 mg/ml \textit{Liu-Jun-Zi-Tang} (Fig. 3) or domperidone,\textsuperscript{16} although even 0.1 mg/ml \textit{Liu-Jun-Zi-Tang} may inhibit the release of ACh, since the ACh-induced contraction was not influenced but the twitch response was inhibited. These findings indicate that \textit{Liu-Jun-Zi-Tang} and domperidone inhibit not only the release of ACh from the cholinergic neuron but also contractile effects by various drugs on the ileal smooth muscle.

Therefore, the effect of ACh-, histamine- and barium chloride-induced contractions, which act directly on the smooth
muscle, in the presence of Liu-Jun-Zi-Tang and domperidone, were investigated. ACh-, histamine- and barium chloride-induced contractions were inhibited in the presence of Liu-Jun-Zi-Tang or domperidone (Figs. 4, 5 and Fig. 6). However, histamine-induced contraction of basal tension in the presence of domperidone showed significantly more inhibition (81%) (Fig. 5 and Fig. 6 middle) than in the presence of Liu-Jun-Zi-Tang (19%) (Fig. 6). These results suggest that domperidone may exert more effect on the histamine-receptor than Liu-Jun-Zi-Tang. However, the effect of ACh on cholinergic receptors, the release of ACh from the cholinergic neurone monitored by the twitch response, and the direct contractile effect induced by barium chloride on smooth muscle showed no significant difference between Liu-Jun-Zi-Tang and domperidone, although Liu-Jun-Zi-Tang and domperidone showed the smooth muscle relaxation. Since the presence of domperidone showed significantly more inhibition of the twitch response of agonists at the concentration which induces the contraction of histamine, without side effect on the histaminergic system, if the effect of domperidone on the histaminergic system is a side effect, may be useful in the treatment of various gastritis, although Liu-Jun-Zi-Tang or domperidone (Figs. 4, 5 and Fig. 6). These results suggest that neither drug affects a single active site or neuron. Therefore, it is suggested that domperidone affects the histaminergic system as a side effect, may be useful in the treatment of histamine-induced disorders in various gastritis. And, Liu-Jun-Zi-Tang, more than domperidone, may clinically be a mild and useful drug for the treatment of the gastritis, without side effect on the histaminergic system, if the effect of domperidone on the histaminergic system is a side effect.

In conclusion, our findings reflect the different characteristics of action between Liu-Jun-Zi-Tang and domperidone: both inhibit the ACh-, histamine- and barium chloride-induced contractions. Especially, domperidone inhibited histamine-induced contraction more strongly than Liu-Jun-Zi-Tang, although both are clinically effective in the treatment of various gastritis. These effects might be considered when domperidone is used for the treatment of patients with functional gastrointestinal disorders.

REFERENCES