Effects of Keishi-ka-shakuyaku-to (Gui-Zhi-Jia-Shao-Yao-Tang) on Diarrhea and Small Intestinal Movement

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The present study was conducted to determine the characteristics of the effects of Keishi-ka-shakuyaku-to (Gui-Zhi-Jia-Shao-Yao-Tang; TJ-60) on diarrhea. Significant repression was noted by TJ-60 at 1000 mg/kg, *p.o.* for diarrhea induced by pilocarpine, barium chloride or castor oil. Under normal conditions, TJ-60 did not influence small intestinal transit by its oral treatment even at 1000 mg/kg, however, it dose-dependently improved the acceleration of such transit caused by neostigmine. TJ-60 did not influence the resting tonus in isolated small intestine, but did selectively inhibit low frequency electrostimulated contractions.

These results indicate that the antidiarrheal effects of TJ-60 may be due to the inhibition of excessively accelerated small intestinal movement, and that the inhibition of acetylcholine release by parasympathetic nerves is partly involved in the mechanism of this antidiarrheal action.

Key words Keishi-ka-shakuyaku-to; diarrhea; small intestinal transit; acetylcholine release

Keishi-ka-shakuyaku-to (Gui-Zhi-Jia-Shao-Yao-Tang; TJ-60), a kampo medicine, is clinically used to treat spastic constipation or diarrhea as well as to treat the diarrhea type or alternate type between constipation and diarrhea in irritable bowel syndrome (IBS).^{1–3)} However, there have been few basic studies which can support the clinical effectiveness of TJ-60.

The aim of the present study was to determine the importance of clinical application of TJ-60 by elucidating the characteristics and mechanism of its effects on diarrhea. For this purpose, we evaluated the actions of TJ-60 on animal models with diarrhea induced by castor oil, barium chloride (BaCl₂) or pilocarpine, on small intestinal transit and on various contractive reactions using the isolated guinea pig ileum.

MATERIALS AND METHODS

Animals Five-week-old male ICR mice (SLC Japan) were used for *in vivo* studies, and male Hartley guinea pigs weighing 400–700 g (SLC Japan) were used for *in vitro* studies.

Drugs TJ-60 is a dried powder, without excipient, of an extract manufactured by Tsumura Co., Ltd. (Tokyo, Japan). Ratios of respective crude drug components were as follows: Peony root (root of *Peony lactiflora* PALL.) (6.0), Cinnamon bark (bark of *Cinnamomum cassia* BL.) (4.0), Jujube (fruit of *Zizyphus jujuba* MILL. var. *inermis* REHD.) (4.0), Glycyrrhiza (root and stolon of *Glycyrrhiza glabra* L., *Glycyrrhiza uralensis* FISCH.) (2.0) and Ginger (rhizome of *Zingiber officinale* ROSC.) (1.0). The yield of the extract was 22%. The extract contained glycyrrhizin (1.22%) and paeoniflorin (1.78%). Indomethacin and neostigmine bromide were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Nicotine tartrate, potassium chloride (KCl) and tetrodotoxin (TTX) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other drugs were described previously.⁴⁾

Effects on Diarrhea The effects of TJ-60 on diarrhea induced by pilocarpine, BaCl₂ or castor oil were examined by

the method described.⁴⁾ Eight to eleven mice were allocated to each test group. Each test drug was given orally 30 min before pilocarpine (10 mg/kg) or BaCl₂ (30 mg/kg) administration, and was administered to mice 1 h before castor oil (10 ml/kg) treatment.

Effects on Small Intestinal Transit The experiment was carried out according to the method described by Yokochi *et al.*⁵⁾ Eight to ten mice, fasted for 24 h, were allocated to each group. Thirty minutes after oral administration of test drugs, 5% of activated charcoal powder suspended in 10% gum arabic was orally administered to each mouse at a volume of 10 ml/kg and drug efficacy was evaluated 20 min later. We also examined the effects on accelerated small intestinal transit. Neostigmine (50 µg/kg, *s.c.*) was administered to mice followed by oral administration of activated charcoal powder 15 min later. Test drugs were given orally to the animals 30 min prior to the neostigmine treatment.

Effects on the Isolated Guinea Pig Ileum This experiment was carried out according to a method described in detail elsewhere.⁴⁾ After removing the mucosae, the isolated guinea pig ileum was suspended along the longitudinal muscle by 0.5 g loading in a Magnus tube with oxygenated (95% O₂–5% CO₂) Krebs solution. The composition of Krebs solution was as follows (mM): NaCl, 118; KCl, 4.8; MgSO₄, 1.2; KH₂PO₄, 1.2; CaCl₂, 2.5; NaHCO₃, 25 and glucose, 11. The experiment was carried out at 37 °C. Field stimulation was added to the suspended ileum *via* platinum electrodes placed parallel to the specimen using square waves (electric current 50 mA, duration 0.5 ms, frequency 0.1 Hz). Contractile responses were recorded isotonicly as described.⁴⁾ The effects of the cumulative dose of TJ-60 (1×10^{–7}–1×10^{–4} g/ml) were examined on the resting tonus. The effects of a single dose of TJ-60 (1×10^{–7}–1×10^{–3} g/ml) administered 5 min before ileum contraction induced by acetylcholine (ACh; 1×10^{–7} g/ml), histamine (1×10^{–7} g/ml), nicotine (5×10^{–6} g/ml), serotonin (3×10^{–7} g/ml) or BaCl₂ (1×10^{–4} g/ml) were evaluated. Also determined were, the effects of a cumulative dose of TJ-60 (1×10^{–7}–1×10^{–3} g/ml)

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on ileum contraction induced by an isotonic high concentration of KCl (3×10^{-3} g/ml) and a low frequency electrostimulated contraction (ESC).

Statistical Analysis Statistical significance of value for diarrhea incidence and intestinal transit were determined as described previously.⁴⁾ The results of the examination of ESC were evaluated by Student's *t*-test.

RESULTS AND DISCUSSION

In this study evaluating the antidiarrheal effects of TJ-60 together with its mechanism, the medicine (250–1000 mg/kg) was found to inhibit diarrhea induced by pilocarpine or BaCl₂ in a dose dependent manner (Table 1). Furthermore, at doses of 500 and 1000 mg/kg it markedly inhibited castor oil-induced diarrhea. Loperamide, a comparative drug used in this study, significantly inhibited diarrhea induced by BaCl₂ or castor oil at a dose of 5 mg/kg. Castor oil-induced diarrhea was also suppressed by indomethacin at 5 mg/kg. The antidiarrheal effects of atropine (1 mg/kg) and papaverine (30 mg/kg) were similar to those reported.⁴⁾ Small intestinal transit rate increased to approximately 80% after the treatment with neostigmine, while it was 60% in normal mice (Fig. 1). Under normal conditions, TJ-60 showed no marked effect on transit rate even at 1000 mg/kg, but at doses of 250 to 1000 mg/kg it normalized accelerated small intestinal transit in a dose-dependent manner. On the other hand, loperamide at 5 mg/kg significantly decreased transit rate under conditions of both normal and neostigmine-induced accelerated transit rate. We therefore speculate that the mode of antidiarrheal action of TJ-60 differs from that of loperamide.

On isolated guinea pig ileum, TJ-60 did not markedly influence the resting tonus nor any drug-induced ileum contraction. Since ESC was eliminated by atropine (1×10^{-7} g/ml) or TTX (1×10^{-6} g/ml) in an electrostimulative experiment, it was confirmed that this contraction was due to the response *via* parasympathetic nerves. TJ-60 significantly inhibited ESC at a dose above 1×10^{-4} g/ml compared to the contraction treated with vehicle.

Diarrhea occurs when bowel contents are passed without being sufficiently digested or absorbed due to accelerated enteric juice secretion or peristalsis after stimulation of the enteric tract.^{6,7)} The development of diarrhea is caused by stress, bacterial infection, cold stimulation, food or drug uptake, and gastrointestinal diseases such as ulcerative colitis.^{8–11)} Our previous report described the mode of action of purgatives used in this study.⁴⁾ It was also reported that diarrhea induced by pilocarpine or castor oil is non-selectively inhibited by noradrenaline or morphine.^{12,13)} BaCl₂ is also known to act on parasympathetic nerves as well as acting directly on smooth muscles of the isolated guinea pig ileum.¹⁴⁾ Furthermore, since the increased intestinal transit by neostigmine was mediated at muscarinic cholinergic receptors,¹⁵⁾ neostigmine is believed to act as well as pilocarpine on small intestine. In fact, atropine at 1 mg/kg improved the transit rate accelerated by neostigmine. These studies indicate that ACh release from parasympathetic nerves may be involved in the action of purgatives and that of neostigmine. *In vitro* studies suggested that ESC of the isolated ileum might be a contraction due to excessively accelerated parasympathetic

Table 1. Effects of Keishi-ka-shakuyaku-to (TJ-60), Loperamide, Atropine, Papaverine and Indomethacin on Diarrhea Induced by Pilocarpine, BaCl₂ or Castor Oil in Mice

Purgative	Drug	Dose (mg/kg)	N	Incidence of diarrhea
Pilocarpine	Control		11	11/11
	TJ-60	250	10	7/10
		500	10	6/10
		1000	11	6/11*
	Loperamide	5	11	7/11
	Atropine	1	11	1/11**
BaCl ₂	Control		9	9/9
	TJ-60	250	9	7/9
		500	9	6/9
		1000	9	5/9*
	Loperamide	5	9	3/9**
	Papaverine	30	9	4/9**
Castor oil	Control		10	10/10
	TJ-60	250	10	7/10
		500	10	4/10*
		1000	10	4/10*
	Loperamide	5	10	2/10**
	Indomethacin	5	10	1/10**

Diarrhea was induced by pilocarpine (10 mg/kg, s.c.), BaCl₂ (30 mg/kg, s.c.) or castor oil (10 ml/kg, p.o.). Each drug was given orally 30 min before the subcutaneous injection of pilocarpine or BaCl₂. In castor oil-induced diarrhea, each drug was given orally 60 min before purgative administration. * and **: significantly different from each control at $p < 0.05$ and $p < 0.01$, respectively.

Fig. 1. Effects of Keishi-ka-shakuyaku-to (TJ-60), Loperamide and Atropine on Neostigmine (50 µg/kg, s.c.)-Accelerated Small Intestinal Transit in Mice

Each column and vertical bar represents the mean $\pm$ S.E. of 8–10 animals. *, ** and ***: significantly different from the control at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

nerves. Based on the results obtained here, the antidiarrheal effect of TJ-60 may be caused by selectively inhibiting contraction due to ACh release after excessive acceleration of parasympathetic nerves.

Maeda and his colleagues reported that the inhibitory effects of water extract of Peony root and Glycyrrhiza on neurogenic contraction are due to the inhibition of ACh release from cholinergic nerves and inhibition of ACh action on ileum smooth muscle, respectively.^{16,17)} The ratio of Peony root is the highest of the crude drug components of TJ-60. Therefore, the effects of water extract of this root may support our present results that TJ-60 selectively inhibits con-

traction by releasing ACh from cholinergic nerves. Using frog sciatic nerve-sartorius muscle preparations and mouse phrenic nerve-diaphragm muscle preparations, it was reported that the combined use of paeoniflorin and glycyrrhizin, a main component of Peony root and Glycyrrhiza, blocked indirectly stimulated twitchings at concentrations which, when used alone, induced no blocking effects.¹⁸⁾ These studies suggest that the effects of TJ-60 might not be able to be elucidated solely by Peony root. Although further investigations are necessary, the antidiarrheal effect of TJ-60 may be due to the inhibition of excessively accelerated movement of the small intestine. We also speculate that the inhibition of ACh release after excessive acceleration of parasympathetic nerves is partly involved in the antidiarrheal effect of the medicine.

In conclusion, TJ-60 may be effective in treating diarrhea and this study provides basic data to support its clinical effectiveness.

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