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Original Article

The Effects of *Jungri-tang Gamibang* on Carbachol-accelerated Mouse Small Intestinal Transit

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Objectives: To clarify the effects of *Jungri-tang Gamibang* on accelerating small intestinal movement induced by the stimulation of cholinergic neurotransmission.
Methods: 500, 250 and 125mg *Jungri-Tang Gamibang* or 20mg domperidone were dissolved or suspended in distilled water and orally pretreated on the carbachol-accelerated small intestinal transit mice once a day for 7 days at a volume of 10ml/kg (of body weight) using a Zonde needle attached to 1 ml syringes containing test drugs.
Result: Significantly ($p<0.01$) increase of % regions of activated charcoal transit in the small intestine was detected in carbachol control compared to that of intact control. However, significant ($p<0.01$) decreases of % regions of activated charcoal transit were dose-dependently observed in all *Jungri-Tang Gamibang* extracts or domperidone-pretreated groups.
Conclusions: it was concluded that *Jungri-tang Gamibang* enhancement in the normal intestinal motility and normalization in the accelerated intestinal motility might interfere with a variety of muscarinic, adrenergic and histaminic receptor activities or with the mobilization of calcium ions required for smooth muscle contraction non-specifically.

Key Words : *Jungri-tang*, antispasmodic effects, domperidone.

Introduction

Gastric motility may be regulated by multiple neural systems. Studies on the mechanisms involving the neuronal systems suggest the involvement of cholinergic[1] and noradrenergic systems[2]. Additionally, various studies have suggested the existence of dopamine neurons and receptors in these tracts[3-4].

Since intestinal motility is reduced following laparotomy, many studies focusing on the mechanisms of enhanced intestinal motility have been reported[5]. However, intestinal obstruction following laparotomy is frequently accompanied by abdominal pain, which is thought to be associated with intestinal "cluster" contractions[6]. Moreover, cramping abdominal pain is usually noted in irritable bowel syndrome (IBS), which is characterized by hypercontractility[7-8]. Although the relationship between abnormal gastrointestinal motility and the cause of abdominal pain remains controversial, muscarinic antagonists act as antispasmodics by reducing gastrointestinal hypermotility in patients. Thus it is thought that hypercontractility is due, at least in part, to stimulation of cholinergic neurotransmission. Therefore, the reducing of hypercontractility has been also regarded as an important mechanism to reduce the irritability in such abnormal intestinal disorder[9].

Jungri-tang is a traditional Korean polyherbal

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medicine is known for its accelerated intestinal motility, reducing gastric pH and gastritis[10] and has been used for gastrointestinal disorders in folk medicine[11]. However, the antispasmodic effects of *Jungri-tang* have not been established yet with the *in vivo* effects on the abnormal intestinal motilities.

In the present study, to clarify the effects of *Jungri-tang Gamibang*, on accelerating small intestinal movement induced by the stimulation of cholinergic neurotransmission, we evaluated the effects of *Jungri-tang Gamibang* on *in vivo* carbachol (an acetylcholinergic agent)-accelerated mouse small intestinal transit. The effects were compared to those of domperidone.

Materials and Methods

1. Preparations of *Jungri-tang Gamibang* extracts

Jungri-tang Gamibang (490g), purchased from Daegu Oriental Medical Hospital of Daegu Haany University after confirming the morphology under microscopy as listed in Table 1, were prepared and an aqueous mixture of *Jungri-tang Gamibang* were extracted (yield: 20.17%) by routine methods using a rotary vacuum evaporator (Lab. Camp, Korea) and programmable freeze dryer (IlShin Lab., Korea). Prepared water extracts of *Jungri-tang Gamibang* were ground using a commercial electronic pulverizer and stored in a desiccator, then protected from light and moisture until used.

2. Carbachol-accelerated mouse small intestinal transit

1) Animals and husbandry

Sixty male ICR mice (6-wk old upon receipt, SLC, Japan) were used after acclimatization for 10 days. Animals were allocated 5 per polycarbonate cage in a temperature (20-25ºC) and humidity (40-45%) controlled room. Light : dark cycle was 12hr : 12hr and feed (Samyang, Korea) and water were supplied free to access. All animals were treated according to the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Resources, Commission on Life Science, National Research Council, USA of 1996, Washington D.C.

Table 1. Composition of *Jungri-tang Gamibang* used in this study.

Herbs	Botanical Name	Dose (g)
Perillae Folium	*Perilla frutescens LINNE*	4
Atractylodis Rhizoma	*Atractylodes japonica KOIDZ*	6
Cyperi Rhizoma	*Cyperus rotundus LINNE*	4
Aurantii Immaturus Fructus	*Poncirus trifoliata RAFIN*	4
Raphini Semen	*Raphanus sativus var. hortensis for. acanthiformis MAKINO*	4
Citri Pericarpium	*Fraxinus rhynchophylla HANCE*	3
Magnoliae Cortex	*Magnolia obovata THUNB*	3
Pinelliae Rhizoma	*Pinellia ternat a (THUNB.) BREIT.*	3
Poria	*Poria cocos (SCHW.) WOLF*	3
Glycyrrhizae Radix	*Glycyrrhiza uralensis FISCH*	3
Pogostemonis Herba	*Agastache rugosa (FISCH. et MEYER) O. KUNIZE*	3
Aucklandiae Radix	*Saussurea lappa CLARKE*	3
Zingiberis Rhizoma Recens	*Zingiber officinale ROSC*	6
Total	13 types	49

2) Grouping and administration of test drugs

Animals were divided into 10 animals per 6 groups as follows:

Intact control: vehicle pretreated non-carbachol-accelerated small intestinal transit group

Vehicle control: vehicle pretreated carbachol-accelerated small intestinal transit group

Jungri-tang Gamibang 500: 500 mg/kg *Jungri-tang Gamibang* pretreated carbachol-accelerated small intestinal transit group

Jungri-tang Gamibang 250: 250 mg/kg *Jungri-tang Gamibang* pretreated carbachol-accelerated small intestinal transit group

Jungri-tang Gamibang 125: 125 mg/kg *Jungri-tang Gamibang* pretreated carbachol-accelerated small intestinal transit group

Domperidone 20: 20 mg/kg domperidone pretreated carbachol-accelerated small intestinal transit group

In the present study, 500, 250 and 125 mg *Jungri-tang Gamibang* or 20 mg domperidone were dissolved or suspended in distilled water and orally pretreated to the carbachol-accelerated small intestinal transit mice once a day for 7 days at a volume of 10ml/kg (of body weight) using a Zonde needle attached to 1 ml syringes containing test drugs.

3) Carbachol treatment and small intestinal transit mouse measurement

To induce the acceleration of mice small intestinal transit, 1mg/kg carbachol was subcutaneously dosed once 15 min before last administration of the test drugs. The animals were then killed 20 min later, and the small intestine completely removed. The small intestinal transit rate was obtained after dividing the migrating length of activated charcoal powder by the total length of the small intestine as previous[9].

4) Body weight changes

Changes of body weight and its gains were calculated at 1 day before test drug administration, at dosing and 1, 2, 5 and 6 days after dosing and at sacrifice. At dosing and sacrifice day, experimental animals were overnight fasted (water was not; about 18hr) to reduce the erratum of feeding. In addition, body weight gains during dosing period were calculated with the following equation.

EQUATION 1.Weight gains (g)

= (Body weight at sacrifice - Body weight at dosing)

3. Statistical analyses

All data was calculated as mean ± SD. Statistical analysis was conducted using Mann-Whitney U-Wilcoxon Rank Sum W test (MW test) with SPSS for Windows (Release 6.1.3., SPSS Inc., USA). The percentage changes compared to that of vehicle control were calculated to help the understanding of the efficacy of test materials on differences between vehicle control and test groups, and the differences between intact and vehicle control were also calculated in case of carbachol-accelerated mice as follows.

EQUATION 2. Percentage Changes *vs. intact control (%)*

= *[((Data of vehicle control - Data of intact control)/Data of intact control) × 100]*

EQUATION 3. Percentage Changes *vs. vehicle control (%)*

= *[((Data of test groups - Data of vehicle control)/Data of vehicle control) × 100]*

Results

1. Results of *in vivo* assays

1) Changes of body weights

No meaningful changes between the body weight and gains were detected in any test drug dosing groups compared to those of the intact and carbachol controls. In addition, no meaningful changes to the body weight and gains were detected between the intact and carbachol controls (Table 2 and 3).

Table 2. Changes on the body weight of carbachol-accelerated mice.

Body weight	1 day before dosing	At dosing(1)	Days after dosing				At sacrifice (1)
			1 day	2 day	5 day	6 day	
Controls							
Intact	34.47±1.72	30.81±1.68	34.68±1.66	34.84±1.34	35.99±0.92	36.41±1.07	32.93±1.56
Carbachol	34.38±2.50	30.60±1.96	34.86±1.93	35.11±1.74	35.90±1.84	36.67±2.21	32.88±2.51
Jungri-tang Gamibang							
500mg/kg	34.43±1.97	30.58±2.08	34.66±1.96	35.16±1.82	35.57±1.66	36.06±1.77	32.52±1.57
250mg/kg	34.98±2.22	31.16±2.28	34.33±2.26	34.59±1.61	35.32±1.70	36.34±1.51	32.77±1.34
125mg/kg	34.48±3.09	30.57±3.08	35.03±3.22	35.54±3.38	36.18±3.54	36.82±3.24	33.16±3.53
Domperidone							
20mg/kg	34.51±2.17	30.89±2.06	34.84±1.88	35.35±1.55	36.02±1.49	36.60±1.43	32.82±1.32

n=10; (Mean ± S.D.), g; (1) overnight fasted

In the intact control, the body weight at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were measured as 34.47 ± 1.72, 30.81 ± 1.68, 34.68 ± 1.66, 34.84 ± 1.34, 35.99 ± 0.92, 36.41 ± 1.07 and 32.93 ± 1.56g/head, respectively (Table 2).

In the carbachol control, the body weights at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were measured as 34.38 ± 2.50, 30.60 ± 1.96, 34.86 ± 1.93, 35.11 ± 1.74, 35.90 ± 1.84, 36.67 ± 2.21 and 32.88 ±2.51g/head, respectively (Table 2).

In the 500 mg/kg *Jungri-tang Gamibang* extracts pretreated group, the body weights at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were measured as 34.43 ± 1.97, 30.58 ± 2.08, 34.66 ± 1.96, 35.16 ± 1.82, 35.57 ± 1.66, 36.06 ± 1.77 and 32.52 ± 1.57g/head, respectively (Table 2).

In the 250 mg/kg *Jungri-tang Gamibang* extracts pretreated group, the body weights at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were measured as 34.98 ± 2.22, 31.16 ± 2.28, 34.33 ± 2.26, 34.59 ± 1.61, 35.32 ± 1.70, 36.34 ± 1.51 and 32.77 ± 1.34g/head, respectively (Table 2).

In the 125 mg/kg *Jungri-tang Gamibang* extracts pretreated group, the body weights at 1 day before

Table 3. Changes on the body weight gains of carbachol-accelerated mice.

Gains	Body weight gains during dosing (at dosing ~ sacrifice)
Controls	
Intact	2.12 ± 1.01
Carbachol	2.28 ± 1.46
Jungri-tang Gamibang	
500mg/kg	1.94 ± 0.91
250mg/kg	1.61 ± 1.55
125mg/kg	2.59 ± 1.30
Domperidone	
20mg/kg	1.93 ± 1.60

n=10; (Mean ± S.D.), g

dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were measured as 34.48 ± 3.09, 30.57 ± 3.08, 35.03 ± 3.22, 35.54 ± 3.38, 36.18 ± 3.54, 36.82 ± 3.24 and 33.16 ± 3.53g/head, respectively (Table 2).

In the 20 mg/kg domperidone pretreated group, the body weights at 1 day before dosing, at dosing, 1, 2, 5, 6 days after dosing and at sacrifice were measured as 34.51 ± 2.17, 30.89 ± 2.06, 34.84 ±1.88, 35.35 ± 1.55, 36.02 ± 1.49, 36.60 ± 1.43 and 32.82 ± 1.32g/head, respectively (Table 2).

The body weight gains during dosing periods (at dosing ~ sacrifice) of the intact and carbachol controls, 500, 250, 125 mg/kg *Jungri-tang Gamibang* extracts- and 20 mg/kg domperidone-pretreated groups were measured as 2.12 ± 1.01, 2.28 ± 1.46, 1.94 ± 0.91, 1.61 ± 1.55, 2.59 ± 1.30 and 1.93 ± 1.60g/head, respectively (Table 3).

2) Changes of small intestinal activated charcoal transit

Significant ($p<0.01$) increase of % regions of activated charcoal transit in the small intestine was detected in the carbachol control compared to that of the intact control. However, significant ($p<0.01$) decreases of % regions of activated charcoal transit were dose-dependently observed in all *Jungri-tang Gamibang* extracts or domperidone-pretreated groups (Table 4).

In the carbachol control, % regions of activated charcoal transit in the small intestine showed 65.41% changes vs. the intact control, and showed % changes vs. carbachol control as -26.43, -17.05, -13.25 and -24.34% in *Jungri-tang Gamibang* extracts 500, 250, 125mg/kg- and domperidone 20mg/kg-pretreated groups, respectively.

The % regions of activated charcoal transit in the small intestine of the intact and carbachol controls, 500, 250, 125 mg/kg *Jungri-tang Gamibang* extracts- and 20 mg/kg domperidone-pretreated groups were measured as 52.13 ± 7.37, 86.22 ± 7.29, 63.64 ± 5.74, 71.52 ± 4.26, 74.80 ± 8.39 and 65.24 ± 7.94%, respectively (Table 4).

Discussion

Plants have been a constant source of drugs and recently, much emphasis has been placed on finding novel therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs[12]. Numerous herb extracts have been shown to have beneficial pharmacological effects on intestinal motilities, among them Viguiera hypargyrea root extracts[13], methanolic extract of Ficus platyphylla[14], Aurantii fructus immaturus extracts[15],

Table 4. Changes on % regions of activated charcoal transit on carbachol-accelerated small intestine transit mice.

% regions	% regions of activated charcoal transit
Controls	
Intact	52.13 ± 7.37
Carbachol	86.22 ± 7.29*
Jungri-tang Gamibang	63.64 ± 5.74*, #
500mg/kg	71.52 ± 4.26*, #
250mg/kg	74.80 ± 8.39*, #
125mg/kg	
Domperidone	
20mg/kg	65.24 ± 7.94*, #

n=10; (Mean ± S.D.), %;
* $p<0.01$ compared to that of sham by MW test;
# $p<0.01$ compared to that of vehicle control by MW test

methanol extract from Monadenium lugardiae[16] and Teucrium polium boiled leaf extract[17]. In addition, poly herbal formulae, DaiKenjung-tang (大建中湯)[9] and Yukkunga-tang (六君子湯)[18] also showed favorable effects on the gastrointestinal motilities, and are used to treated various gastrointestinal disorders. The *Jungri-tang* was first mentioned in the book 'Clinical Prescriptionology in Oriental Medicine[19],' by Yun. This medicine has medical actions on Anwigeoseup (安胃祛濕), Sanhanchegyul (散寒除結) so that it can treat Sikche (食滯), Cheokchui (積聚), all kinds of Kikyul (氣結), etc. Since the first report until now, the medicine has been applied clinically to treat constipation, acute or chronic gastritis, appendicitis, pleuritis, pulmonary tuberculosis, hypertension, liver cirrhosis and nephritis.

In this study to prove the virtues of *Jungri-tang*, we use *Jungri-tang Gamibang* composed of Raphani Semen (蘿葍子) for Sosikhwadam (消食化痰), Pogostemonis Herba (藿香) for Banghwanghwaseup (芳香化濕) and Aucklandiae Radix (木香) for Hangkigitong (行氣止痛).

To clarify the effects of *Jungri-tang Gamibang* on accelerating small intestinal movement induced by the stimulation of cholinergic neurotransmission, we evaluated the effects of *Jungri-tang Gamibang* on *in vivo* carbachol (an acetylcholinergic agent)-accelerated mouse small intestinal transit.

The effects were compared to those of domperidone. Domperidone, a dopamine D2-receptor antagonist[20], is also clinically effective in treating functional gastrointestinal disorders such as gastroesophageal reflux, gastritis, gastric atony, gastroptosis, dyspepsia, anorexia, nausea and vomiting[21], and has been used as a reference drug for detecting antispasmodic effects[18].

To induce the acceleration of mouse small intestinal transit, 1mg/kg carbachol was dosed once subcutaneously 15 min before last administration of the test drugs. In the present study, 500, 250 and125 mg/kg *Jungri-tang Gamibang* or domperidone 20mg /kg were orally pretreated on the carbachol accelerated mouse small intestinal transit once a day for 7 days and the small intestinal transit rate of activated charcoal powder was monitored.

In changes of body weights, no meaningful changes on the body weight and gains were detected in any test drug dosing groups compared to those of intact and carbachol controls, respectively. In addition no meaningful changes on the body weight and gains were detected between intact and carbachol controls (Table 2 and 3).

In changes of small intestinal activated charcoal transit, significant ($p<0.01$) increase of % regions of activated charcoal transit in the small intestine was detected in the carbachol control compared to that of the intact control. However, significant ($p<0.01$) decreases of % regions of activated charcoal transit were dose-dependently observed in all *Jungri-tang Gamibang* extracts or domperidone-pretreated groups (Table 4).

Clinical conditions such as ileus and IBS exhibit various symptoms depending on critical factors. For example, in relation to adhesive ileus, it is known that the intestinal motility is different on the proximal side compared with the distal side at the obstruction[22]. Intestinal dysmotility due to dysfunction of the autonomic nervous system is considered one etiology of postoperative ileus and IBS[10]. Previous and the present results seem to indicate that *Jungri-tang Gamibang* has a dual effect on intestinal motility: enhancement in the normal condition; and normalization in the accelerated condition. Hence, the prescription of *Jungri-tang Gamibang* is useful from the viewpoint of clinical treatment of digestive tract disorders.

Based on the results, although the exact molecular mechanism was not proved and which herbs or compound in *Jungri-tang Gamibang* are responsible for actions has not yet been analyzed, it was concluded that *Jungri-tang Gamibang* enhanced the normal intestinal motility; normalization in the accelerated

intestinal motility might interfere with a variety of muscarinic, adrenergic and histaminic receptor activities or with the mobilization of calcium ions required for smooth muscle contraction non-specifically. Therefore, it is expected that *Jungri-tang Gamibang* will be promising as a clinical prescription for digestive tract disorders, not only constipation but also the relief of pain from IBS and ileus.

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