J Neurogastroenterol Motil, Vol. 22 No. 2 April, 2016
pISSN: 2093-0879 eISSN: 2093-0887
http://dx.doi.org/10.5056/jnm15131
Journal of Neurogastroenterology and Motility

Original Article

Effect of DA-9701 on the Normal Motility and Clonidine-induced Hypomotility of the Gastric Antrum in Rats

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Background/Aims
DA-9701 is a novel prokinetic agent. In the present study, we investigated the effect of DA-9701 on the motility of the gastric antrum in the normal and clonidine-induced hypomotility in an in vivo animal model.

Methods
A strain gauge force transducer was sutured on the gastric antrum to measure the contractile activity in rats. A total of 28 rats were subclassified into the 4 groups: (1) the placebo group, (2) the DA-9701 group, (3) the placebo group in the clonidine-pretreated rats, and (4) the DA-9701 group in the clonidine-pretreated rats. After the basal recording, either placebo (3% [w/v] hydroxypropylmethyl cellulose) or DA-9701 was administered. Contractile signals were measured after the administration and after a meal. In the clonidine-pretreated rats, either placebo or DA-9701 was administered. Contractile signals were measured after the administration and after a meal.

Results
Oral administration of DA-9701 did not significantly alter the motility index of the gastric antrum in the preprandial and postprandial periods, compared with the placebo group. The administration of clonidine decreased the motility index of the gastric antrum in the preprandial and postprandial periods, compared with the administration of placebo. This reduction of the antral motility by the administration of clonidine was not observed in the clonidine-pretreated DA-9701 group. The percentage of the motility index in the postprandial period was significantly greater in the clonidine-pretreated DA-9701 group, compared with the clonidine-pretreated placebo group.

Conclusions
DA-9701 improves the hypomotility of the gastric antrum induced by clonidine, suggesting its gastroprokinetic effect in the pathologic condition.
(J Neurogastroenterol Motil 2016;22:304-309)

Key Words
Antrum; DA-9701; Motility; Prokinetic

Received: August 13, 2015 Revised: December 6, 2015 Accepted: December 20, 2015

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Introduction

Functional dyspepsia (FD) is characterized by the presence of recurrent or persistent symptoms thought to originate in the gastroduodenal region without any organic, systemic or metabolic disease that is likely to explain the symptoms.[1] Diverse pathophysiologic mechanisms are known to be involved in FD. Delayed gastric emptying has been proposed to be an important pathophysiologic mechanism of FD.[2,3] The term prokinetic refers to a group of medications accelerating gastrointestinal motility. The pathophysiologic mechanism related to the effectiveness of prokinetics in FD is considered to be mainly delayed gastric emptying. Delayed gastric emptying is observed in approximately 30-40% of patients with FD.[4,5] Actually, prokinetic drugs are commonly prescribed for the treatment of dyspeptic patients suspected to have delayed gastric emptying. However, currently available gastroprokinetic agents have some demerits such as adverse effects or conflicting efficacy data.[6-8]

DA-9701 is a novel prokinetic agent formulated with the extracts of Pharbitis Semen and Corydalis Tuber. These plants have been used in oriental traditional medicine. Pharbitis Semen, the seed of *Pharbitis nil* Choisy, is believed to have analgesic effects. Corydalis Tuber, the root of *Corydalis yahusuo* W.T. Wang (Papaveraceae), is known to have analgesic and anti-ulcer effects.[9-11] DA-9701 was reported to not only significantly enhance gastric emptying in healthy rats, but also normalize delayed gastric emptying induced by apomorphine, cisplatin or stress.[12,13] These findings suggest that DA-9701 may become a new therapeutic option for the treatment of patients with delayed gastric emptying. However, the effect of DA-9701 on the normal motility and hypomotility, a pathologic condition, and of the gastric antrum, has yet to be fully investigated.

Thus, in the present study, we aimed to investigate the effect of DA-9701 on the motility of the gastric antrum in the normal and clonidine-induced hypomotility in an in vivo animal model.

Materials and Methods

Preparation of DA-9701

DA-9701, the standardized extract of Pharbitis Semen and Corydalis Tuber, was prepared as previously reported.[14] Briefly, dried herbs (water content of < 10%) were mixed in a specific ratio of the 2 herbs and extracted with 50% aqueous ethanol at room temperature for 48 hours. After filtration, the aqueous ethanol extract was evaporated under reduced pressure to yield a brown extract. The quality was evaluated using the standard method involving quantitative high-performance liquid chromatography, as previously reported.[12]

Animals

Male Sprague-Dawley rats aged 6 weeks were purchased and housed in individual cages under controlled conditions of temperature (22-24°C) and illumination (12-hour light cycle starting at 6 AM) for at least 1 week. The rats were given *ad libitum* access to food and water. The rats underwent surgical procedure to place the strain gauge force transducer, and were subsequently allowed at least 1 week to acclimate to that condition. All the experiments were performed in 28 rats (n = 7 in each group) aged 8 weeks (200-250 g). The experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Ajou University. All efforts were made to minimize animal suffering and to reduce the number of animals in experiments.

Measurement of Gastric Antral Motility in Conscious Rats

After fasting for 24 hours, the rats were anesthetized with intraperitoneal administration of Zoletil (tiletamine + zolazepam, 0.06 mL/100 g) and Rompun (xylazine, 0.04 mL/100 g). A midline incision was made in the abdomen under anesthesia, and the stomach was exteriorized. Strain gauge transducers (F-061S; Star Medical, Inc, Tokyo, Japan) were sutured onto the serosal surface of the gastric antrum to measure the contractile activity of the circular muscle. The abdomen was then closed, and the wires from transducer were exteriorized through the abdominal wall, ran under the skin toward the back. Wires were protected by a protective jacket (Star Medical, Inc, Tokyo, Japan). After the surgery, rats were housed individually and were allowed to recover for 1 week before the experiments. On the experimental day, the wires from the transducer were connected to the recording system (Power-Lab model 4SP; ADI instruments, Colorado Springs, CO, USA), and motility recording was started at a constant time in the morning.

Study Protocols for the Effect of DA-9701 on the Normal Motility of the Antrum

After the basal recording for 20 minutes in the fasting state, either placebo (3% [w/v] hydroxypropylmethyl cellulose) (Sigma, St. Louis, MO, USA) or DA-9701 (3 mg/kg) was administered orally. Contractile signals were measured for 20 minutes from 30 minutes

after the administration (the preprandial period). Subsequently, an oropharyngeal feeding tube was inserted, and then the standardized liquid meal was administered through the feeding tube. The recording continued for 20 minutes after the oral infusion of the meal (the postprandial period) (Fig. 1A).

Study Protocols for the Effect of DA-9701 on the Clonidine-induced Hypomotility

After the basal recording for 20 minutes in the fasting state, clonidine hydrochloride (clonidine), an α2-adrenoceptor agonist, at a dose of 100 μg/kg was subcutaneously administered. The contractile signals were measured for 20 minutes after the administration of clonidine (after clonidine). Subsequently, either placebo (3% [w/v] hydroxypropylmethyl cellulose) or DA-9701 (3 mg/kg) was administered orally. After 30 minutes, the contractile signals were recorded for 20 minutes (the preprandial period). Subsequently, oropharyngeal feeding tube was inserted, and then the standardized liquid meal was administered through the feeding tube. The recording continued for 20 minutes after the oral infusion of the meal (the postprandial period) (Fig. 1B).

Statistical Methods

All data are expressed as the mean ± standard deviation. Motility index and the area under the curve was calculated using a computer-assisted system (PowerLab, ADInstruments, Colorado Springs, CO, USA). The motility index was determined as the area under the curve (AUC) in the antrum for a 20-minute period, and is shown as a percentage (% motility index = 100 × [AUC post-treatment/AUC pretreatment or basal period]). These percentages were compared between groups using Student's *t* test, and among groups using one-way ANOVA with multiple comparisons. Statistical analyses were performed using SPSS version 18.0 (IBM Corp, Armonk, NY, USA). A P-value of less than 0.05 was considered to be statistically significant.

Results

Effects of Placebo or DA-9701 on the Normal Motility of the Antrum

Oral administration of DA-9701 did not significantly alter the motility index of the gastric antrum in the preprandial ($P = 0.675$) and postprandial periods ($P = 0.632$), compared with the placebo group (Fig. 2A and 2B) (Table 1).

Effects of Clonidine on the Antral Motility of the Stomach

The administration of clonidine decreased the motility index of the gastric antrum in the preprandial ($P = 0.053$) and postprandial periods ($P = 0.017$), compared with the administration of placebo (Fig. 2C and Table 2).

Effects of DA-9701 on the Clonidine-induced Hypomotility

In the clonidine-pretreated DA-9701 group, the reduction of the antral motility by the administration of clonidine was not observed in the preprandial ($P = 0.601$) and postprandial periods ($P = 0.673$), compared with the placebo group. The percentage motility index in the postprandial period was significantly greater in the clonidine-pretreated DA-9701 group, compared with the clonidine-

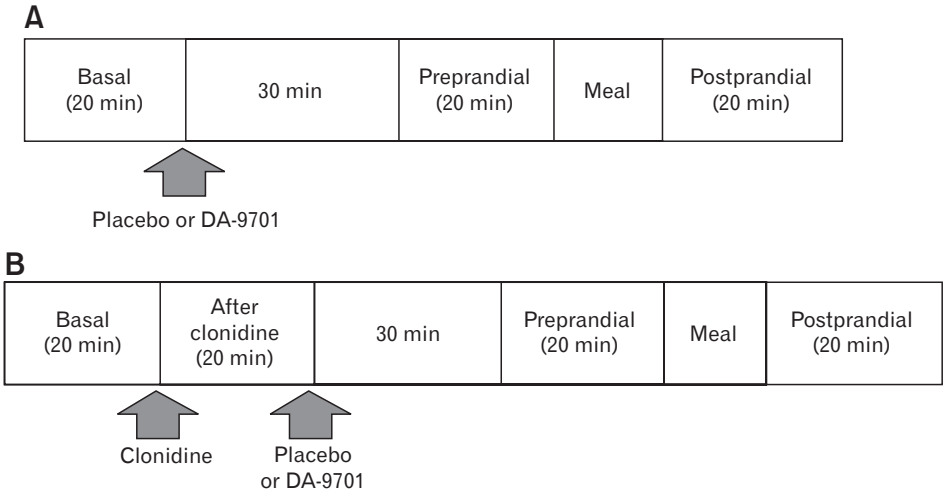

Figure 1. Study protocols. (A) After the basal recording for 20 minutes, either placebo (3% [w/v] hydroxypropylmethyl cellulose) or DA-9701 (3 mg/kg) was administered orally, and then the standardized liquid meal was administered through the feeding tube. (B) After the basal recording for 20 minutes, clonidine hydrochloride (clonidine) at a dose of 100 μg/kg was subcutaneously administered. Subsequently, either placebo (3% [w/v] hydroxypropylmethyl cellulose) or DA-9701 was administered orally, and then the standardized liquid meal was administered through the feeding tube.

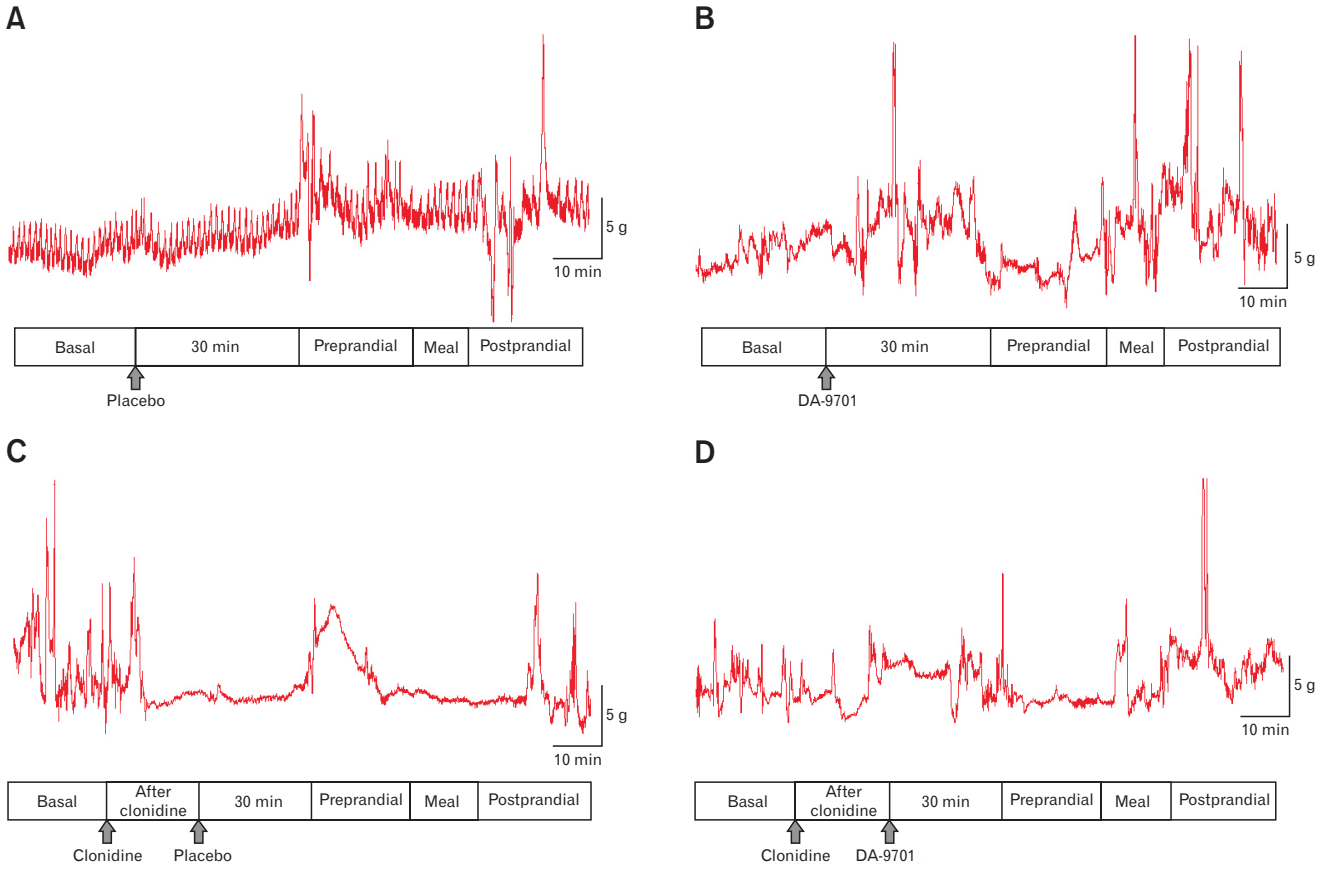

Figure 2. Effects of placebo or DA-9701 on normal motility and hypomotility of the gastric antrum. Compared with the placebo group (A), the motility index of the gastric antrum was not significantly changed after the administration of DA-9701 (B), both in the preprandial and postprandial periods. In the clonidine-pretreated placebo group, the motility index of the gastric antrum was significantly reduced, both in the preprandial and postprandial periods (C), but this hypomotility was not observed in the clonidine-pretreated DA-9701 group (D).

Table 1. Effects of Placebo or DA-9701 on the Normal Motility of the Gastric Antrum

	% motility index compared to the basal period	
	Preprandial period	Postprandial period
Placebo	112.5 ± 36.2	127.9 ± 40.1
DA-9701	125.2 ± 62.5	142.7 ± 60.9
P-value	0.675	0.632

Table 2. Effects of DA-9701 on the Clonidine-induced Hypomotility

	% motility index compared to the basal period	
	Preprandial period	Postprandial period
Placebo	112.5 ± 36.2	127.9 ± 40.1
Clonidine + placebo	65.8 ± 24.3[a]	63.5 ± 37.9[b]
Clonidine + DA-9701	100.4 ± 19.9	117.4 ± 23.1[c]

[a]$P = 0.05$, compared with the placebo group.
[b]$P < 0.05$, compared with the placebo group.
[c]$P < 0.05$, compared with the clonidine + placebo group.

pretreated placebo group ($P = 0.041$) (Fig. 2D and Table 2).

Discussion

DA-9701 is a currently available prokinetic agent in South Korea, named Motilitone (Dong-A ST, Seoul, South Korea). This agent is more like a herbal drug rather than a chemical drug, because it is formulated with the extracts of Pharbitis Semen and Corydalis Tuber. The present study demonstrated that DA-9701 improved clonidine-induced hypomotility of the gastric antrum. This finding suggests a beneficial role of DA-9701 in the treatment of hypomotility of the gastric antrum observed in a subset of patients with FD.

A recent study in rats demonstrated that DA-9701 improved

stress-induced delay in gastric emptying.[13] In addition, animal studies have shown the beneficial effect of DA-9701 on gastric accommodation and visceral sensitivity.[14,15] Since DA-9701 is a herbal compound formulated with Pharbitidis Semen and Corydalis Tuber, it has an affinity to multiple receptors associated with gastroduodenal function.[16] This agent is suggested to be involved in dopamine D2, serotonin receptor 4 (5-HT_4), 5-HT_{1A}, and adrenergic α2 receptors. Its action on dopamine D_2 and 5-HT_4 receptors explains the prokinetic effect of DA-9701. This mechanism appears to be important in enhancing upper gastrointestinal motility. Moreover, the affinity of DA-9701 to 5-HT_{1A} and adrenergic α2 receptors is likely to be associated with the fundus relaxing effect of this drug. A phase III study in patients with FD demonstrated the comparable effect of DA-9701 to itopride. DA-9701 showed noninferior efficacy to itopride that is known to increase acetylcholine concentrations by inhibiting dopamine D_2 receptors and acetylcholinesterase.[17] Actually, itopride was reported to stimulate gastric motility and accelerate gastric emptying.[18] In several Asian countries such as South Korea and Japan, mosapride citrate (mosapride), a serotonin 5-HT_4 receptor agonist, as well as itopride has been used in patients with FD as a prokinetic drug. A recent animal study showed the comparable effect of DA-9701 to mosapride in reversing delayed gastric emptying induced by acute stress.[13] However, itopride and mosapride have shown variable efficacy in the treatment of FD, particularly in placebo-controlled randomized studies.[19,20] To date, there is no report regarding a placebo controlled trial of DA-9701 in patients with FD. Therefore, the efficacy of DA-9701 in FD needs to be confirmed.

Delayed gastric emptying has been reported to be a major pathophysiologic mechanism of FD.[2-4] Gastric antral hypomotility is suggested to contribute to delayed gastric emptying.[21] Clonidine, an adrenergic α2 receptor agonist, is known to induce hypomotility and delayed transit of the stomach and intestine, via the inhibition of acetylcholine release from cholinergic nerve terminals.[22,23] A delayed gastric emptying model induced by α2-adrenoceptor agonists has been used to demonstrate the effect of gastroprokinetic agents in animals.[24,25] In the present study, we showed that the pretreatment of clonidine significantly reduced motility of the gastric antrum, that is keeping with the previous observations.[26,27] Hypomotility was observed both in the preprandial and postprandial periods. The gastroprokinetic activity of prokinetics may be different among experimental models of gastric dysfunction. Given that the administration of DA-9701 recovered antral hypomotility induced by clonidine, the prokinetic effect of DA-9701 through dopamine D_2 and 5-HT_4 receptors might be stronger than its relaxing effect via adrenergic α2 receptors.

In the present study, the effect of DA-9701 on the normal motility was not remarkable. No significant change in the motility index of the gastric antrum was observed in the preprandial and postprandial periods, compared with the placebo group. This may indicate that it does not increase motility in patients with normal motility of the gastric antrum. The reason for that cannot be explained exactly. However, it might be attributed to the feature of a herbal drug. In addition, the probability of a type II error cannot be excluded. In contrast to the negative effect of DA-9701 in the normal condition, its effect on clonidine-induced hypomotility, a pathologic condition, was distinct. The recovery of antral hypomotility by DA-9701 may result in improvement of delayed gastric emptying. Therefore, our results suggest that DA-9701 is a potent prokinetic agent that can be used for the treatment of gastric hypomotility. However, studies revealing the prokinetic effect of DA-9701 in humans are lacking. Thus, further studies to elucidate the beneficial effect of DA-9701 on the pathophysiologic mechanisms of FD are required. Since this agent is a herbal extract, no serious adverse events related to DA-9701 have been reported in clinical settings. Therefore, DA-9701 appears to be a promising prokinetic agent for the treatment of FD.

The present study has several limitations. The number of rats included in the experiments may not be appropriate for the analysis. Therefore, the possibility of type II errors in the results cannot be completely excluded. In addition, the artifacts produced by motion, tension, stress, operations, manipulations, etc. might not be completely removed in the analysis due to technical limitations.

In conclusion, DA-9701 improves the hypomotility of gastric antrum induced by clonidine. Our results suggest that the prokinetic activity of DA-9701 may improve delayed gastric emptying or meal-related symptoms of FD. Placebo-controlled randomized trials of DA-9701 in patients with postprandial distress syndrome are expected. In addition, the effects of DA-9701 on the gastric emptying and accommodation to a meal in humans need to be studied.

Financial support: This study was financially supported by Dong-A ST.

Conflicts of interest: None.

Author contributions: Je Wook Kang acquired data and analyzed data; Dae Kyeong Han and Ock Nyun Kim performed experiments and acquired data; and Kwang Jae Lee made the concept of the study, analyzed data, wrote the paper, and revised the manuscript.

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