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Original Article

Effect of DA-9701, a Novel Prokinetic Agent, on Post-operative Ileus in Rats

Sang Pyo Lee,[1] Oh Young Lee,[2]* Kang Nyeong Lee,[2] Hang Lak Lee,[2] Ho Soon Choi,[2] Byung Chul Yoon,[2] and Dae Won Jun[2]

[1]Department of Internal Medicine, Konkuk University School of Medicine, Seoul, Korea; and [2]Department of Internal Medicine, Hanyang University College of Medicine, Seoul, Korea

Background/Aims
Post-operative ileus (POI) is a common complication of abdominal surgery. DA-9701, an extract of Pharbitis Semen and Corydalis Tuber, is a new prokinetic agent that also alleviates visceral pain. The aim of this study was to investigate whether DA-9701 can ameliorate POI in rats.

Methods
A total of 32 rats were divided into 4 groups: no surgery/no medication (NSNM), no surgery/medication (NSM), surgery/no medication (SNM), and surgery/medication (SM). Gastrointestinal transit (GIT), which is assessed by migration of charcoal, and cumulative stool weight were measured at 24 hours after surgery.

Results
GIT was significantly more delayed in the SNM group than in the other groups (SNM vs NSNM, $P < 0.001$; SNM vs NSM, $P < 0.001$; SNM vs SM, $P = 0.005$). Cumulative stool weight in that group was also lower than in the no surgery groups (SNM vs NSNM, $P = 0.007$; SNM vs NSM, $P = 0.033$), and there was no significant difference between the SM group and the no surgery groups (SM vs NSM, $P = 0.703$; SM vs NSNM, $P = 0.347$).

Conclusion
DA-9701 can ameliorate POI by reducing delayed GIT and improving defecation in a rat model of POI.
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Key Words
Corydalis; DA-9701; Gastrointestinal transit; Ghrelin; Ileus

Introduction

Post-operative ileus (POI) is a common complication of abdominal surgery.[1,2] Previous studies have shown that POI lasts more than four days in more than half of the patients after major abdominal surgery, and it lasts for more than 6 days in 25%.[3] Although POI is not life-threatening, it involves additional medical cost and pain. Therefore, there has been much effort to reduce the severity and duration of the complication. Widely approved methods for reducing the duration of POI include nil per os, early ambulation, minimal use of opioids, and aggressive correction of

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*Correspondence: Oh Young Lee, MD, PhD
Department of Internal Medicine, Hanyang University Medical Center, 22 Wangsimni-ro, Seongdong-gu, Seoul 04763, Korea
Tel: +82-2- 2290-8343, Fax: +82-2- 2298-9183, E-mail: leeoy@hanyang.ac.kr

electrolyte imbalance.[1] Despite all these efforts, POI is still a problematic issue.

DA-9701 (Motilitone; Dong-A Pharmaceutical Co, Seoul, South Korea), a herbal agent obtained from extracts of Pharbitidis Semen and Corydalis Tuber, has been reported to have potent prokinetic effects and a good safety profile.[4-7] DA-9701 has agonistic activity on serotonergic receptors, and antagonistic activity on dopaminergic receptors; thus it is known to increase gastric emptying and accommodation, and to reduce visceral hypersensitivity.[8-17]

Pain-induced neural reflexes and postoperative intestinal inflammation can induce POI.[1] Abdominal pain after surgery can lead to the release of endogenous neuromuscular inhibitors, such as corticotropin-releasing hormone, norepinephrine, and nitric oxide synthase. These inhibitors cause neurogenic bowel inhibition, which seems to play a leading part in the pathogenesis of POI. On the other hand, surgical manipulation of the intestine can cause localized inflammation and bowel wall edema by activating macrophages and mast cells. DA-9701 may reduce the duration and severity of POI, via a prokinetic effect in the proximal gastrointestinal (GI) tract and by helping to alleviate visceral pain.

Ghrelin is a peptide synthesized in endocrine cells of the gastric mucosa with orexigenic, gastroprokinetic, and anti-inflammatory effects.[18-21] Circulating ghrelin levels, especially active ghrelin levels, decrease under conditions of abdominal surgery[22,23] and gradually normalize over 24 hours.[22,24,25] Therefore, the decrease of ghrelin after surgery could be a key driver of POI. Therapeutic effects of ghrelin and ghrelin receptor agonists have been shown on surgery-induced POI.[26-29] Meanwhile, a recent study found that acute stress can increase plasma acylated ghrelin levels and that DA-9701 normalized the levels.[30] On the basis of these observations, we thought that DA-9701 might have a normalizing effect on the reduced ghrelin levels after surgery.

The aim of this study was to investigate, using rats, whether DA-9701 can ameliorate POI. Gastrointestinal function was indirectly assessed by measuring GI transit (GIT) and stool weight, because sustained ileus can result in disturbed stool passage and delayed GIT. In addition, we assessed the effect of DA-9701 on ghrelin levels by measuring serum ghrelin in the rat model of POI.

Materials and Methods

Animals

All procedures were approved by the Institutional Animal Care and Use Committee of Dong-A ST (I-1312011). Male Sprague-Dawley rats (6-week-old) were purchased from Orient Bio, weighing 200-220 g. Rats were randomized with 4 rats per cage, and maintained in 12 hours alternating light and dark with free access to normal rat chow and water. They were kept at constant temperature (24 ± 1°C) and 40-60% relative humidity, and were acclimatized for 1 week to minimize stress induced by manipulation. After adaptive feeding for 1 week, the rats were grouped according to their weights and fasted for 12 hours with free access to water. The water supply was stopped 3 hours before initiating the experiment (Fig. 1).

Experimental Groups and Medication

A total of 32 rats were randomly divided into 4 experimental groups: no surgery/no medication (NSNM), no surgery/medication (NSM), surgery/no medication (SNM), and surgery/medication (SM). The NSNM group served as the control and received neither oral administration of DA-9701 nor surgery. The NSM group received the drug but did not undergo surgery. The SNM

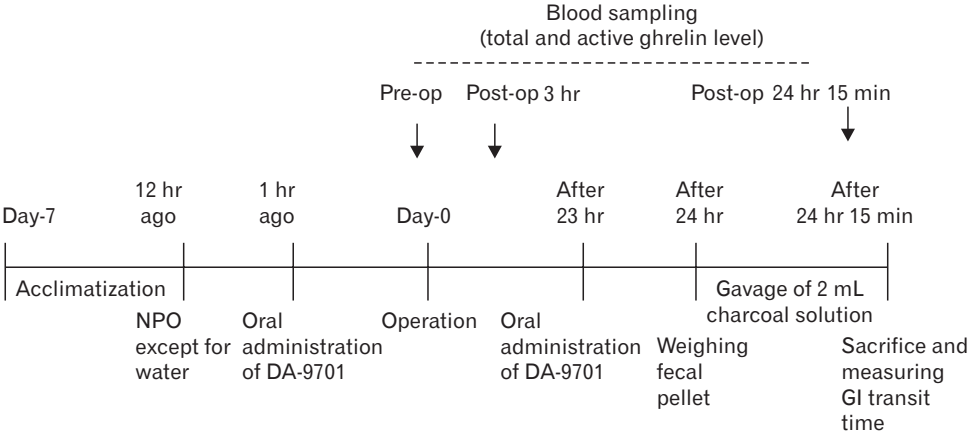

Figure 1. Schematic diagram of the experimental protocol. Pre-op, pre-operative; Post-op, post-operative; NPO, nil per os; GI, gastrointestinal.

group did not receive the drug but underwent the surgery, and the SM group received the drug and underwent surgery.

The novel prokinetic agent, DA-9701, was administered orally to the medication groups (the NSM and SM groups) 1 hour before and 23 hours after surgery. The dose of the test drug was 3 mg/kg; this dose has been shown to accelerate GIT in previous studies.[5,7,30] Three percent hydroxypropyl methylcellulose, the vehicle, was administered to the rats in the no medication groups (the NSNM and SNM groups) at the same time. The rats received the drug by intragastric feeding catheter (oral zonde needle).

The Operation Protocol

The animals in the surgery groups received an abdominal operation, which was carried out according to the following modified program.[2] After anesthesia with ether,[31,32] the surgical site was shaved and disinfected with povidone iodine. A 3 cm midline laparotomy was made just lateral to the linea alba. The small intestine from the distal duodenum to the distal ileum was exteriorized on moist gauze, and was manipulated extensively for 5 minutes. It was rolled gently using gloved fingers, enough to compress and move the luminal contents but not enough to totally occlude the lumen and induce bleeding. Then, the small intestine was covered with saline-soaked gauze, and left for 10 minutes. Thereafter, it was returned to the abdominal cavity, and the abdomen and peritoneum was sutured with an easy clip applier and disinfected with povidone iodine. The animals were allowed to recover from anesthesia on a heating pad before being returned to their cages, where they had free access to water and rat chow. The rats not subjected to surgery were not anesthetized, because ether anesthesia has no effect on subsequent gastrointestinal function.[33]

Stool Weight

After the abdominal operation, each animal was removed from its home cage and placed in a clean and clear metabolic cage. Fecal pellets were collected and weighed at 24 hours after surgery. Stool weights of the rats not subjected to surgery were measured at the same time. The temperature and humidity in the cages remained constant for the whole period.

Measuring Gastrointestinal Transit

After measuring stool weights, GIT was measured. The GIT protocol was modified from published procedures.[34,35] Intestinal transit was studied in all animals by gavage of 2 mL semisolid charcoal solution (5% charcoal and 5% gum arabicum in distilled water). The time at which the animals were killed was based on previous tests on normal rats. These tests showed that it took 15 minutes for the charcoal to reach 50 percent of the total length of the intestine. Therefore, fifteen minutes after the charcoal meal, the rats were killed by neck dislocation and the entire small intestine was removed en bloc. The length of the entire small intestine was measured by laying it out on a measuring tape and measuring the distance from the pyloric sphincter to the ileo-cecal valve. The distance traveled by the charcoal was also measured from the pyloric sphincter to the most caudal edge of the charcoal. The results are expressed as the proportion of the length of the entire small intestinal traversed by the charcoal at the time of tissue harvest.

Measurement of Serum Ghrelin

Blood samples (0.5 mL) were taken from the infraorbital veins of the rats before and 3 hours after surgery, and finally by cardiac puncture at 24 hours 15 minutes after surgery, just before the animals were killed. To minimize stress, the first blood samples were obtained under anesthesia immediately before the operation. The rats whose blood was sampled 3 hours after being operated were isolated from those whose blood were not yet sampled, because the stressed rat can lead to anxiety in other rats. Whole blood was drawn directly into a tube that contained no anti-coagulant, and Pefabloc was added immediately to a final concentration of 1 mg/mL. The blood samples were allowed to clot for 30 minutes at room temperature, then centrifuged at 5000 rpm for 15 minutes. The supernatants were transferred to separate tubes and acidified with HCl to 0.05 N. Total ghrelin and active ghrelin levels in the sera were measured with a commercial assay kit (Millipore, ELISA Kit, Rat/Mouse Ghrelin, Germany), according to the manufacturer's instructions.

Statistical Methods

All results were analyzed with SPSS 19.0 (SPSS, Chicago, IL, USA). Total and active ghrelin levels were compared using repeated measures ANOVA and paired t tests. If values did not follow a normal distribution, we used nonparametric tests, including the Wilcoxon signed rank test. Stool weights and GIT were analyzed by two-way ANOVA and one-way ANOVA with post-hoc Tukey's test. If the values did not follow a normal distribution, we used nonparametric tests, including the Kruskal-Wallis test with post-hoc Tukey's test with ranks. A $P < 0.05$ was considered to indicate statistical significance.

Results

Gastrointestinal Transit

The mean length of the small intestines was 111.8 ± 4.9 cm (range 103-120 cm), and charcoal solution reached 48.2 ± 6.0% of the length of the small intestine (range 33-56 cm) in 15 minutes. Surgery significantly reduced GIT (main effect of surgery: $F\ [1, 25] = 18.43$, $P < 0.001$) and there was a main effect of DA-9701 on GIT ($F\ [1, 25] = 5.17$, $P = 0.032$). GIT in the SNM group was more delayed than in the other groups (Fig. 2A) (SNM vs NSNM, $P < 0.001$; SNM vs NSM, $P < 0.001$; SNM vs SM, $P = 0.005$). There was no difference between the SM group and the no surgery (NSNM and NSM) groups (SM vs NSM, $P = 0.739$; SM vs NSNM, $P = 0.536$). The individual values of GIT are shown in the table.

Stool Weight

The mean cumulative stool weight was 1.3 ± 1.0 g (range 0.0-3.2 g). Surgery significantly reduced cumulative stool weight (main effect of surgery: $F\ [1, 27] = 9.88$, $P = 0.004$) and there was no main effect of DA-9701 on the cumulative stool weight ($F\ [1, 27] = 0.36$, $P = 0.551$). The stool weight in the SNM group was less than in the no surgery groups (Fig. 2B) (SNM vs NSNM, $P = 0.007$; SNM vs NSM, $P = 0.033$). There was no difference between the SM group and the no surgery groups (SM vs NSM, $P = 0.703$; SM vs NSNM, $P = 0.347$). Four of the 8 rats in the SNM group did not pass stool at all for 24 hours, compared with only 1 out of 7 rats in the SM group (Table).

Active Ghrelin Levels

There were no inter-group differences in active ghrelin levels before the operations (means 128.7/121.5/125.2/126.1 pg/mL in the NSNM/NSM/SNM/SM groups, respectively; $P = 0.237$). There were no differences of active ghrelin level according to the measurement time ($P = 0.288$), and there were no interactions between time and the groups ($P = 0.270$). Supplementary Figure 1 shows that active ghrelin levels had decreased by 3 hours after surgery in the surgery groups. The decrease was statistically significant in the SNM group (pre-operative [pre-op] vs post-operative [post-op] 3 hours, $P = 0.013$), but not in the SM group (pre-op vs post-op 3 hours, $P = 0.109$). Moreover, the level of active ghrelin had not recovered by 24 hours after surgery in the SNM group (pre-op vs post-op 24 hours, $P = 0.007$), whereas it had at least partially recovered at 24 hours after surgery in the SM group, and the difference between the pre-op and the post-op 24 hours level was not significant ($P = 0.250$).

Total Ghrelin Levels

There were no inter-group differences in total ghrelin levels before the operations (means 191.1/179.9/182.7/185.0 pg/mL in the NSNM/NSM/SNM/SM groups, respectively; $P = 0.575$). There were differences of active ghrelin level according to the mea-

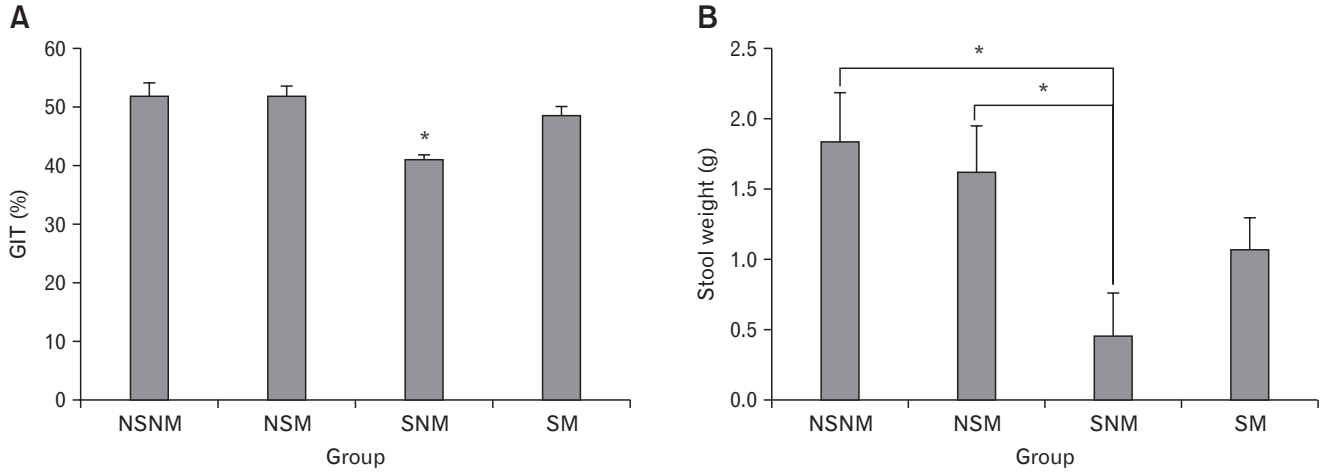

Figure 2. Gastrointestinal transit (%) and cumulative stool weights (g) by group. (A) Gastrointestinal transit (GIT) was significantly delayed in the surgery/no medication (SNM) group compared with the other groups. (B) Cumulative stool weight was significantly lower in the SNM group than in the no surgery/no medication (NSNM) and no surgery/medication (NSM) groups. SM, surgery medication. The asterisk (*) indicates a statistically significant difference.

Table. Individual Values of Gastrointestinal Transit and Cumulative Stood Weight in Each Group

Group	Rat (case No.)	GIT				Stool weight	
		Total length (cm)	Length moved (cm)	GIT (%)	Mean (%)	Weight (g)	Mean (g)
NSNM	1	-	-	-		1.92	
	2	120	65	54.2		0.83	
	3	-	-	-		2.77	
	4	110	55	50.0		3.24	
	5	115	60	52.2		2.29	
	6	110	61	55.5		1.76	
	7	120	62	51.7		1.70	
	8	120	61	50.8	52.4	0.23	1.8
NSM	9	105	50	47.6		1.28	
	10	108	61	56.5		2.46	
	11	118	57	48.3		3.22	
	12	110	55	50.0		1.21	
	13	113	58	51.3		0.93	
	14	113	59	52.2		0.36	
	15	103	53	51.5		1.27	
	16	105	58	55.2	51.6	2.20	1.6
SNM	17	115	45	39.1		2.43	
	18	110	47	42.7		0	
	19	110	53	48.2		0.35	
	20	110	37	33.6		0	
	21	120	48	40.0		0.74	
	22	110	36	32.7		0	
	23	115	58	50.4		0	
	24	110	45	40.9	41.0	0.16	0.5
SM	25	115	55	47.8		1.38	
	26	117	53	45.3		0.64	
	27	-	-	-		-	
	28	110	58	52.7		1.31	
	29	110	50	45.5		0.82	
	30	110	58	52.7		0	
	31	105	58	55.2		1.50	
	32	105	47	44.8	49.1	1.76	1.1

GIT, gastrointestinal transit; NSNM, no surgery/no medication; NSM, no surgery/medication; SNM, surgery/no medication; SM, surgery medication.

surement time ($P < 0.001$), and there were significant interactions between time and the groups ($P = 0.041$). Supplementary Figure 2 shows that the total ghrelin levels at 3 hours after the operations had declined in the SM group (pre-op vs post-op 3 hours, $P = 0.018$) and the NSM group (pre-op vs post-op 3 hours, $P = 0.011$). In the SM group only, total ghrelin levels had recovered at 24 hours after surgery (post-op 3 hours vs post-op 24 hours, $P = 0.001$).

Discussion

This study showed that there was a main effect of DA-9701 on GIT. In addition, GIT in the SNM group was slower than in the SM group. This means that DA-9701 can reduce the delay in GIT after abdominal operations. Although there was no main effect of DA-9701 on the cumulative stool weight, the cumulative stool weight in the SNM group was significantly lower than in the no surgery groups (NSNM + NSM) and there was no difference between the SM group and the no surgery group. That is, DA-9701 may also stimulate the passage of stool after abdominal operations. During the first post-surgery 24 hours, half of the rats in the SNM group did not pass stool at all, compared with only one rat in the SM group. This suggests that DA-9701 not only decreases the

severity of POI, but also reduces its duration.

POI can occur due to various factors. Neurogenic bowel inhibition and localized postoperative inflammation are the main causes.[1] The pain-induced neural reflex can contribute to GI inhibition by generating endogenous opioids and increasing sympathetic hyperactivity. Localized tissue trauma and postoperative inflammation can also impair GI motility. In addition, the use of opioids in the perioperative period has a negative effect on GI motility. The clinical features of POI include nausea, vomiting, abdominal distension, inability to tolerate oral feeding, and delayed passage of flatus and stool.[3] Similarly, the clinical criteria used to define the resolution of POI are the passage of stool, return of flatus, absence of abdominal distension and pain, absence of nausea or vomiting, presence of bowel sounds, and tolerance of an oral diet. In our study, we assessed the resolution of POI by measuring GIT and stool weights, because judging levels of pain would be highly subjective.

Many studies have shown that DA-9701 ameliorates visceral hypersensitivity, improves bowel movement, and accelerates gastric fundus relaxation.[5-7,9-15,30] We have already proven that DA-9701 decreases visceral pain in rats by down-regulating the level of phosphorylated extracellular signal-regulated kinase in the dorsal root ganglion and spinal cord.[12] Kim et al[11] also observed that the drug increased the pain threshold in rats with colorectal distension-induced visceral hypersensitivity. Other studies have shown that DA-9701 enhances gastric emptying via stimulation of the serotonin type 4 receptor and inhibition of the dopamine D_2 receptor.[5,6,13-15,30] These effects of the drug would probably have helped to improve POI in our experiment.

There has been only one previous study of the effect of DA-9701 on delayed GIT.[5] However, the POI model used involved only a 3 cm laparotomy and the incision was closed immediately. Our study was designed to overcome this limitation. As we noted in the methods section, our operation protocol was intended to provide an approximation to a real abdominal operation, and we succeeded in demonstrating an effect of DA-9701. In addition, we believe that our experimental method was fairly well designed. Thus, we clearly observed delayed GIT and reduced volume of stool in the surgery groups. In other words, our method of surgery clearly caused POI. In addition, there was no problem with the acclimatization of the rats in this study, because the groups had very similar mean baseline ghrelin levels. This indirectly indicates that identical environmental conditions were provided for the rats and they were under similar level of stress.

Our experimental results about serum ghrelin were not encouraging. Previous studies have revealed that circulating ghrelin levels, especially acyl ghrelin levels, fall in response to abdominal surgery and that the levels gradually recover over 24 hours.[22-25] We also found that ghrelin levels followed this pattern. However, our results were more complex and difficult to interpret.

Active ghrelin levels were reduced at 3 hours after surgery in the SNM group and they were not restored by 24 hours after surgery. Whereas, in the SM group, they had decreased at 3 hours after surgery and partially recovered by 24 hours after surgery, but in fact the differences among basal level, 3 hour level, and 24 hour level was not statistically significant. At the same time, in the SM group, total ghrelin levels were significantly decreased at 3 hours after surgery but had recovered by 24 hours. Thus, DA-9701 administration reversed the reduction in ghrelin levels after surgery within 24 hours. However, we cannot account for the decrease in total ghrelin levels at 3 hours after surgery in the NSM group and the absence of a decrease of total ghrelin level at 3 hours after surgery in the SNM group. Therefore, more work is mandatory to determine the relationship between ghrelin and DA-9701. Since ghrelin is not the only gut hormone determining GIT, it may be helpful to research different types of gut hormones, such as nesfatin, motilin, and leptin.

Our study has some limitations. First, there were some missing values. As one rat (No. 27) was killed by mistake during blood collection, we could not obtain its GIT and stool weight. Likewise, the GIT of 2 other rats could not be measured because the infusion syringe became clogged up during gavage of the charcoal solution (No. 1) and the small intestine was removed by mistake during autopsy (No. 3). Second, the sample sizes were not large enough. Further experiments with a larger number of rats are necessary to obtain statistically significant results, especially on serum ghrelin levels. Third, despite our efforts to minimize environmental effects, blood sampling may have been stressful for the animals. Therefore, we cannot exclude the possibility that stress affected serum ghrelin levels.

In conclusion, DA-9701 can ameliorate POI by reducing delayed GIT and improving defecation in a rat model of POI. Therefore, DA9701 might be useful as a prokinetic agent for preventing POI. These effects of DA-9701 may be associated with alterations in ghrelin levels, but further studies are needed.

Supplementary Materials

Note: To access the supplementary figures mentioned in this article, visit the online version of *Journal of Neurogastroenterol-*

ogy and Motility at http://www.jnmjournal.org/, and at https://doi.org/10.5056/jnm16003.

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Conflicts of interest: The authors disclose no conflicts.

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References

1. Doorly MG, Senagore AJ. Pathogenesis and clinical and economic consequences of postoperative ileus. Surg Clin North Am 2012;92:259-272, viii.
2. Chapelle SL, Bove GM. Visceral massage reduces postoperative ileus in a rat model. J Bodyw Mov Ther 2013;17:83-88.
3. Vather R, Trivedi S, Bissett I. Defining postoperative ileus: results of a systematic review and global survey. J Gastrointest Surg 2013;17:962-972.
4. Choi S, Choi JJ, Jun JY, et al. Induction of pacemaker currents by DA-9701, a prokinetic agent, in interstitial cells of Cajal from murine small intestine. Mol Cells 2009;27:307-312.
5. Lee TH, Choi JJ, Kim DH, et al. Gastroprokinetic effects of DA-9701, a new prokinetic agent formulated with Pharbitis Semen and Corydalis Tuber. Phytomedicine 2008;15:836-843.
6. Lee TH, Kim KH, Lee SO, Lee KR, Son M, Jin M. Tetrahydroberberine, an isoquinoline alkaloid isolated from corydalis tuber, enhances gastrointestinal motor function. J Pharmacol Exp Ther 2011;338:917-924.
7. Kwon YS, Son M. DA-9701: a new multi-acting drug for the treatment of functional dyspepsia. Biomol Ther (Seoul) 2013;21:181-189.
8. Ji HY, Liu KH, Jeong JH, et al. Effect of a new prokinetic agent DA-9701 formulated with corydalis tuber and pharbitidis semen on cytochrome P450 and UDP-glucuronosyltransferase enzyme activities in human liver microsomes. Evid Based Complement Alternat Med 2012;2012:650718.
9. Jung JW, Kim JM, Jeong JS, et al. Pharmacokinetics of chlorogenic acid and corydaline in DA-9701, a new botanical gastroprokinetic agent, in rats. Xenobiotica 2014;44:635-643.
10. Kim ER, Min BH, Lee SO, Lee TH, Son M, Rhee PL. Effects of DA-9701, a novel prokinetic agent, on gastric accommodation in conscious dogs. J Gastroenterol Hepatol 2012;27:766-772.
11. Kim ER, Min BH, Lee TH, Son M, Rhee PL. Effect of DA-9701 on colorectal distension-induced visceral hypersensitivity in a rat model. Gut Liver 2014;8:388-393.
12. Lee SP, Lee KN, Lee OY, et al. Effects of DA-9701, a novel prokinetic agent, on phosphorylated extracellular signal-regulated kinase expression in the dorsal root ganglion and spinal cord induced by colorectal distension in rats. Gut Liver 2014;8:140-147.
13. Lee TH, Son M, Kim SY. Effects of corydaline from corydalis tuber on gastric motor function in an animal model. Biol Pharm Bull 2010;33:958-962.
14. Lim CH, Choi MG, Park H, Baeg MK, Park JM. Effect of DA-9701 on gastric emptying in a mouse model: assessment by ^{13}C-octanoic acid breath test. World J Gastroenterol 2013;19:4380-4385.
15. Min YW, Ko EJ, Lee JY, et al. Nitrergic pathway is the major mechanism for the effect of DA-9701 on the rat gastric fundus relaxation. J Neurogastroenterol Motil 2014;20:318-325.
16. Oh KH, Nam Y, Jeong JH, Kim IK, Sohn UD. The effect of DA-9701 on 5-hydroxytryptamine-induced contraction of feline esophageal smooth muscle cells. Molecules 2014;19:5135-5149.
17. Park CH, Kim HS, Lee SK. Effects of the new prokinetic agent DA-9701 formulated with corydalis tuber and pharbitis seed in patients with minimal change esophagitis: a bicenter, randomized, double blind, placebo-controlled study. J Neurogastroenterol Motil 2014;20:338-346.
18. Camilleri M, Papathanasopoulos A, Odunsi ST. Actions and therapeutic pathways of ghrelin for gastrointestinal disorders. Nat Rev Gastroenterol Hepatol 2009;6:343-352.
19. Delhanty PJ, Neggers SJ, van der Lely AJ. Mechanisms in endocrinology: ghrelin: the differences between acyl- and des-acyl ghrelin. Eur J Endocrinol 2012;167:601-608.
20. De Smet B, Mitselos A, Depoortere I. Motilin and ghrelin as prokinetic drug targets. Pharmacol Ther 2009;123:207-223.
21. Poitras P, Tomasetto C. The potential of ghrelin as a prokinetic. Regul Pept 2009;155:24-27.
22. Stengel A, Goebel-Stengel M, Wang L, et al. Abdominal surgery inhibits circulating acyl ghrelin and ghrelin-O-acyltransferase levels in rats: role of the somatostatin receptor subtype 2. Am J Physiol Gastrointest Liver Physiol 2011;301:G239-G248.
23. Stengel A, Taché Y. Ghrelin: new insight to mechanisms and treatment of postoperative gastric ileus. Curr Pharm Des 2011;17:1587-1593.
24. Maruna P, Gürlich R, Rosická M. Ghrelin as an acute-phase reactant during postoperative stress response. Horm Metab Res 2008;40:404-409.
25. Jeon TY, Lee S, Kim HH, et al. Changes in plasma ghrelin concentration immediately after gastrectomy in patients with early gastric cancer. J Clin Endocrinol Metab 2004;89:5392-5396.
26. Sibilia V, Lattuada N, Rapetti D, et al. Ghrelin inhibits inflammatory pain in rats: involvement of the opioid system. Neuropharmacology 2006;51:497-505.
27. Poitras P, Polvino WJ, Rocheleau B. Gastrokinetic effect of ghrelin analog

RC-1139 in the rat. Effect on post-operative and on morphine induced ileus. Peptides 2005;26:1598-1601.
28. Venkova K, Fraser G, Hoveyda HR, Greenwood-Van Meerveld B. Prokinetic effects of a new ghrelin receptor agonist TZP-101 in a rat model of postoperative ileus. Dig Dis Sci 2007;52:2241-2248.
29. Popescu I, Fleshner PR, Pezzullo JC, Charlton PA, Kosutic G, Senagore AJ. The ghrelin agonist TZP-101 for management of postoperative ileus after partial colectomy: a randomized, dose-ranging, placebo-controlled clinical trial. Dis Colon Rectum 2010;53:126-134.
30. Jung YS, Kim MY, Lee HS, Park SL, Lee KJ. Effect of DA-9701, a novel prokinetic agent, on stress-induced delayed gastric emptying and hormonal changes in rats. Neurogastroenterol Motil 2013;25:254-259, e166.
31. Ciechanownicz R, Sein-Anand J, Chodorowski Z, Bitel M, Petrusewicz J, Korolkiewicz RP. Salutary effects of tachykinin receptor antagonists in a rat model of postoperative ileus. J Surg Res 2006;133:197-202.
32. Huge A, Kreis ME, Jehle EC, et al. A model to investigate postoperative ileus with strain gauge transducers in awake rats. J Surg Res 1998;74:112-118.
33. De Winter BY, Boeckxstaens GE, De Man JG, Moreels TG, Herman AG, Pelckmans PA. Effect of adrenergic and nitrergic blockade on experimental ileus in rats. Br J Pharmacol 1997;120:464-468.
34. Goetz B, Benhaqi P, Müller MH, Kreis ME, Kasparek MS. Changes in beta-adrenergic neurotransmission during postoperative ileus in rat circular jejunal muscle. Neurogastroenterol Motil 2013;25:154-e84.
35. De Winter BY, Boeckxstaens GE, De Man JG, et al. Effect of different prokinetic agents and a novel enterokinetic agent on postoperative ileus in rats. Gut 1999;45:713-718.