Prokinetic effects of LD02GIFRO on functional gastrointestinal disorder in rats

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Abstract. LD02GIFRO is a novel prokinetic agent formulated with *Poncirus fructus* and *Zanthoxylum sp*. fruits. The aim of the present study was to evaluate the effect of LD02GIFRO on delayed gastrointestinal transit (GIT) and colorectal hypersensitivity. To investigate the effect of LD02GIFRO, a rat model of delayed GIT was induced via three mechanisms; postoperative ileus (POI), morphine, and POI plus morphine. Visceromotor responses (VMR) to colorectal distension (CRD) were also evaluated. POI was induced by laparotomy surgery and manipulation of the small intestine under anesthesia, and GIT was calculated by measuring the length that Evans Blue travelled through the gastrointestinal tract in a given time. Oral administration of 260 mg/kg LD02GIFRO caused Evans Blue to migrate significantly further in the delayed GIT models induced by POI, morphine and POI plus morphine compared with the control ($P<0.05$). This effect was inhibited by atropine, a muscarinic receptor antagonist, and completely abolished by GR125487, a 5-HT_4-receptor antagonist. Furthermore, intraperitoneal administration of 600 and 900 mg/kg LD02GIFRO significantly reduced VMR to CRD in acute and chronic colorectal hypersensitive rat models, induced by acetic acid and trinitrobenzenesulfonic acid, to almost normal levels ($P<0.01$). In the present study, LD02GIFRO successfully ameliorated delayed GIT models and colorectal hypersensitivity models, suggesting that LD02GIFRO may be an effective therapeutic treatment for patients with functional gastrointestinal disorders and abnormalities in GIT.

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Introduction

Functional gastrointestinal disorder (FGID) is one of the most common gastrointestinal disorders in humans, encompassing functional dyspepsia and irritable bowel syndrome (IBS) (1). The pathophysiology of FGID is complicated and there are various factors to consider, including abnormal motility, visceral hypersensitivity, psychological factors, and disturbed brain-gut interaction (1). Disturbed digestive motility is often responsible for the manifestation of gastrointestinal symptoms in various diseases (2).

Postoperative ileus (POI) typically occurs following abdominal surgery and is characterized by a transient hypomotility of the gastrointestine, which may prolong hospital stays and increase mortality (3,4). Surgical stress stimulates the release of endogenous opioids, resulting in impaired post-surgical gastrointestinal function (5,6). Previous studies have suggested μ-opioid receptor antagonists, cholinergic agonists or 5-HT_4 receptor agonists as targets for the development of a drug to improve management of POI (7,8).

IBS is considered to be a functional gastrointestinal disorder characterized by chronic abdominal pain and discomfort associated with alteration in bowel habits in the absence of demonstrable pathology (9). Evidence suggests that increased visceral sensitivity is primarily responsible for the manifestation of symptoms of IBS (10). At present, it has been suggested that abnormal interactions between normal and disordered motility or visceral hypersensitivity may be responsible for IBS symptoms (11,12).

Prokinetic drugs are commonly prescribed for gastrointestinal disorders in humans (13). However, two gastroprokinetic agents, domperidone and tegaserod, are associated with serious side effects (14,15), and the efficacy data on two others, mosapride and itopride, are conflicting (16,17). Therefore, there is a requirement for safer and more effective gastroprokinetic agents to be developed. LD02GIFRO is a novel prokinetic agent obtained from extracts of *Poncirus fructus* (PF) and *Zanthoxylum* sp. fruits (ZF), both of which have been used in Asian traditional medicine for the treatment of gastrointestinal disorders. PF has been used as a traditional medicine for the treatment of abnormal gastrointestinal motility and gastric

secretion (18-20). ZF, which belongs to the Rutaceae family, has been used as a traditional medicine to alleviate stomach pain, diarrhea, and jaundice (21,22). Additionally, ZF extracts have been demonstrated to have a prokinetic effect that is able to ameliorate delayed gastrointestinal transit (GIT) in rats, and have been reported to stimulate duodenal and ileal motility (23,24). Based on these traditional herbal medicinal theories and clinical records, PF and ZF were selected for use in the present study.

Materials and methods

Preparation of LD02GIFRO. LD02GIFRO is the standardized extract of two herbs. PF is the immature fruit of *Poncirus trifoliate*, and *Zanthoxyli fructus* is the pericarp of *Zanthoxylum piperitum*. These herbs were purchased at a Chinese herb medicine shop in Kyung-dong Market (Seoul, South Korea) and washed with distilled water to remove adulterations prior to use. A total of 100 g of dried *Ponciri fructus* was added to 800 ml of 70% ethanol and 250 g of dried *Zanthoxyli fructus* was added to 1,750 ml of 30% ethanol. The herbs were subsequently extracted at room temperature for 24 h, concentrated at 55-65˚C under 25.5 MPa pressure and dried to obtain the solvent extracts of *Ponciri fructus* and *Zanthoxyli fructus*. The 70% ethanol extract of *Ponciri fructus* and 30% ethanol extract of *Zanthoxyli fructus* were mixed at a ratio of 1:12 by weight to prepare the complex extracts of *Ponciri fructus* and *Zanthoxyli fructus*.

Chemicals. Atropine sulfate, mosapride, phenol red, Evans Blue and trinitrobenzenesulfonic acid (TNBS) were purchased from Sigma-Aldrich (Merck Millipore, Darmstadt, Germany). Acetic acid was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). Alvimopan and morphine were purchased from GlaxoSmithKline PLC (Brentford, UK) and BC World Pharm Co., Ltd. (Seoul, Korea), respectively. GR125487 was purchased from Tocris Bioscience (Bristol, UK).

Experimental animals. A total of 296 male Sprague-Dawley rats (age, 6 weeks; weight, 180-200 g) were obtained from Orient Bio, Inc. (Gyeonggi, Korea). Animal care and experimental procedures were conducted in accordance with the approval and guidelines of the Institutional Animal Care and Use Committee of the Medical School of Inha University (Incheon, Korea; approval ID, INHA 140618-302, INHA 141126-340). Rats were provided standard rat chow and tap water *ad libitum* and were maintained at room temperature (25-30˚C) and at 45-55% relative humidity in a 12-h light/dark cycle. Rats were fasted for 24 h prior to experiments, with *ad libitum* access to water. Rats were randomly distributed into experimental groups (37 groups; n=8 per group).

Surgical preparation. Rats were anesthetized via intraperitoneal injection of 100 mg/kg ketamine (Yuhan Co., Ltd., Seoul, Korea) and 4.4 mg/kg xylazine (Bayer AG, Leverkusen, Germany). Teflon-coated electrodes (A-M Systems, Inc., Sequim, WA, USA) were implanted in the external oblique muscles and externalized to the back of the neck to record electromyography (EMG). The experiment was initiated three days post-surgery.

POI. POI was induced according to the previously reported method (25). Control and treatment groups were anesthetized with 3% isoflurane (Hana Pharm Co., Ltd., Seoul, Korea) and the abdomen was opened via a 2-cm midline laparotomy. The small intestine and caecum were gently pulled out of the abdominal cavity and the small intestine was gently manipulated with the fingers for 5 min. Following manipulation, the small intestine and caecum were replaced in the abdominal cavity, and the surgical wound was sutured. The normal group underwent isoflurane anesthesia only.

GIT. To study the effects of POI on GIT, 0.2 ml Evans Blue (50 mg/ml) in saline was orally administered to each rat 4 h post-operatively. Rats (n=8 per group) were sacrificed under 100 mg/kg ketamine (Yuhan Co., Ltd.) and 4.4 mg/kg xylazine (Bayer AG) anesthesia 20 min after the administration of Evans Blue, and the rate of GIT was calculated by dividing the distance of Evans Blue migration by the total length of the small intestine.

Study 1: In the POI model group, LD02GIFRO (65, 130 or 260 mg/kg) or alvimopan (10 mg/kg) was orally administered 30 min prior to Evans Blue. Control and normal rats were orally administered the same volume (5 ml/kg) of vehicle (5% Pluronic F-68; Sigma-Aldrich; Merck Millipore). Rats were randomly distributed into six groups (n=8 per group): Normal group, 3% isoflurane anesthesia + vehicle; control group, POI + vehicle; LD02GIFRO groups (65, 130 or 260 mg/kg), POI + LD02GIFRO; and alvimopan group, POI + alvimopan.

Study 2: To investigate whether LD02GIFRO was able to ameliorate delayed GIT induced by morphine (3 mg/kg), LD02GIFRO was administered 30 min after a subcutaneous (s.c.) injection of morphine (3 mg/kg; BC World Pharm Co., Ltd.). Rats were randomly distributed into six groups (n=8 per group): Normal group, 3% isoflurane anesthesia + vehicle; control group, POI + vehicle; LD02GIFRO groups (65, 130 and 260 mg/kg), POI + LD02GIFRO; and alvimopan group, POI + alvimopan.

Study 3: As presurgical treatment, LD02GIFRO was administered 30 min prior to morphine (1 mg/kg, s.c.), which was in turn administered 15 min prior to the intestinal manipulation to induce POI. Two h post-surgery, Evans Blue was orally administered, and rats were deeply anesthetized 20 min later under 100 mg/kg ketamine (Yuhan Co., Ltd.) and 4.4 mg/kg xylazine (Bayer AG) anesthesia. Subsequently, the abdominal aorta was transected to exsanguinate the rats. Rats were randomly distributed into five groups (n=8 per group): Normal group, 3% isoflurane anesthesia + vehicle; control group: vehicle + morphine + POI; LD02GIFRO groups (130 and 260 mg/kg): LD02GIFRO + morphine + POI; and alvimopan group, alvimopan + morphine + POI.

Study 4: As postsurgical treatment, morphine (1 mg/kg, s.c.) was administered 15 min prior to POI surgery, and LD02GIFRO was administered 30 min post-surgery. At 2 h following the induction of POI, rats were administered with Evans Blue by gavage, and were sacrificed 20 min later. Rats were randomly distributed into five groups (n=8 per group): Normal group, 3%

isoflurane anesthesia + vehicle; control group, morphine + POI + vehicle; LD02GIFRO groups (130 and 260 mg/kg), morphine + POI + LD02GIFRO; and alvimopan group, morphine + POI + alvimopan.

Study 5: To investigate whether cholinergic or serotonergic receptors mediate the stimulatory effect of LD02GIFRO on POI, rats were administered with saline (1 ml/kg), atropine (2 mg/kg) or GR125487 (a 5-HT_4 receptor antagonist; 2 mg/kg) via s.c. injection 15 min prior to LD02GIFRO administration. The group that underwent both surgical and morphine treatment was designed to mimic the clinical situation of patients receiving opioids following abdominal surgery. Rats were randomly distributed into five groups (n=8 per group): Normal group, 3% isoflurane anesthesia + saline + vehicle; control group, POI + saline + vehicle; saline group, POI + saline + LD02GIFRO; atropine group: POI + atropine + LD02GIFRO; and GR125487 group, POI + GR125487 + LD02GIFRO.

Visceromotor responses (VMR) to colorectal distension (CRD). Rats were anesthetized with 3% isoflurane and a barostat balloon (Mui Scientific, Mississauga, ON, Canada) was subsequently inserted into the distal colon and held in place by taping the balloon catheter to the base of the tail. The catheter was attached to a programmable rigid piston barostat with zero intrinsic compliance (Distender Series II; G&J Electronics, Inc., Toronto, ON, Canada). The Teflon coated silver wire was connected to an alternating current (AC) amplifier system (Grass Instrument Co., West Warwick, RI, USA). The AC amplifier was connected to data acquisition equipment and data were analyzed using Powerlab 8/35 (PL3508; AD Instruments, Colorado Springs, CO, USA). The rats were left for 30 min to recover completely. The stimulus-response of the external oblique muscle during CRD was recorded with computerized signals in the holding cage. The CRD procedure consisted of two series of CRDs for 3 min at constant pressures of 15, 30, 45, 60 and 80 mmHg, separated by 3-min intervals without distension. The stimulus-response during each distension period was expressed as the integral.

Colonic hypersensitivity. Study 6: Acute colonic hypersensitivity was induced as described by Langlois *et al* (26) and Plourde *et al* (27). Briefly, male SD rats (300-400 g) were fasted overnight, anesthetized with 3% isoflurane and administered with an intracolonic infusion of dilute acetic acid (0.6%; 1.5 ml). Rats were randomly distributed into five groups (n=8, each group): 0.6% AA group (0.6% acetic acid + vehicle), 0.6% AA + LD02GIFRO groups (300, 600, 900 mg/kg); Normal control group: saline + vehicle.

Study 7: Chronic hypersensitivity was induced as described by Greenwood *et al* (28). Briefly, male SD rats (180-200 g) were anesthetized with 3% isoflurane and administered with an intracolonic infusion of TNBS (50 mg/kg; 0.5 ml in 25% ethanol). The experiment was initiated 30 days after infusion. Rats were randomly distributed into five groups (n=8, each group): 50 mg/kg TNBS group (50 mg/kg TNBS + vehicle), 50 mg/kg TNBS + LD02GIFRO groups (300, 600, 900 mg/kg); Normal control group: 25% ethanol + vehicle.

Experimental design and drug treatment of colonic hypersensitivity. Experiments were designed to examine the effect of LD02GIFRO on acute acetic acid-induced colonic sensitivity and postinflammatory rat models. The VMRs to an initial series of CRDs were recorded 10 min prior to the administration of LD02GIFRO or the vehicle. A second series of CRDs was performed 10 min after intraperitoneal administration of LD02GIFRO (300, 600 or 900 mg/kg) or vehicle (50% propylene glycol) and the VMRs recorded.

Data analysis and statistics. All values are expressed as the mean ± standard error of the mean. Differences among groups were examined using a Mann-Whitney U test. $P<0.05$ was considered to indicate a statistically significant difference. Data analyses were performed using SPSS software (version 19.0; IBM SPSS, Armonk, NY, USA). Abdominal muscular contractions in response to CRD, were monitored via EMG recording to determine the response to visceral pain. The degree of EMG response was positively correlated to the CRD volume. The EMG response was calculated as follows: Integral of EMG activity during CRD/80 mmHg control EMG response x100. Responses were measured for two consecutive series of CRD at 15, 30, 45, 60 and 80 mmHg.

Results

Effects of LD01GIFRO on GIT. The aim of the present study was to investigate whether LD02GIFRO was capable of restoring delayed GIT. The rate of GIT was significantly reduced in rats post-surgery ($P<0.01$); however, administration of ≥65 mg/kg LD02GIFRO was able to ameliorate the delayed GIT, and a significant improvement was observed in the rats treated with 260 mg/kg ($P<0.05$; Fig. 1A). Furthermore, administration of 10 mg/kg morphine significantly decreased the GIT compared with the normal group ($P<0.05$). The maximum effect was presented at the dose of 260 mg/kg, with GIT of 60.7±3.5% ($P<0.05$; Fig. 1B). These results suggest that LD02GIFRO is able to accelerate GIT in rats with delayed GIT induced by laparotomy and morphine administration.

Pre-treatment with LD02GIFRO for morphine and POI induced delayed GIT. Rats were administered with LD02GIFRO 30 min prior to 1 mg/kg morphine injection (1 mg/kg, s.c.), which was administered 15 min prior to mechanical stimulation. GIT was significantly decreased in the control group with presurgical treatment for vehicle and morphine rats (Fig. 1C; $P<0.01$). However, administration of LD02GIFRO increased GIT by 32.7±9.3 and 30.2±5.7% at the doses of 130 and 260 mg/kg, respectively. Notably, treatment with 260 mg/kg LD02GIFRO significantly restored delayed GIT ($P<0.01$), whereas 10 mg/kg alvimopan had no significant effect on GIT.

Post-treatment with LD02GIFRO for morphine and POI induced delayed GIT. Rats were treated with 1 mg/kg morphine 15 min prior to surgery and LD02GIFRO was administered 45 min post-surgery. As demonstrated in Fig. 1D, GIT was significantly diminished in the control rats (30.5±3.3%; $P<0.01$); however, LD02GIFRO significantly improved GIT at the dose of 260 mg/kg compared with the controls ($P<0.05$). Treatment with alvimopan also induced a significant increase

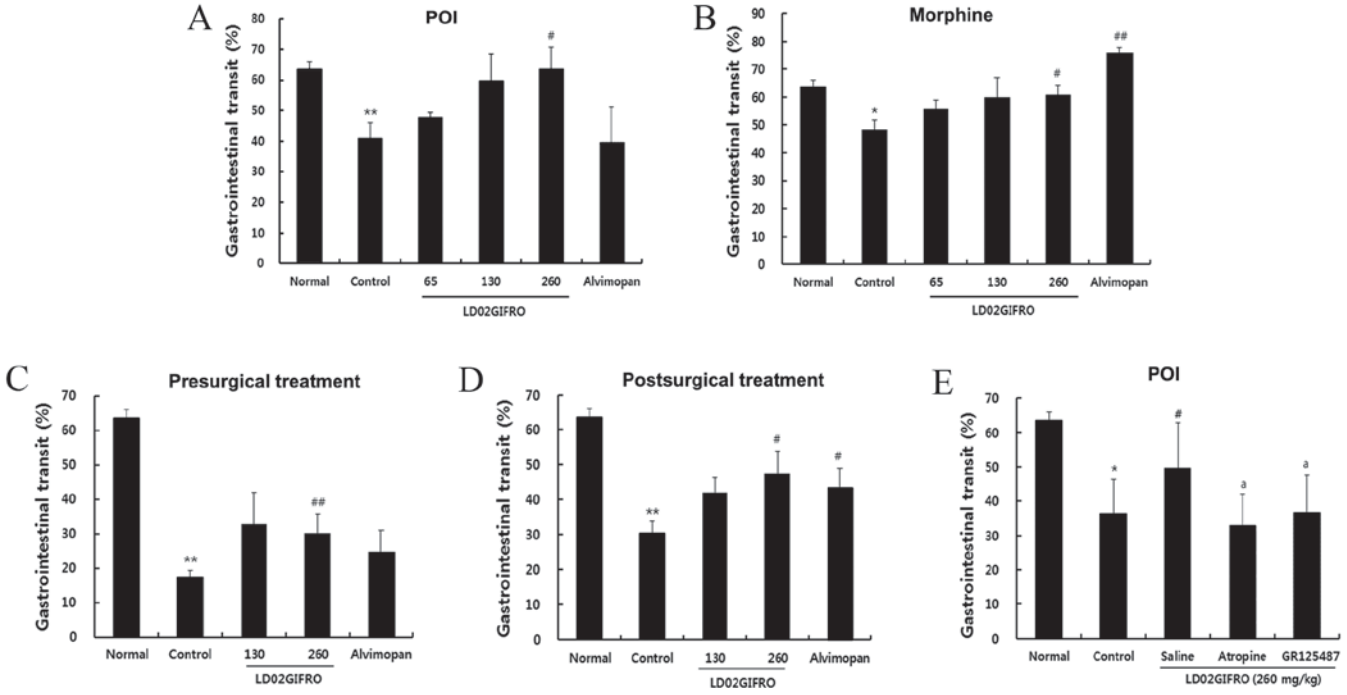

Figure 1. Effects of LD02GIFRO on GIT in rats. Following 24 h fasting, rats (n=8 each group) were orally administered with LD02GIFRO at indicated doses in combination with the following; alvimopan (10 mg/kg), atropine (2 mg/kg), GR125487 (2 mg/kg) and 5% Pluronic F-68 as a vehicle (control). GIT in (A) POI-induced rats, (B) morphine-induced rats, (C) in presurgical treatment with morphine rats, (D) in postsurgical treatment with morphine rats, and (E) in POI-induced rats with atropine and GR125487 treatment. Values are expressed as the mean ± standard error of the mean. *P<0.05 and **P<0.01 vs. normal group. $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. control group ^{a}P<0.05 vs. saline control group. GIT, gastrointestinal transit; POI, post-operative ileus.

in GIT rate compared with the controls (P<0.05). As a result of these findings, all subsequent experiments were performed using LD02GIFRO at a dosage of 260 mg/kg.

Influence of atropine and GR125487 on the effect of LD02GIFRO on delayed GIT in POI. GIT was markedly delayed in rats with POI compared with normal rats (P<0.05; Fig. 1E). Administration of 260 mg/kg LD02GIFRO significantly ameliorated the delayed GIT (P<0.05); however, this amelioration was prevented by co-administration of 2 mg/kg atropine, a muscarine-receptor antagonist (32.9±9.1%; P<0.05) or GR125487 (2 mg/kg), a 5-HT_4 receptor antagonist (49.2±5.7%; P<0.05).

Effects of LD02GIFRO in rats with acute colonic hypersensitivity. Acute colonic hypersensitivity in rats induced by intra-colorectal infusion of 0.6% acetic acid was assessed by comparing the exaggerated VMR to graded pressure of CRD with the control rats (Fig. 2A-C). Rats with acute colonic hypersensitivity developed significant visceral hyperalgesia compared with the control group (P<0.01); this hypersensitivity was evident for all CRD pressures (Fig. 2C). To investigate whether LD02GIFRO had an inhibitory effect on the VMR in rats with hypersensitivity to colonic stimulation, the rats were treated with LD02GIFRO (300, 600 or 900 mg/kg). The vehicle for LD02GIFRO, propylene glycol, had no significant effect on the VMR of acetic acid-treated rats (Fig. 2A). In contrast, when rats were administered with LD02GIFRO, the VMR level decreased in a dose-dependent manner. Rats treated with 300 mg/kg, LD02GIFRO had a significantly lower VMR at CRD of 80 mmHg compared with the acetic acid-treated response (P<0.05). At doses of 600 mg/kg the VMR was significantly reduced at all CRD pressures to a level similar to the corresponding control rats (P<0.01). Notably, the highest dose of 900 mg/kg induced a significantly greater reduction in VMR level at all distension pressures, with responses being reduced below the VMR level recorded in normal control rats (P<0.01; Fig. 2C). Fig. 2B demonstrates a representative EMG response during the distension period to pressures from 15-80 mmHg of vehicle, acetic acid-induced and following LD02GIFRO.

Effects of LD02GIFRO in rats with postinflammatory colonic hypersensitivity. It was investigated whether postinflammatory colonic hypersensitivity may be verified by recording electrical activity in response to CRD in rats (Fig. 3A-C). Rats developed significant visceral hyperalgesia compared with the control 30 days following the induction of TNBS colitis (P<0.01; Fig. 3C). Hypersensitive rats were treated with LD02GIFRO or vehicle. The vehicle had no significant effect on the VMR at any CRD pressures (Fig. 3A), whereas LD02GIFRO (300, 600 or 900 mg/kg) suppressed the VMR levels. At a dosage of 600 or 900 mg/kg, the VMR induced by all distension pressures were significantly reduced to a VMR level below or similar to that recorded at pressures in normal control rats (P<0.01; Fig. 3C). A representative EMG response to pressures from 15 to 80 mmHg during a distension period in each treatment group is displayed in Fig. 3B.

Discussion

FGID is one of the most prevalent gastrointestinal disorders in humans (1). Several herbal medicinal products have been

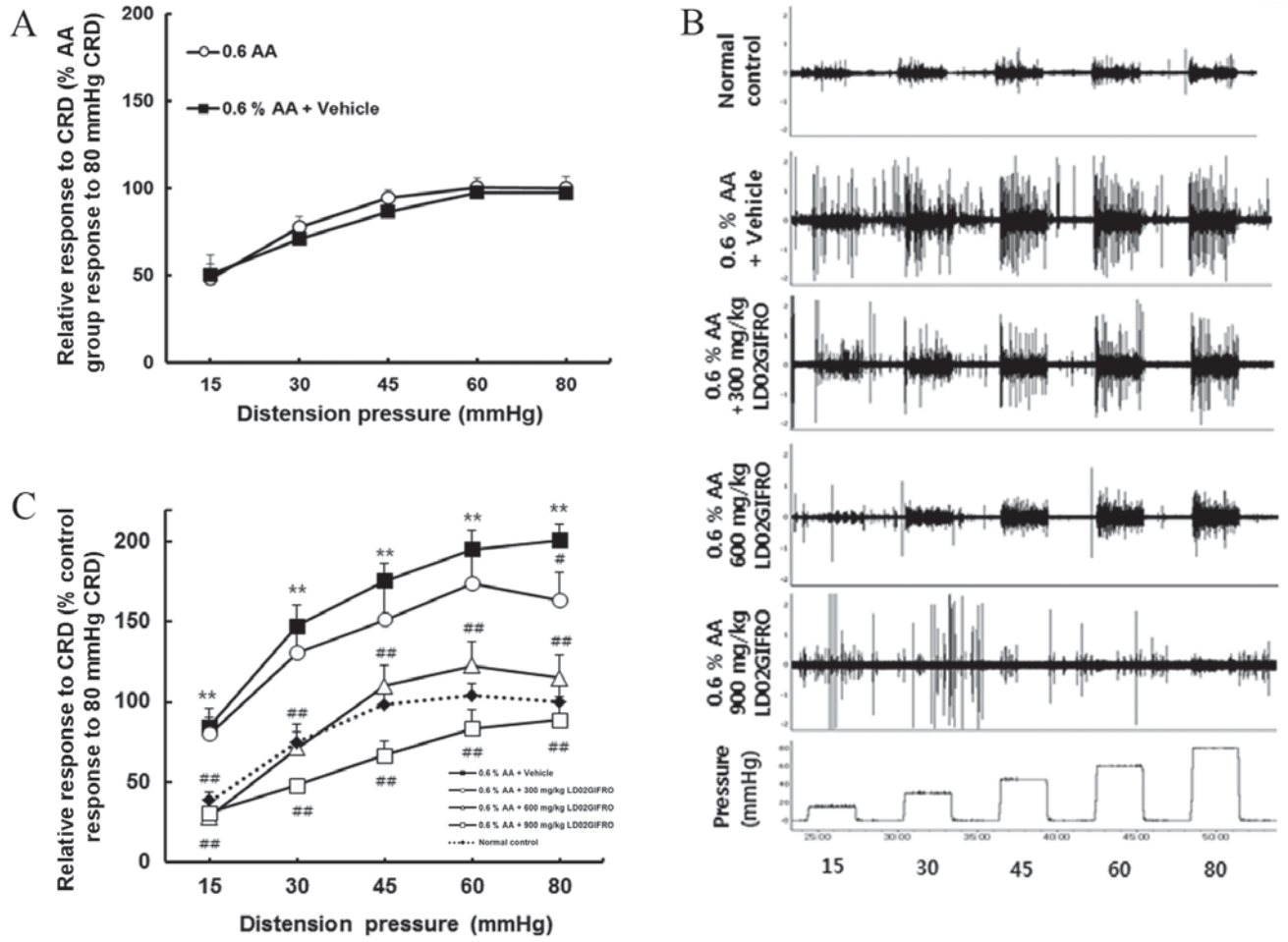

Figure 2. Effect of LD02GIFRO on VMR to CRD in rats with acute hypersensitivity. (A) VMR to CRD in rats with 0 AA-induced colonic hypersensitivity (n=8). (B) VMR to CRD represented electromyography. Values are presented as the mean ± standard error of the mean. (C) Effect of LD02GIFRO on VMR to CRD in rats with AA-induced colonic hypersensitivity (n=8).**P<0.01 vs. normal control. $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. 0.6% AA plus vehicle. VMR, visceromotor response; CRD, colorectal distension; PG, propylene glycol; AA, acetic acid.

assessed to determine whether they may be used as an effective therapeutic treatment for patients with FGID (29). For example, a Chinese herbal medicine, known as Rikkunshi-to and TJ-43, has been reported to enhance gastric emptying (30) and gastric adaptive relaxation (31). It has been demonstrated that daiken-chuto, a Japanese herbal medicine comprised of four herbs, is able to ameliorate morphine-induced inhibition of intestinal and colonic transit (32). However, the optimal therapeutic strategy for FGID remains to be determined due to the poorly defined pathogenesis of the condition. In the present study, the prokinetic agent LD02GIFRO was developed and its pharmacological effects evaluated. The results suggest that LD02GIFRO may have a prominent effect on gastrointestinal motility by ameliorating GIT and reducing visceral hypersensitivity.

Various mechanisms have been proposed for the pathogenesis of POI, although there is not yet a clear consensus. Experimental and clinical ileus has been demonstrated to be associated with the degree of surgical manipulation performed (33), suggesting that surgical manipulation of the intestine serves an important role in mediating POI (34,35). Additionally, a typical factor that contributes to post-surgical gastrointestinal dysregulation is the use of opioids for pain management during the peri- and post-operative periods (36). Typically, morphine inhibits relaxation of the circular muscle and contraction of the longitudinal muscle, resulting in delayed GIT (37). In the present study, treatment with 260 mg/kg LD02GIFRO was demonstrated to significantly improve POI-induced delayed GIT. Morphine-induced delayed GIT was also eliminated by administration of 260 mg/kg LD02GIFRO.

Differences in the stimulatory effect were observed between pre- and post-treatment of LD02GIFRO with morphine and POI. GIT significantly improved with pre- and post-treatment of LD02GIFRO, whereas administration of alvimopan had a significant effect only when POI was accompanied with morphine.

Neural reflex pathways have previously been implicated in postoperative dysmotility; cholinergic agonists and 5-HT_4 receptor agonists are able to ameliorate postoperative GIT dysmotility (7,38), suggesting that activation of the cholinergic nervous system or 5-HT_4 receptor agonist improves dysmotility in POI. LD02GIFRO ameliorated the hypoperistalsis from POI, which was completely inhibited by treatment with atropine or GR125487. Therefore, the cholinergic nerves and 5-HT_4 receptors may be involved in this action of LD02GIFRO. Based on these results, LD02GIFRO may be suggested as a superior treatment to conventional therapeutics, particularly for GIT in abnormal conditions.

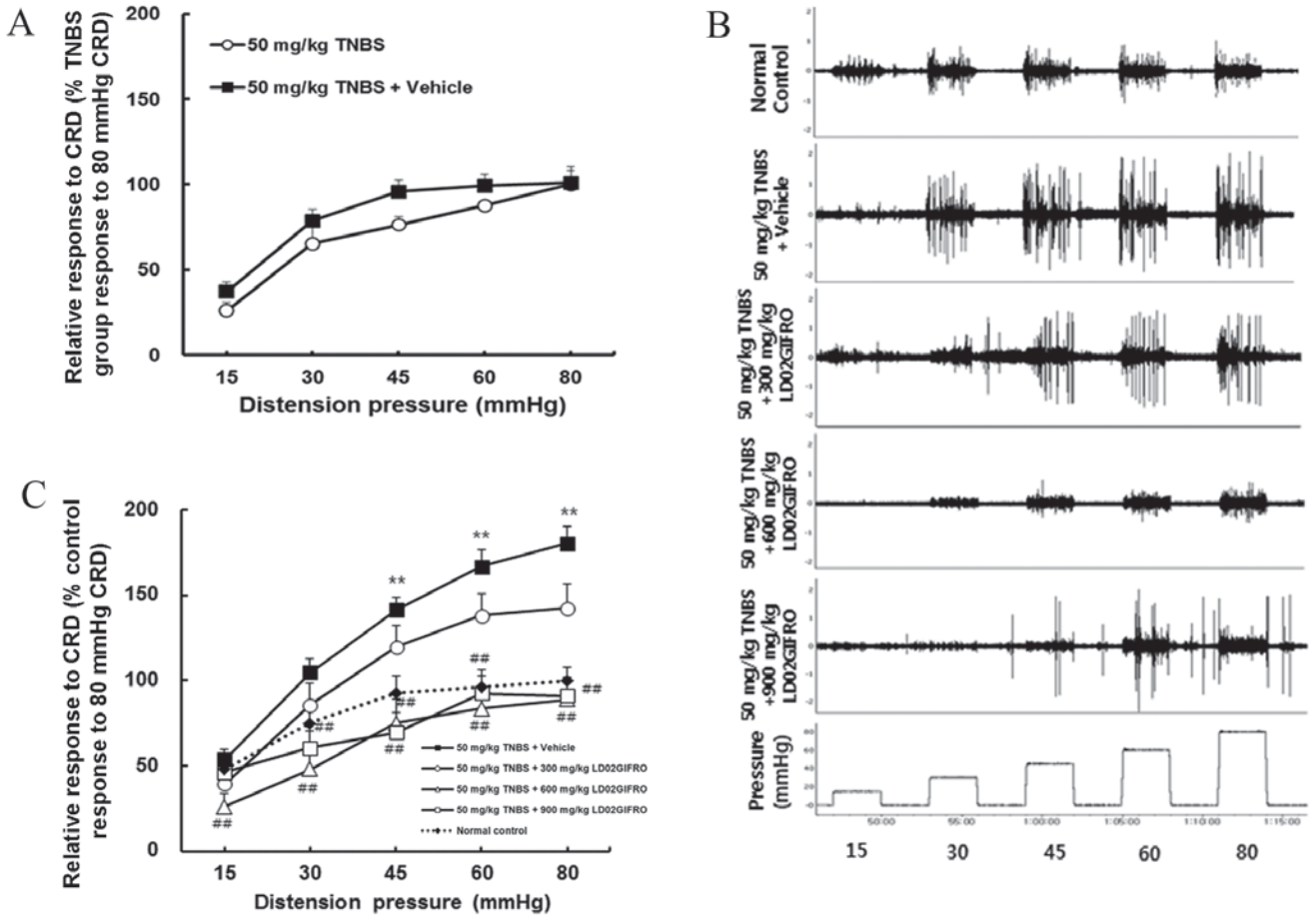

Figure 3. Effect of LD02GIFRO on VMR to CRD in rats with chronic hypersensitivity. (A) VMR to CRD in rats with TNBS-induced colonic hypersensitivity (n=8). (B) VMR to CRD represented as electromyography activity. (C) Effect of LD02GIFRO on VMR to CRD in rats with TNBS-induced colonic hypersensitivity (n=8). Values are presented as the mean ± standard error of the mean. **P<0.01 vs. normal control (vehicle). $^{\#\#}$P<0.01 vs. 50 mg/kg TNBS plus vehicle. VMR, visceromotor response; CRD, colorectal distension; TNBS, trinitrobenzenesulfonic acid; PG, propylene glycol.

Several studies have demonstrated that 5-HT has a pivotal role in the control of gastrointestinal motility and visceral sensations (39-41). Visceral hypersensitivity may be induced by abnormal afferent nervous function or central integration (42) and may be associated with digestive dysmotility (43,44). Several visceral hypersensitivity models have been investigated, including that induced by acetic acid. In this model, mucosal inflammation was induced via mucosal exposure to acetic acid, inducing visceral hypersensitivity to rectal distension, which was suggested to be due to the higher degranulation rate of mast cells in the mucosa (45,46). In addition, another study previously reported that postinflammatory visceral hypersensitivity was induced 30 days following infusion of TNBS (28). In the present study, LD02GIFRO was able to significantly ameliorate VMR to CRD in the acetic acid- and TNBS-induced hypersensitivity rat model.

PF exerts its prokinetic activity via influencing the 5-HT_3 and 5-HT_4 receptor mediated pathways (47,48). In addition, ZF, has been reported to ameliorate delayed GIT as an agonist of cholinergic nerves and the 5-HT_4 receptor (22,23), which was confirmed in the present study using LD02GIFRO.

In conclusion, the results of the present study demonstrate that LD02GIFRO may have potential as an effective prokinetic agent capable of ameliorating gastrointestinal disorders and for an enhanced quality of life in patients with FGID.

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