Enhancement by KW-5092, a Novel Gastroprokinetic Agent, of the Gastrointestinal Motor Activity in Dogs

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ABSTRACT—KW-5092 (\(1\)-[2-[\(5\)-(piperidinomethyl)-2-furanylmethyl]amino\]ethyl\]-2-imidazolidinylidene propanedinitrile fumarate) is a novel gastroprokinetic agent with acetylcholinesterase (AChE) inhibitory activity and acetylcholine (ACh) release facilitatory activity. The present study examined the effects of KW-5092 on gastrointestinal (GI) motor activity in dogs. In anesthetized dogs, KW-5092 at 0.03 to 1 mg/kg, i.v. dose-dependently enhanced the gastric antral and the colonic motor activity. Neostigmine, an AChE inhibitor, enhanced the motor activity at 0.03 and 0.1 mg/kg, i.v. Ranitidine, a histamine H2-receptor antagonist with AChE inhibitory activity and ACh release facilitatory activity, enhanced the motor activity but decreased blood pressure at 1 to 10 mg/kg, i.v. In conscious dogs, KW-5092 at 0.03 to 1 mg/kg, i.v. or 1 to 10 mg/kg, p.o. dose-dependently enhanced the gastric antral, duodenal, ileal and the colonic motor activities. Neostigmine at 0.1 mg/kg, i.v. or 3 mg/kg, p.o. enhanced the duodenal, ileal and colonic motor activities, but induced excitement, slavering, vomiting and diarrhea. Ranitidine at 3 mg/kg, i.v. enhanced the gastric antral and colonic motor activities, but induced collapse or akinesia. The present results suggest that KW-5092 enhances the GI motor activity in a wide range from the gastric antrum to the colon and does not induce behavioral and cardiovascular side effects. KW-5092 may be a useful drug for the treatment of GI motility dysfunctions.

Keywords: KW-5092, Neostigmine, Ranitidine, Gastrointestinal motility, Choline esterase inhibitor

Dysfunctions of gastrointestinal (GI) motility associated with gastroparesis, gastric stasis, postoperative ileus or gastroesophageal reflux disease have been treated effectively with gastroprokinetic agents, including domperidone (1), metoclopramide (2) and cisapride (3). These agents are known to exert gastroprokinetic effects via different mechanisms of action. Domperidone enhances gastrointestinal coordination via blockade of a specific dopamine receptor (1). Metoclopramide exerts the gastroprokinetic effect via an antidopaminergic property and/or an increase in acetylcholine (ACh) release from postganglionic nerve endings (2). On the other hand, cisapride enhances ACh release without affecting dopamine receptors (3). KW-5092 (\(1\)-[2-[\(5\)-(piperidinomethyl)-2-furanylmethyl]amino\]ethyl\]-2-imidazolidinylidene propanedinitrile fumarate) is a newly synthesized gastroprokinetic agent that enhances GI motility in anesthetized rabbits in a dose-dependent manner (1–10 mg/kg, i.v.) (4). In the in vitro study examining the effect on the AChE inhibitory activity of the enzyme derived from rat brain, KW-5092 was a potent AChE inhibitor, whose inhibitory activity was equipotent to that of neostigmine and more potent than that of ranitidine (4). In the in vitro study examining the ACh release facilitatory activity in the isolated longitudinal muscle-myenteric plexus preparation of the guinea pig ileum, KW-5092 concentration-dependently enhanced ACh release at 10 nM to 3 \(\mu\)M in the electrically stimulated preparation and at 10 nM to 100 \(\mu\)M in the unstimulated preparation (5). These results suggest that KW-5092 stimulates GI motility through the AChE inhibition and the ACh release facilitation. Ranitidine, a histamine H2-receptor antagonist, also concentration-dependently enhanced ACh release at higher concentrations of 30 \(\mu\)M to 300 \(\mu\)M in the electrically stimulated preparation (6). KW-5092 is a ranitidine derivative with a negligible histamine H2-receptor blocking activity (H. Nonaka et al., unpublished observation). At up to 10 mg/kg, p.o., KW-5092 did not significantly affect gastric acid
secretion in the pylorus-ligated rat (T. Yokoyama et al., unpublished observation). GI motor activity in conscious dogs can be divided into two main patterns (7): the interdigestive state in which the interdigestive migrating contractions occur at regular intervals in the stomach and migrate through the small intestine to the terminal ileum and the digestive state that is characterized by irregular contractile activity. Similarly, these patterns of GI motor activity are observed in humans (8).

In the present study, we investigated the effects of KW-5092 on the GI motor activity in anesthetized dogs and in conscious dogs in the digestive state and compared them with those of neostigmine and ranitidine. Moreover, the cardiovascular and the behavioral effect were also determined, and the implications were discussed.

MATERIALS AND METHODS

Drugs
KW-5092 and ranitidine were synthesized in our laboratories. Atropine sulfate was purchased from Nacalai Tesque, Inc. (Kyoto). Neostigmine methyl sulfate was purchased from Sigma Chemical Co. (St. Louis, MO, USA). When test drugs were given i.v., they were dissolved in saline and administered to dogs at a volume of 0.2 ml/kg. When test drugs were given p.o., they were enclosed in gelatin capsules and orally administered to dogs.

Preparation of animals
The GI motor activity in anesthetized dogs was determined with a modification of the reported procedure (9). Seventeen mongrel dogs of both sexes weighing 8.0 to 17 kg were anesthetized with sodium pentobarbital (Nembutal, 25 mg/kg, i.v.; Abbott Laboratories, Chicago, IL, USA) and the abdominal cavity was opened. Rubber balloons were inserted into the gastric antrum and the colon. For the measurement of respiration, a glass tube was placed into the trachea. For the measurement of blood pressure, a polyethylene tube was placed into the femoral artery. Each test drug was administered as a slow (10 sec) i.v. bolus injection through the tube placed into the cephalic antebrachium vein. To measure the motor activity quantitatively, the motor index was calculated by integrating the area between the baseline and the contractile wave. When the intragastrointestinal pressure was over 20 cmH2O, it was regarded as 20 cmH2O and calculated.

The experiments using conscious dogs were performed about 2 weeks after the operation. GI motor activity was recorded on a polygraph (WR3701-8L; Graphtech, Tokyo) by connecting the lead wires of the transducers to the connecting cables and the amplifiers (FS-08M, Star Medical). The data on each parameter were simultaneously stored in a visceral motor activity analyzing system (ESC-800, Star Medical). Each test drug was administered as a slow (10 sec) i.v. bolus injection into the cephalic antebrachium or administered p.o. about 2 hr after feeding. To measure the motor activity quantitatively, the motor index was calculated, using the visceral motor activity analyzing system, by integrating the area between the baseline and the contractile wave.

Statistical analyses
All data are represented as means±S.E.M. Statistical significance was estimated by the Sign-Wilcoxon test or paired t-test. A P-value of less than 0.05 was considered statistically significant.
Fig. 1. Typical tracings of the effects of KW-5092 (A), neostigmine (B) and ranitidine (C) on the motor activity of gastric antrum and colon, blood pressure and respiration in anesthetized dogs. KW-5092, neostigmine or ranitidine was intravenously administered at the point indicated by the arrow.
RESULTS

Effects of KW-5092 given i.v. on the GI motor activity in anesthetized dogs

Figure 1A shows the typical tracings of the effects of KW-5092, and Fig. 2 summarizes the dose-response relationship. The motor index of the gastric antrum and the colon for the 10-min period before the administration of KW-5092 were 23.9±1.85 cmH2O·min and 9.75±1.38 cmH2O·min (mean±S.E.M., n=20), respectively. KW-5092 at 0.03 to 1 mg/kg, i.v. dose-dependently and significantly enhanced the gastric antral and colonic motor activities (Fig. 2), and did not affect respiration or

Fig. 2. Effects of KW-5092 (■: 0.03 mg/kg, i.v., ■: 0.1 mg/kg, i.v., ■: 0.3 mg/kg, i.v., ■: 1 mg/kg, i.v.) on the motor activity of gastric antrum and colon in anesthetized dogs. The motor index for the 10-min period after intravenous administration of KW-5092 is expressed as a percentage of that for the 10-min period before the administration. Each point represents the mean±S.E.M. of 5 dogs. *P<0.05, compared with the value in the 10-min period before the administration (Sign-Wilcoxon test).

Fig. 3. Effects of neostigmine (A: ■, 0.03 mg/kg, i.v.; ■, 0.1 mg/kg, i.v.; ■, 0.3 mg/kg, i.v., ■: 1 mg/kg, i.v.) or ranitidine (B: ■, 1 mg/kg, i.v.; ■, 3 mg/kg, i.v.; ■, 10 mg/kg, i.v.) on the motor activity of gastric antrum and colon in anesthetized dogs. The motor index for the 10-min period after intravenous administration of neostigmine or ranitidine is expressed as a percentage of that for the 10-min period before the administration. Each point represents the mean±S.E.M. of 5~7 dogs. *P<0.05, compared with the value in the 10-min period before the administration (Sign-Wilcoxon test).
Fig. 4. Typical tracings of the effects of KW-5092 (A), neostigmine (B) and ranitidine (C) on GI motor activity in conscious dogs in the digestive state. KW-5092, neostigmine or ranitidine was intravenously administered at the point indicated by the arrow.
blood pressure (Fig. 1A). The enhancement of the motor activity by KW-5092 was mainly due to the increase in the amplitude of contractions (Fig. 1A). The effect of KW-5092 at 0.03 mg/kg, i.v. disappeared within 10 min; however, at 0.1 to 1 mg/kg, i.v., the enhanced GI motor activity lasted for longer than 20 min (Fig. 2).

Figure 1 (B and C) shows the typical tracings of the effects of neostigmine and ranitidine, and Fig. 3 summarizes the dose-response relationship. Neostigmine at 0.03 and 0.1 mg/kg, i.v. dose-dependently and significantly enhanced the motor activity (Fig. 3A). Neostigmine at 0.03 and 0.1 mg/kg, i.v. tended to increase blood pressure and to induce reduced amplitude and decreased frequency of respiration (Fig. 1B). Ranitidine at 1 mg/kg, i.v. significantly enhanced only the gastric antral motor activity, and this drug at 3 and 10 mg/kg, i.v. significantly enhanced the gastric antral and colonic motor activities (Fig. 3B). Ranitidine at doses higher than 3 mg/kg, i.v. decreased blood pressure, and it induced reduced amplitude and increased frequency of respiration (Fig. 1C).

**Effects of KW-5092 given i.v. on the motor activity in conscious dogs**

Figure 4A shows the typical tracings of the effects of KW-5092, and Fig. 5 summarizes the dose-response relationship. The motor index of the gastric antrum, duodenum, ileum and the colon for the 10-min period before the administration of KW-5092 were 145±13.8 g·min, 49.5±8.25 g·min, 58.0±8.69 g·min and 130±15.7 g·min (mean±S.E.M., n=24), respectively. KW-5092 at 0.03 to 1 mg/kg, i.v. dose-dependently enhanced the gastric antral, duodenal, ileal and the colonic motor activities. The enhancement of the motor activity by KW-5092 was mainly due to the increase in the amplitude of contractions (Fig. 4A). KW-5092 significantly enhanced the motor activity of the gastric antrum at 0.1 to 1 mg/kg, i.v.; the duodenum and the colon at 0.3 and 1 mg/kg, i.v.; and the ileum at 1 mg/kg, i.v., respectively (Fig. 5). At up to 1 mg/kg, i.v., KW-5092 did not induce behavioral side effects. The duration of the gastric antral motor activity enhanced by KW-5092 was dose-dependently prolonged, and the effect of KW-5092 at 1 mg/kg, i.v. lasted for 30 min (Fig. 5). Moreover, KW-5092 at 0.3 and 1 mg/kg, i.v. significantly enhanced the duodenal and the colonic motor activity for 10 min; and at 1 mg/kg, i.v., it significantly enhanced the ileal motor activity for 10 min (Fig. 5).

Figure 4 (B and C) shows the typical tracings of the effects of neostigmine and ranitidine, and Figs. 6 and 7 summarize the dose-response relationship. Neostigmine...
Fig. 6. Effects of neostigmine (○, 0.01 mg/kg, i.v.; ■, 0.03 mg/kg, i.v.; △, 0.1 mg/kg, i.v.) on GI motor activity in conscious dogs in the digestive state. The motor index for the 10-min period after intravenous administration of neostigmine is expressed as a percentage of that for the 10-min period before the administration. Each point represents the mean ± S.E.M. of 6 dogs. *P < 0.05, **P < 0.01, compared with the value in the 10-min period before the administration (paired t-test).

Fig. 7. Effects of ranitidine (○, 1 mg/kg, i.v.; ■, 3 mg/kg, i.v.) on GI motor activity in conscious dogs in the digestive state. The motor index for the 10-min period after intravenous administration of ranitidine is expressed as a percentage of that for the 10-min period before the administration. Each point represents the mean ± S.E.M. of 6 dogs. *P < 0.05, **P < 0.01, compared with the value in the 10-min period before the administration (paired t-test).
Fig. 8. Typical tracings of the effects of KW-5092 on GI motor activity (A) and the inhibitory effect of atropine on the excitatory response to KW-5092 (B) in conscious dogs in the digestive state. KW-5092 was orally administered, and atropine was intravenously administered at the point indicated by the arrow.
Effects of KW-5092 given p.o. on the GI motor activity in conscious dogs

Figure 8A shows the typical tracings of the effects of KW-5092, and Fig. 9 summarizes the dose-response relationship. The motor index of the gastric antrum, duodenum, ileum and the colon for the 30-min period before the administration of KW-5092 were 393±33.7 g·min, 125±17.6 g·min, 154±18.4 g·min and 235±21.4 g·min (mean ± S.E.M., n=15), respectively. KW-5092 at 1 to 10 mg/kg, p.o. dose-dependently enhanced the gastric antral, duodenal, ileal and colonic motor activities (Fig. 9). The enhancement of the motor activity by KW-5092 was mainly due to the increase in the amplitude of contractions (Fig. 8A). KW-5092 at 1 to 10 mg/kg, p.o. significantly enhanced the duodenal and ileal motor activities; and at 3 and 10 mg/kg, p.o., it significantly enhanced the gastric antral and colonic motor activities (Fig. 9). At up to 10 mg/kg, p.o., KW-5092 did not induce behavioral side effects. The GI motor activity enhanced by KW-5092 at 10 mg/kg, p.o. was completely suppressed by atropine (0.05 or 0.1 mg/kg, i.v.), an anticholinergic agent, treated 10 min before the administration of KW-5092. Figure 8B shows the typical tracings of the inhibitory effect of atropine (0.05 mg/kg, i.v.) on the excitatory response to KW-5092.

Figure 10 shows the typical tracings of the effects of neostigmine and ranitidine, and Figs. 11 and 12 summarize the dose-response relationship. Neostigmine at 1 mg/kg, p.o. significantly enhanced the gastric antral motor activity, but, at 3 mg/kg, p.o., it did not significantly enhance the motor activity. Moreover, neostigmine significantly enhanced the motor activities of the duodenum and the ileum at 1 and 3 mg/kg, p.o., and that of the colon at 3
mg/kg, p.o., respectively (Fig. 11). However, among the 6 dogs examined, neostigmine at 3 mg/kg, p.o. suppressed the gastric antral and the duodenal motor activity in 3 dogs, induced slavering and diarrhea in all dogs, excitement in 5 dogs, and vomiting in 4 dogs. Ranitidine at 10 and 30 mg/kg, p.o. significantly enhanced the gastric antral and ileal motor activities (Fig. 12), and it did not induce behavioral side effects.

**DISCUSSION**

It is reported that domperidone at 1 mg/kg, i.v. and metoclopramide at 0.5 mg/kg, i.v. enhance only the antral and the duodenal motor activities in conscious dogs (10, 11). On the other hand, cisapride at 0.5 mg/kg, i.v. enhances the GI motor activity in a wide range of organs from the gastric antrum to the colon in conscious dogs (11). The present study in anesthetized dogs and con-
Fig. 11. Effects of neostigmine (○, 1 mg/kg, p.o.; ●, 3 mg/kg, p.o.) on GI motor activity in conscious dogs in the digestive state. The motor index for the 10-min period after oral administration of neostigmine is expressed as a percentage of that for the 10-min period before the administration. Each point represents the mean ± S.E.M. of 5–6 dogs. *P<0.05, **P<0.01, compared with the value in the 10-min period before the administration (paired t-test).

Fig. 12. Effects of ranitidine (○, 10 mg/kg, p.o.; ●, 30 mg/kg, p.o.) on GI motor activity in conscious dogs in the digestive state. The motor index for the 10-min period after oral administration of ranitidine is expressed as a percentage of that for the 30-min period before the administration. Each point represents the mean ± S.E.M. of 5–6 dogs. *P<0.05, **P<0.01, compared with the value in the 30-min period before the administration (paired t-test).
scious dogs in the digestive state demonstrated that KW-5092 enhances the GI motor activity at similar doses than those of domperidone (10), metclopramide (11) and cisapride (11), and that KW-5092, like cisapride, but in contrast to domperidone and metoclopramide, enhances the GI motor activity in a wide range of organs from the gastric antrum to the colon. The present study in conscious dogs also demonstrated that KW-5092 given p.o., as well as given i.v., enhances the GI motor activity, suggesting that KW-5092 could be an orally active gastroprokinetic agent. Moreover, in conscious dogs in the interdigestive state, KW-5092 at 10 mg/kg, p.o. also enhanced the GI motor activity in a wide range of organs from the gastric antrum to the colon (A. Tomaru et al., unpublished observation). These results suggest that KW-5092, unlike domperidone and metoclopramide, may be a useful drug for the treatment of GI motility dysfunctions in a wide range of organs from the stomach to the colon.

In the previous in vitro study, KW-5092 inhibited the activity of acetylcholinesterase (AChE) derived from rat brain. The IC50 values of KW-5092, neostigmine and ranitidine were 30 nM, 22 nM and 650 nM, respectively (4). In addition to the AChE inhibition, KW-5092 enhanced ACh release from the longitudinal smooth muscle of the guinea pig ileum (5). In the electrically stimulated preparation, the EC50 value of KW-5092 was 49 nM. Thus, KW-5092 inhibited AChE and enhanced ACh release at almost the same concentrations, suggesting that the AChE inhibition and the ACh release facilitation of KW-5092 equally contribute to the stimulation of GI motility. In the present study, the GI motor activity enhanced by KW-5092 in conscious dogs was completely suppressed by atropine. These results suggest that KW-5092 stimulates the GI motility through the inhibition of AChE and/or the facilitation of ACh release, resulting in the activation of cholinergic neurons.

In the present study, KW-5092 dose-dependently enhanced the GI motor activity when it did not induce apparent behavioral and cardiovascular side effects. Domperidone (1), metclopramide (2) and cisapride (3) are also reported to be safe drugs in terms of behavioral and cardiovascular effects. On the other hand, neostigmine and ranitidine enhanced the GI motor activity, but these drugs also induced behavioral and cardiovascular side effects. The present results suggest that KW-5092 could be a novel gastroprokinetic drug with a safer profile than those of neostigmine and ranitidine.

In conclusion, the present results demonstrate that KW-5092 enhances the GI motor activity in a wide range of organs from the gastric antrum to the colon and does not induce apparent side effects. KW-5092 may be a useful drug of a novel type for the treatment of GI motility dysfunctions.

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