Effects of SK-896, a New Human Motilin Analogue ([Leu¹³]motilin-Hse), on Postoperative Ileus in Dogs after Laparotomy

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The effects of SK-896, a new human motilin analogue ([Leu¹³]motilin-Hse), on digestive tract motility in postoperative ileus were evaluated in a dog model of ileus after laparotomy. SK-896 was intravenously administered at 0.17, 0.33 and 0.67 $\mu\text{g/kg}$ starting soon after operation and then at 6-h intervals, for a total of 9 times. SK-896 progressively, dose-dependently and significantly increased the duodenal motility from 1 h after operation. The recovery time of the gastrointestinal-interdigestive migrating complex (GI-IMC) activity, which is an indicator of normal gastrointestinal tract activity after laparotomy, was 56.5 ± 5.0 h in the control group. SK-896 significantly shortened this recovery time. On the other hand, the plasma SK-896 concentrations declined exponentially after administration, and can be described by a linear pharmacokinetic model within the dose range used. In addition, the pharmacokinetics of SK-896 did not change significantly at any postoperative time. There was no correlation between the plasma SK-896 concentrations and the intensity of duodenal motility, because the activity in the duodenum decreased transiently 13 h after laparotomy and increased with time thereafter. The changes in the activity are considered to reflect the progressive changes in the state of ileus. In conclusion, SK-896 increased the duodenal motility significantly, shortening the recovery time of GI-IMC-like activity in dogs with post-laparotomy ileus. Therefore, it is expected from these results that SK-896 would be useful and effective for the treatment of gastroparesis after abdominal surgery.

Key words SK-896; human motilin analogue; pharmacokinetics; pharmacodynamics; postoperative ileus; dog

Motilin, which has a molecular weight of about 2700, is a polypeptide of 22 amino acids. It was isolated and purified from hog gastroduodenum mucosa by Brown *et al.* in 1972,¹⁾ and its structure was determined conclusively by Schubert and Brown in 1974.²⁾ Since then, motilin and motilin analogues have been extensively synthesized, and their properties have been elucidated. It is known that in dog, motilin increases the motility of the stomach and small intestine by stimulating the cholinergic neurons,^{3–5)} whereas in rabbit and human it produces the contractions of the stomach, pylorus and duodenum by direct action on the smooth muscle cells.^{6–11)} The mechanisms of the increase in gastrointestinal (GI) motility differ with the animal species.

Motilin is considered to be a peptide hormone involved in regulation of the digestive tract activity during the interdigestive period,¹²⁾ and the administration of exogenous motilin has been reported to have biological activities limited to the digestive system such as the induction in dogs of interdigestive migrating complex (IMC), which has a marked contractile activity that is transmitted from the upper to the lower digestive tract in the interdigestive period,¹³⁾ and an increase in gastric ejection in humans.¹⁴⁾ Clinically, it has been also reported that the blood motilin concentration is reduced in patients after laparotomy and postoperative ileus is improved as soon as the blood motilin concentration begins to recover from the early postoperative levels.^{15,16)}

The amino acid sequence of human motilin remained unclear for a long time after the discovery of porcine motilin, but in 1987 was found to be identical to that of porcine motilin.¹⁷⁾

We thought that motilin may be useful for the treatment of gastroparesis due to the pharmacological actions mentioned above. Therefore, various methods of synthesizing of motilin analogues were investigated in our laboratory, and a

new high-yield, low-cost method for synthesis of a new human motilin analogue, SK-896 ([Leu¹³]motilin-Hse), was established using recombinant DNA techniques. SK-896 (Phe-Val-Pro-Ile-Phe-Thr-Gly-Glu-Leu-Gln-Arg-Leu-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-Hse), in which leucine replaces methionine at position 13 of human motilin and homoserine is added at the C-terminal, is a new human motilin analogue. A pharmacological profile of SK-896 in rabbits, rats and dogs was developed from tests using *in vitro* techniques, and it was revealed that SK-896 bound to motilin receptors in the rabbit duodenum and induced the contraction of smooth muscle preparations isolated from the rabbit gastrointestinal tract but not those isolated from the rat or the dog.¹⁸⁾ In addition, from an *in vivo* study it was reported that treatment with SK-896 significantly shortened the time to the first appearance of IMC in the stomach as well as the gastric emptying time in dogs with operative ileus, as compared with treatment with prostaglandin F_{2 α} , which is currently used to treat of gastroparesis.¹⁹⁾

SK-896 has the same pharmacological profile as human motilin *in vitro*¹⁸⁾ and is more effective than prostaglandin F_{2 α} *in vivo*.¹⁹⁾ Clinically, intravenously administered motilin or motilin analogue enhances GI motility without serious side effects.^{14,20)} Therefore, it is expected that SK-896 may be useful and effective for the treatment of gastroparesis after abdominal surgery.

In the present study, we evaluated the effects of SK-896 on postoperative ileus with regard to digestive tract motility, using dogs after laparotomy as a model of postoperative ileus. In addition, we determined the plasma concentrations of SK-896 and endogenous dog motilin after intravenous administration of SK-896, and evaluated the relation between the effects and plasma concentrations.

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MATERIALS AND METHODS

Animals Male beagle dogs (OBC, Shizuoka, Japan) weighing 9.0–11.8 kg were used in the experiments. The animals were housed in an air-conditioned room at $22 \pm 3^\circ\text{C}$ with a 12-h light cycle, fed standard laboratory diet (Canine Diet #4360, Purina Japan, Tokyo, Japan) and given water *ad libitum*. The animals were fasted for 24 h before laparotomy (*ad libitum* intake of water was permitted).

Materials SK-896 ([Leu¹³]motilin-Hse), dog motilin (Phe–Val–Pro–Ile–Phe–Thr–His–Ser–Glu–Leu–Gln–Lys–Ile–Arg–Glu–Lys–Glu–Arg–Asn–Lys–Gly–Gln) and dog motilin-tyrosine were synthesized at Sanwa Kagaku Kenkyusho, Co., Ltd. Rabbit anti-human motilin antiserum and rabbit anti-dog motilin antiserum were prepared in our laboratory. The anti-human motilin antiserum was shown to react mainly with the C-terminal portion of SK-896 but never with dog motilin or other known gastrointestinal hormones, while the anti-dog motilin antiserum was shown to react mainly with the C-terminal portion of dog motilin but never with SK-896 or other known gastrointestinal hormones. Other drugs used were: sodium pentobarbital (Nembutal[®], Dinabot, Osaka, Japan), atropine (atropine sulfate injection tanabe[®], Tanabe Seiyaku, Osaka, Japan), ceftriaxone sodium (Rocephin[®], Japan Roche, Tokyo, Japan), KN[®] (Otsuka Pharmaceutical, Tokushima, Japan), saline (Otsuka Normal Saline, Otsuka Pharmaceutical), aprotinin (Sigma Chemical Co., St. Louis, U.S.A.), albumin bovine fraction V (Seikagaku Corporation, Tokyo, Japan), polyoxyethylene hydrogenated castor oil 60 (HCO-60) (Nikko Chemicals, Co., Ltd., Tokyo, Japan), lactoperoxidase (Sigma Chemical Co.), Amerlex-M donkey anti-rabbit (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, England), Na¹²⁵I (Amersham), and Norit[®] EXW (Nakarai Tesque, Inc., Kyoto, Japan).

Laparotomy Laparotomy was performed according to the method of Tsukamoto *et al.*¹⁹⁾ In brief, the dogs were anesthetized with 35 mg/kg intravenous injection of sodium pentobarbital, and the laparotomy was performed aseptically. The site of surgery in the dog was clipped with an electric clipper, and the animal was fixed on the surgical table. The neck and the abdominal region were disinfected with a surgical Iodine solution, and unclean parts were covered with a sterilized sheet. The external jugular vein was catheterized (Anthon[®]; Toray Medical, Tokyo, Japan) to obtain a route for drug administration. Median laparotomy was performed, 1 mg atropine sulfate was administered intravenously, and a force transducer (F-12IS; Star Medical, Tokyo, Japan) was sutured with a silk thread to the serosal surface of the antrum of the stomach, duodenum, jejunum (3 points at 70, 170, and 220 cm anally of the entire length of 300 cm from the ligament of Treitz to the ileocecum), and colon to record contractile activities of the annular muscle. The leads from the force transducers were passed under the skin to an incision between the bilateral scapular regions, where they were brought out of the body and fixed. To collect gastric fluid, a gastric tube (KN-365 D-14C, Natsume Seisakusyo Co., Ltd., Tokyo, Japan) was attached to the incisura angularis, the tip of the tube was advanced through the abdominal wall, and the tube was fixed in the left flank. The duration of laparotomy from opening to closing of the abdomen was 2.5–3 h.

Postoperatively, a jacket was put on the dog to protect the

leads and cannula, and 1 g of sodium ceftriaxone (Rocephin[®]) was administered intravenously at the end of surgery. In addition, 500 ml of KN fluid, in which 0.5 g of sodium ceftriaxone for injection was dissolved, was infused at a rate of 500 ml/d. The dogs were given no food or water during the recording of digestive tract activities.

SK-896 Administration SK-896 was administered through the catheter placed in the external jugular vein at 0.17, 0.33, and 0.67 $\mu\text{g/kg}$ over 2 min. In the control group, physiological saline was administered. SK-896 and physiological saline were administered continuously at 6-h intervals from 1 h to 49 h after laparotomy, for a total of 9 times.

Blood samples (1 ml) were collected on an EDTA anticoagulant by venipuncture at 2, 4, 6, 8, 10, 15 and 30 min after injection. Aprotinin was added to the blood samples to a final concentration of 1000 kallikrein inactivator units (KIU)/ml, and the plasma was immediately separated from blood by centrifugation and stored at -80°C until analysis.

Record of Digestive Tract Motility The motility of the digestive tract was recorded continuously starting 1 h after the surgical incision was closed using an organ activity analysis system (ESC-820; Star Medical, Tokyo, Japan).

Iodination of SK-896 or Dog Motilin SK-896 was labeled with ¹²⁵I by a modified lactoperoxidase method.²¹⁾ Briefly, 5 μl of SK-896 solution (80 $\mu\text{g/ml}$), 5 μl of Na¹²⁵I (37 MBq/10 μl), 1 μl of lactoperoxidase (50 $\mu\text{g/ml}$) and 1 μl of hydrogen peroxide solution (0.9 mM) were incubated in a glass tube (precoated by 1% HCO-60 solution) for 10 min at 37°C . Furthermore, 2 μl of hydrogen peroxide solution (0.45 mM) were added to the reaction mixture and incubated for 10 min at 37°C . Iodination was terminated by addition of 100 μl of NaN₃ solution (100 mg/ml) and 100 μl of potassium iodide (16.6 mg/ml). After iodination, ¹²⁵I-SK-896 was further purified on a PD-10 column (Sephadex G-25M, Pharmacia Biotech) and eluted with phosphate buffer saline containing 1% BSA and 1000 KIU/ml aprotinin. ¹²⁵I-SK-896 solution was stored at -20°C .

Since, tyrosine is not included in the amino acid sequence of dog motilin, it was added at the C-terminal of dog motilin, and iodination was performed according to the method for SK-896.

Preparation of Plasma without Motilin (Motilin Free Plasma) Two grams of charcoal (Norit EXW) were added to every 10.0 ml of plasma and this was agitated for 12 h at 4°C . After centrifuging for 1 h at 4°C and 105000 *g*, the supernatant was filtered with a 20 μm filter. Aprotinin was added to the motilin free plasma to a final concentration of 1000 KIU/ml.

Determination of Plasma Concentration of SK-896 or Endogenous Dog Motilin The concentrations of SK-896 were determined by radioimmunoassay (RIA). One hundred microliters of sample and 100 μl of 10 mM phosphate buffer saline (pH 7.4) containing 1% BSA, 0.1% sodium azide (sample, buffer A) or 100 μl of standard (motilin free plasma) and 100 μl of SK-896 standard solution (0, 0.025, 0.050, 0.125, 0.50, 1.0, 2.0 ng/ml) were added to the assay tube. Then, 100 μl of ¹²⁵I-SK896 (14000 cpm) and 100 μl of rabbit anti-human motilin antiserum at 1/20000 dilution with buffer A were added and incubated for 48–72 h at 4°C . Antibody bound SK-896 was precipitated using 5000 μl of Amerlex-M donkey anti-rabbit. The tubes were incubated for

3 h at 4 °C and centrifuged for 20 min at 1600 *g*. Precipitate was counted in a gamma counter (Cobra Auto Gamma, Packard). All samples were assayed in duplicate. The calibration curve was analyzed with a 4-parameter logistic using the gamma-counter program, and a concentration of SK-896 was obtained.

On the other hand, the concentrations of endogenous dog motilin were determined according to the method for SK-896, using the anti-dog motilin antiserum and ¹²⁵I-dog motilin. The determination limit was 0.050 ng/ml.

Pharmacodynamic Data Analysis In various mammals, including humans, gastrointestinal motility is divided into two principal stages, termed the interdigestive and the digestive periods. During the digestive period, continuous weak contractions are observed in the gastrointestinal tract from stomach to intestine. During the interdigestive period, giant contraction wave groups traveling from the gastroduodenum to the lower part of the gastrointestinal tract are observed periodically. This giant contraction wave group is called IMC and is observed at 100 min intervals in both humans and dogs. The activities that occurred in the stomach and were transmitted through the duodenum to a point 170 cm (if the entire length is 300 cm) from the Treitz ligament of the small intestine were thus defined as GI-IMC-like activities. The time when GI-IMC-like activities were first noted after SK-896 administration was determined on the chart, and was regarded as the recovery time for the GI-IMC-like activities. In the control group, the time when the natural GI-IMC first appeared after laparotomy was regarded as the recovery time for the GI-IMC.

Increases in the digestive tract activities due to the administration of SK-896 were measured as follows. An integral of activities (group contraction waves were excluded) during 1 h before the beginning of administration was calculated and converted to a value for a 40-min period (A). A was subtracted from the integral of activities for the 40 min after the beginning of the administration, and the difference was regarded as the increase in the activity level due to the administration of SK-896, or the motility index/40 min.

Pharmacokinetic Data Analysis The elimination half-life ($t_{1/2}$) of the elimination phase was estimated using the least square method of actual data. The areas under the curve (AUC) and under the moment curve (AUMC) of concentration versus time were determined by the trapezoidal rule and by extrapolating to infinity. Plasma clearance (CL_p), mean residence time (MRT) and volume of distribution at steady state (Vd_{ss}) were computed by noncompartmental methods.²²⁾

Statistical Analysis The value of each evaluation item was expressed as the mean and standard error. The statistical analysis of pharmacodynamic parameters was performed by one-way analysis of variance and Fischer's least significance difference procedure (LSD). *p* values less than 0.05 were considered significant. Also, a statistical analysis of pharmacokinetic parameters was performed using the one-way layout analysis of variance and Scheffé multiple comparison test concomitantly. *p* values less than 0.05 were considered significant.

RESULTS

Preparation of the Post-Laparotomy Ileus Model

Figure 1 shows typical changes in gastrointestinal motility of a dog with postoperative ileus. During the interdigestive period in a normal dog, giant contraction wave groups traveling from the gastroduodenum to the lower part of the gastrointestinal tract (GI-IMC) are observed periodically. In the laparotomized dog, the entire digestive tract was paralyzed, and weak gastrointestinal motility was observed until 13 h after laparotomy. At 25 h after laparotomy, weak contractile activities were observed in the lower small intestine, but the stomach and duodenum were still paralyzed. At 37 h or more after laparotomy, contractile activities were observed also in the stomach and duodenum. The GI-IMC was first observed at 56.5 ± 5.0 h ($n=8$) after laparotomy.

Digestive Tract Motility Induced by the Administration of SK-896 Figure 2 shows the serial effects of intravenous administration of SK-896 (0.33 μ g/kg) on the digestive tract motility of a laparotomized dog (data at 0.17 and 0.67 μ g/kg not shown). Contractile activities were induced in the duodenum until 1 h after laparotomy at either dose. However, these activities were not transmitted below the small intestine until 19 h after laparotomy.

On the other hand, SK-896 simultaneously induced contractions in the stomach and duodenum, and GI-IMC-like activities, which were transmitted downward in the small intestine and below, from 31 h or more after laparotomy at the dose of 0.17 μ g/kg and from 25 h or more after laparotomy at the doses of 0.33 and 0.67 μ g/kg.

Temporal Effects of SK-896 on the Gastric and Duodenal Motor Activities The contractile activities induced in the stomach and duodenum by intravenous administration of SK-896 were terminated within 40 min after the beginning of the administration. Therefore, we evaluated temporal effects of SK-896 on the stomach and duodenum by using the integral of contraction waves over 40 min from the beginning of the administration at various doses (Fig. 3).

In the gastric antral region, SK-896 slightly increased the motor activity, but this increase was not significant. In the duodenum, SK-896 significantly and dose-dependently increased the motor activity in the groups administered doses of 0.67 μ g/kg or more from 1 h onward after the administration, and the activity reached a plateau from 31 h or more after laparotomy in the group treated with 0.17 μ g/kg. The responses decreased transiently until 13 h after laparotomy and increased with time thereafter.

The spontaneous gastric and duodenal motility observed during fasting in healthy dogs in the control group 2 weeks or more after laparotomy were measured, and the ranges of the motility index/40 min in these dogs were regarded as the normal ranges. Since the normal range was 236–952 ($g \times \text{min}$) in the antral region and 262–951 ($g \times \text{min}$) in the duodenum, SK-896 (0.67 μ g/kg) is considered to have induced motility of normal strength in the duodenum 37 h or more after laparotomy.

Effects of SK-896 on the Recovery Time of GI-IMC-Like Activities Figure 4 shows the effects of SK-896 on the recovery time of GI-IMC-like activities. In the SK-896-administered group, the mean value of the earliest time at which the GI-IMC-like activity was first observed was regarded as the recovery time of the GI-IMC-like activity. SK-896 induced the GI-IMC-like activity in the stomach starting from 25 h after laparotomy at the earliest and significantly

Fig. 1. Typical Change in Gastrointestinal Motility at 1, 13, 25, 37, 49 and 54 h after Laparotomy and the Implantation of Force Transducers in the Gastrointestinal Tract of a Dog with Postoperative Ileus

Small intestine-1 and -2 represent the points on the small intestine 70 and 170 cm distal from the ligament of Treitz (per 300 cm from the ligament of Treitz to the cecum), respectively.

Fig. 2. Effect of Intravenous Injection of SK-896 (0.33 µg/kg) at 1, 7, 13, 19, 25, 31, 37, 43 and 49 h on Gastrointestinal Motility in a Dog with Postoperative Ileus

shortened its recovery time compared with the control group. The recovery times of groups administered SK-896 at the doses of 0.17, 0.33, and 0.67 µg/kg were 33.4±1.5, 28.6±3.1, and 26.5±2.9 h, respectively.

Plasma Concentrations of Dog Motilin before and after Laparotomy Figure 5 shows plasma concentrations of the endogenous dog motilin in the control group before and after laparotomy. Mean plasma concentration of dog motilin was

Fig. 3. Effect of Intravenous Injection of SK-896 on Motor Activity of Gastric Antrum and Duodenum in Dogs with Postoperative Ileus
Each bar represents the mean $\pm$ S.E.M., * p <0.05, ** p <0.01, *** p <0.001 for the comparison with control, as analyzed by 1-way ANOVA followed by LSD.

Fig. 4. Effect of Intravenous Injection of SK-896 on the Time Required for the First Appearance of IMC-Like Contraction in Dogs with Postoperative Ileus

Each bar represents mean $\pm$ S.E.M., ** p <0.01, *** p <0.001 for comparison with control, as analyzed by 1-way ANOVA followed by LSD.

0.254 ng/ml before laparotomy, decreased to 0.0868 ng/ml at 13 h after operation, and thereafter increased gradually until 49 h.

Pharmacokinetics of SK-896 and the Induction of Endogenous Dog Motilin Figure 6 and Table 1 show the time

Fig. 5. Plasma Concentration of Endogenous Dog Motilin after Operation in Dogs with Postoperative Ileus

Each point represents the mean $\pm$ S.E.M. (n=8).

courses of plasma immunoreactive SK-896 concentration and pharmacokinetic parameters after intravenous administration of SK-896 of the dogs with postoperative ileus at 1, 13, 25, 37 and 49 h after laparotomy, respectively. Figure 7 shows the endogenous dog motilin concentrations after dosing.

Plasma concentrations of immunoreactive SK-896 after

Fig. 6. Plasma Concentrations of SK-896 after Intravenous Injection of SK-896 in Dogs with Postoperative Ileus
Each point represents the mean±S.E.M.

Table 1. Pharmacokinetic Parameters of SK-896 after Intravenous Injection of SK-896 in Dogs with Postoperative Ileus

Dose (μg/kg)	Time (h)	$AUC_{0-\infty}$ (ng eq·min/ml)	CL_p (ml/min/kg)	MRT (min)	Vd_{ss} (ml/kg)	$t_{1/2}$ (min)
0.17*	1	11.7±1.6	15.2±1.6	4.04±0.42	59.1±4.0	7.83±0.80
	13	9.87±1.01	17.6±1.6	3.92±0.68	64.7±5.0	6.81±1.11
	25	12.4±1.4	14.1±1.5	4.83±0.51	65.8±6.2	8.60±1.12
	37	15.5±1.7	11.4±1.3	5.12±0.18	58.7±8.0	7.19±0.86
	49	14.2±1.4	12.3±1.3	5.43±0.24	66.2±6.5	9.12±1.08
0.33*	1	27.2±1.8	12.5±0.9	5.01±0.33	62.1±5.2	7.83±0.78
	13	25.3±2.8	13.8±1.3	4.41±0.24	59.6±3.6	8.35±0.59
	25	28.4±1.4	11.8±0.6	5.04±0.28	59.6±4.2	7.63±0.79
	37	31.7±3.0	10.9±1.2	5.33±0.50	56.5±4.0	8.18±0.51
	49	32.1±1.2	10.4±0.4	5.80±0.46	60.4±5.2	7.74±0.56
0.67**	1	53.1±10.4	13.7±2.0	3.65±0.47	48.2±6.8	6.90±1.36
	13	45.1±11.5	17.0±3.0	3.83±0.59	60.3±5.3	7.23±0.78
	25	39.7±3.3	17.1±1.3	5.00±0.59	83.7±4.5	8.08±0.41
	37	48.2±4.0	14.1±1.2	5.51±0.58	76.6±6.2	7.93±0.56
	49	62.0±11.3	11.8±1.9	5.31±0.51	61.1±8.8	7.97±0.23

Each value represents the mean±S.E.M. (*: n=5, **: n=4).

Fig. 7. Plasma Concentrations of Endogenous Dog Motilin after Intravenous Injection of SK-896 in Dogs with Postoperative Ileus

Each point represents the mean $\pm$ S.E.M.

the intravenous administration at the doses of 0.17, 0.33 and 0.67 $\mu\text{g/kg}$ were eliminated biexponentially at half lives at 6.81–9.12 h. Within the dose range of 0.33–0.67 $\mu\text{g/kg}$, $AUC_{0-\infty}$ increased in proportion to the dose, whereas MRT , CL_p , Vd_{ss} and $t_{1/2}$ were 3.65–5.80 min, 10.4–17.6 ml/min/kg, 48.2–83.7 ml/kg and 6.81–9.12 min, respectively, and were dose independent, indicating that SK-896 plasma levels can be described by a linear pharmacokinetic model within the dose range of 0.33–0.67 $\mu\text{g/kg}$. In addition, no significant difference was observed in the pharmacokinetic parameters at any postoperative time. On the other hand, endogenous dog motilin concentrations increased immediately after the administration and reached a preoperative level at 2–4 min, although it was not dose-dependent. The dog endogenous motilin decreased to the preoperative level at 30 min after the administration.

DISCUSSION

Normal digestive tract contractions consist of “those in the

postprandial period, *i.e.* contractile activities for mixing of food and digestive juice and transport of food to the lower parts of the digestive tract observed on ingestion of food,” and “those in the interdigestive period, *i.e.* strong contractions appearing periodically in the stomach and duodenum and transmitted to the lower parts of the digestive tract at a fixed rate (IMC).”²³⁾ These normal digestive tract activities are known to be arrested after laparotomy, and this state is called postoperative ileus.²⁴⁾

Yokoyama *et al.* directly recorded digestive tract motility, using the same method as in this study, and reported that the reappearance time of phase III contractions in the stomach treated with saline in their model was 105.8 h.²⁵⁾ Morris *et al.* also observed the disappearance of phase III contractions for two days after laparotomy, and their reappearance three days after the laparotomy.²⁶⁾ In our experimental model using dogs, digestive tract motility was temporarily halted after laparotomy, then reappeared first primarily in the lower small intestine. The normal GI-IMC originating in the stomach and duodenum was restored a mean of 56.9 ± 5.0 h after laparo-

tomy. Therefore, our model appear to be than that of Yokoyama *et al.*, closer to the model of Morris *et al.*

We intravenously administered SK-896 a total of 9 times from 1 to 49 h after laparotomy in a dog model of postoperative ileus. After intravenous administration, plasma immunoreactive SK-896 concentration-time profiles did not change at either postoperative time and plasma SK-896 concentrations can be described by a linear pharmacokinetic model indicating that the degree of the restoration of the body did not influence the pharmacokinetics of SK-896. The $V_{d_{ss}}$ was 48.2–83.7 ml/kg and was not distributed widely, presumably due to the high protein binding of SK-896 (91.4–94.2%).²⁷⁾

On the other hand, endogenous dog motilin was induced to preoperative levels by SK-896 administration. Mochiki *et al.* reported that exogenous porcine motilin (identical to human motilin) stimulates endogenous motilin release through muscarinic receptors on motilin-producing cells *via* preganglionic pathways involving 5-hydroxytryptamine 3 receptors.²⁸⁾ It is conceivable that SK-896 induces endogenous motilin by the same mechanism because SK-896 is a human motilin analog. The endogenous dog motilin was eliminated rapidly after it reached a maximum concentration, and induction was transient. The SK-896 and the endogenous motilin exist in the plasma after administration of SK-896. It has been reported that dog and porcine motilins induce a comparable contraction of the smooth muscle preparations isolated from rabbit duodenum.^{29,30)} It has been also reported that dog and porcine motilins have an equal potency in inducing premature phase III of the migrating motor complex after intravenous injection to conscious dogs.³¹⁾ Hence, these results suggest that endogenous motilin of dog and SK-896 have an equal potency in stimulating the contractile activities of the gastrointestinal tract. From a comparison of the plasma concentrations of SK-896 and endogenously induced dog motilin after intravenous administration of SK-896 to dogs with post-laparotomy ileus, it is conceivable that the contractile activities of the gastrointestinal tract may be induced by both SK-896 and endogenous dog motilin.

The intravenous administration of SK-896 did not increase the motility of the stomach significantly, but did increase that of the duodenum significantly and dose-dependently from immediately after laparotomy. Therefore, it is considered that SK-896 induces duodenal motility from early after laparotomy. In addition, the recovery time of the GI-IMC activity after laparotomy was 31 h or more after laparotomy at the dose of 0.17 μ g/kg and from 25 h or more after laparotomy at the doses of 0.33 and 0.67 μ g/kg. These results show that SK-896 administration induced the GI-IMC-like activity earlier than was seen in the control group, and indicate that SK-896 may be useful in the treatment of postoperative ileus.

Although the pharmacokinetics of SK-896 did not change at any postoperative time and the endogenous dog motilin was induced to preoperative levels, the responses in the duodenum were not uniform, decreased transiently 13 h after laparotomy and increased with time thereafter. It is postulated the changes in the responses to SK-896 can be explained by progressive changes in the state of ileus, which may be the strongest at 13 h after laparotomy in this model. In addition, in the control group, the endogenous dog motilin concentration reached a nadir 13 h after laparotomy, the same as the

activity in the duodenum, and thereafter increased gradually to near the preoperative level at 49 h. The fact that the spontaneous GI-IMC activity occurred after the restoration of endogenous motilin levels suggests that the reduction of the endogenous motilin may be related to the postoperative ileus.

The reason the response of the gastrointestinal tract to SK-896 changed with postoperative time is unclear. It has been demonstrated that SK-896 induces gastrointestinal motility during the interdigestive period in dogs with regulation of acetylcholine release from the cholinergic nerve terminal *via* the parasympathetic nervous system.³²⁾ It is postulated from this finding that a factor exists which regulates this parasympathetic nervous system activity. In addition, the fact that the GI-IMC-like activity does not occur in the early postoperative period may be a biophylaxis reaction to hasten the healing by restraining the enterokinesis. Further investigation of these possible explanations is needed.

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