Comparison of the Motor-Stimulating Action of EM523, an Erythromycin Derivative, and Prostaglandin F\textsubscript{2α} in Conscious Dogs

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ABSTRACT—The effect of EM523 [de(N-methyl)-N-ethyl-8,9-anhydroerythromycin A 6,9-hemiacetal], an erythromycin derivative, on gastrointestinal motility was investigated using conscious dogs in the fasting state, and it was compared with those of motilin and prostaglandin F\textsubscript{2α} (PGF\textsubscript{2α}). EM523 and motilin given as i.v. infusions induced strong contractions in the stomach that migrated along the intestine. On the other hand, PGF\textsubscript{2α} stimulated intestinal contractions, but its effect on gastric motility was weak. EM523 had 1/50 the potency of motilin and 6 times the potency of PGF\textsubscript{2α} for stimulation of intestinal motility. Atropine at 0.1 mg/kg, i.v. strongly inhibited gastrointestinal contractions induced by EM523 or motilin and partly inhibited PGF\textsubscript{2α}-induced intestinal motility. ICS-205-930, a 5HT\textsubscript{3}-receptor antagonist, at a dose of 1 mg/kg, i.v. strongly inhibited EM523 or motilin-induced gastric contractions but did not affect the action of PGF\textsubscript{2α}. Infusion of EM523 at 100 μg/kg/hr induced strong migrating contractions even when motility was depressed by dopamine infusion or laparotomy. Infusion of PGF\textsubscript{2α} at 300 μg/kg/hr stimulated intestinal but not gastric motility under these conditions. The results of this study indicate that the cholinergic pathway and 5HT\textsubscript{3} receptors are involved in EM523 and motilin-induced migrating gastrointestinal contractions, whereas the cholinergic pathway seems to be only partly involved in PGF\textsubscript{2α}-induced intestinal contractions.

Keywords: EM523, Motilide, Erythromycin, Prostaglandin F\textsubscript{2α}, Motility

Recent studies have shown that erythromycin, a macrolide antibiotic, mimics the effect of motilin, a gastrointestinal polypeptide hormone, and induces strong gastrointestinal contractions in dogs (1) and humans (2). Erythromycin has been shown to accelerate gastric emptying in patients with diabetic gastroparesis or postvagotomy gastroparesis (3, 4). However, administration of erythromycin may cause a change in the intestinal bacterial flora and a selection of erythromycin-resistant bacteria. Thus, we synthesized many erythromycin derivatives and found that EM523 has more potent gastrointestinal motor-stimulating activity than erythromycin, while being devoid of antibacterial activity (5). Itoh and Omura (6) named these macrolide compounds "motilides", meaning motilin-like macrolides. We have reported that the gastric motor-stimulating activity of EM523 is stronger than those of cisapride, trimebutine maleate and metoclopramide in conscious dogs and that its action resembles that of motilin. However, unlike motilin, EM523 is also active by the oral and intraduodenal routes (7). In vitro, EM523 caused contractions that were similar to those induced by motilin (8) and inhibited the binding of motilin to its receptors in antral smooth muscle in rabbits and humans (9).

In this study, we investigated the effect of EM523 on gastrointestinal motility in conscious dogs and compared it with those of motilin and PGF\textsubscript{2α}, a therapeutic agent for postoperative ileus. The possible mechanism of action was also studied.

MATERIALS AND METHODS

Drugs

EM523 was synthesized at Takeda Chemical Industries, Ltd. The following drugs were used: motilin (Peptide Institute Inc., Osaka), PGF\textsubscript{2α} (Ono Pharmaceuticals, Osaka), phentolamine mesylate (Ciba-Geigy, Basel, Switzerland), propranolol hydrochloride (Sumitomo, Osaka), methysergide (Sandoz, Berne, Switzerland), famotidine
(Yamanouchi, Tokyo), ICS-205-930 (Research Biochemicals Inc., Natick, MA, USA), atropine sulfate, dopamine hydrochloride, hexamethonium chloride (Wako Pure Chemical, Osaka), naloxone, mepyramine (Sigma Chemical Co., St. Louis, MO, USA) and ketanserin (Junsei Chemical, Tokyo). EM523 was dissolved in ethanol and lactobionic acid (EM523 : lactobionic acid = 1 : 1.1 on a molar basis), freeze dried and then dissolved in saline. Famotidine, ketanserine and ICS-205-930 were dissolved in 0.1 N HCl or acetic acid and then neutralized with NaOH. Other agents were dissolved in saline.

**Preparation of animals**

Fifteen male Beagle dogs weighing 7.0–12.0 kg were used. Under pentobarbital (30 mg/kg, i.v.) anesthesia, a Silastic® tube (OD: 0.85 inch, ID: 0.40 inch; Dow Corning, Corning, NY, USA) was inserted into the superior vena cava through a branch vein of the right external jugular vein, and the outer end was sutured onto the adjacent skin. This tube was filled with heparin-containing saline, tipped with a plastic stopper and used as a route for intravenous injection of drugs. The abdomen was opened, and force transducers (F-12 IS-60; Star Medical, Tokyo) were sutured to the serosa of the gastrointestinal tract to measure circular muscle contractions. In four dogs, the transducers were placed on the gastric antrum 3 cm proximal to the pyloric ring and on the small intestine 30 cm distal to the ligament of Treitz and 50 cm proximal to and 20 cm distal to the caecum. In one dog, force transducers were also placed on the greater curvature of the stomach 15 cm proximal to the pyloric ring, on the mid-duodenum, on the small intestine 90 cm distal to the ligament of Treitz and on the colon. In other dogs, force transducers were implanted on the gastric corpus, antrum, duodenum and jejunum. A force transducer was also implanted on the colon in one dog. The leads of the force transducers and the Silastic tube were run subcutaneously along the costal flank to an incision between the scapulae and fixed onto the adjacent skin with silk sutures. After the operation, a jacket was placed on each dog to protect and keep the lead wires and the Silastic tubing in place.

During the experiments, dogs were housed in individual experimental cages, fed a dry-type dog meal (20 g/kg) once a day and given water ad libitum.

![Fig. 1. The effect of intravenous infusion of EM523 on gastrointestinal motility in a conscious dog in the fasting state. EM523 at a dose of 10 μg/kg/hr (A) or 30 μg/kg/hr (B) induced strong contractions in the stomach, and the strong contractions migrated along the intestine.](image-url)
Experimental procedures

Gastrointestinal motor activity was recorded on a polygraph (EMR3701; Graphtech, Tokyo) by connecting the leads of the force transducers to the connecting cables from the amplifiers under the protective jacket. The test drugs were given intravenously, using a syringe pump (STC-521; Terumo, Tokyo), starting 10 min after the spontaneous interdigestive migrating contractions (IMC) in the stomach terminated. To measure the motility quantitatively, the signals from the small intestine were input into a signal processor (7T18; Nihon Denki-Sanei, Tokyo) every 100 msec. The motor index of contractions induced by the drug was calculated according to the procedures described previously (7).

In experiments in which the effect of receptor antagonists were examined, drugs were given intravenously during the infusion of EM523, motilin or PGF$_{2a}$. In experiments in which the effect of EM523 and PGF$_{2a}$ on gastrointestinal motility was studied under dopamine infusion, dopamine was infused for 75 min, and the test drugs were given intravenously for 30 min beginning 15 min after the start of the dopamine infusion.

Statistics

The results are shown as the mean and standard error for 2 observations in each of 3 or 4 dogs. Potencies among drugs were calculated using a parallel line assay.

RESULTS

Effect of drugs in the fasting state

In the fasting state, strong contractions occurred in the stomach at about 100-min intervals and migrated caudad along the small intestine to the terminal ileum (Fig. 1). EM523 given intravenously at a dose of 3 $\mu$g/kg/hr or higher for 30 min induced contractions similar to the IMC in the stomach, which migrated caudad along the small intestine to the ileum (Fig. 1). The jejunal motility increased in a dose-dependent manner, and the peak increase was observed 30 min after beginning the infusion (Figs. 2 and 3). Two of the eight dogs given 100 $\mu$g/kg/hr, i.v. of EM523 vomited within 5 min of the start of the infusion, but doses of up to 30 $\mu$g/kg/hr did not cause vomiting. Motilin at 0.1 $\mu$g/kg/hr or higher induced IMC-like contractions (Fig. 4A). The jejunal motility increased in a dose-dependent manner, and the peak increase was observed 30 or 40 min after starting the infusion (Figs. 2 and 3). One of the six dogs given 0.3 and 1 $\mu$g/kg/hr of motilin vomited, but no vomiting occurred at 0.1 $\mu$g/kg/hr. PGF$_{2a}$ at 30 $\mu$g/kg/hr or higher induced contractions in the duodenum, intestine and colon simultaneously and caused slight contractions in the stomach.

Fig. 2. The effect of porcine motilin, EM523 and PGF$_{2a}$ on intestinal motor indexes in conscious dogs in the fasting state. The motor index is indicated as the total motor index during the 30 min infusion of the drugs. The mean IMC is the mean of the motor indexes during naturally occurring interdigestive migrating contractions of all the animals used in the experiment. The results are shown as the mean and standard error of 6 or 8 experiments.

Fig. 3. The effect of intravenous infusion of EM523 (30 $\mu$g/kg/hr), porcine motilin (0.3 $\mu$g/kg/hr) and PGF$_{2a}$ (100 $\mu$g/kg/hr) on intestinal motility in conscious dogs in the fasting state. The results show the mean and standard error of 6 or 8 experiments.
The jejunal motility increased in a dose-dependent manner, and the peak increase was observed 20–30 min after starting the infusion (Figs. 2 and 3). Two out of the six dogs given 300 μg/kg of PGF₂α vomited, but doses up to 100 μg/kg/hr did not cause vomiting. The dose-response curves for the effect of EM523, motilin and PGF₂α on the jejunal motility are shown in Fig. 2. EM523 had 1/50 the potency of motilin and 6 times the potency of PGF₂α for stimulation of intestinal motility.

**Effect of receptor antagonists**

Atropine at a dose of 0.1 mg/kg, i.v. completely inhibited the gastrointestinal contractions induced by EM523 (30 μg/kg/hr) or motilin (1 μg/kg/hr) in 3 dogs. Even the contractions induced by a high dose of EM523 (100 μg/kg/hr) were completely inhibited by 0.1 mg/kg of atropine (Fig. 5A). The contractions in the duodenum induced by 300 μg/kg/hr of PGF₂α were strongly inhibited, but jejunal and ileal contractions were only partially inhibited by 0.1 mg/kg of atropine (Fig. 5B). Hexamethonium at a dose of 3 mg/kg strongly inhibited the EM523 (30 μg/kg/hr) and motilin (1 μg/kg/hr)-stimulated gastrointestinal motility. Hexamethonium inhibited duodenal but not jejunal or ileal contractions induced by PGF₂α. ICS-205-930, a 5HT₃ receptor antagonist, at a dose of 1 mg/kg strongly inhibited the EM523- or motilin-induced gastric contractions but not the duodenal or intestinal contractions in 3 dogs (Fig. 6). ICS-205-930 did not affect PGF₂α-induced intestinal contractions. Phenotolamine (2 mg/kg), propranolol (1 mg/kg), mepyramine (3 mg/kg), famotidine (1 mg/kg), methysergide (2 mg/kg), ketanserin (1 mg/kg) and naloxone (1 mg/kg) given i.v. did not affect the EM523- or motilin-stimulated gastrointestinal motility.

**Effect of EM523 and PGF₂α during dopamine administration**

Dopamine given i.v. at 10 μg/kg/min to 3 dogs induced vomiting in 2 of the 3 dogs within several minutes after the start of the infusion and inhibited gastrointestinal motility, especially gastric motility (Fig. 7A). Under these conditions, EM523 at 30 μg/kg/hr caused a marked increase in motility both in the stomach and small intestine in all 3 dogs (Fig. 7B). PGF₂α given at 100 μg/kg/hr stimu-
lated intestinal motility although its effect on gastric motility was slight.

Effect of EM523 and PGF₂α on gastrointestinal motility in postoperative ileus

Three dogs that were subjected to laparotomy and the implantation of force transducers in the stomach, duodenum, jejunum and ileum (in two dogs, a force transducer was also implanted in the colon) showed ileus for 2 or 3 days after the operation. One day after the operation, almost no motility was observed in the stomach or intestine. On the second day, irregular contractions with a low amplitude were observed in the stomach and intestine, and the amplitude and frequency of the irregular contractions tended to increase on the third day.

One day after the operation, EM523 given intravenously at 30 μg/kg/hr stimulated gastric and duodenal contractions slightly, but the effect on the intestine was not clear in 2 dogs. In one dog, gastric and intestinal motility was not stimulated by infusion of 30 μg/kg/hr of EM523. EM523 at 100 μg/kg/hr induced contractions in the stomach, duodenum and intestine in all 3 dogs, but the amplitude of contractions was small. EM523 given at 100 μg/kg/hr on the second day after the operation produced stronger contractions in the stomach, duodenum and intestine; and migration of the contractions was observed in all 3 dogs (Fig. 8A). The effect of EM523 was more marked on the third day. The strong contractions induced by EM523 migrated along the gastrointestinal tract (Fig. 9A).

PGF₂α given at 100 μg/kg/hr one day after the operation slightly stimulated intestinal motility, but not gastric motility in 2 dogs and did not stimulate gastric or intestinal motility in one dog. PGF₂α at 300 μg/kg/hr slightly stimulated intestinal motility in all 3 dogs. The second
Fig. 6. The effect of ICS-205-930 on EM523 or motilin-induced contractions in a conscious dog in the fasting state. ICS-205-930 (1 mg/kg, i.v.) strongly inhibited gastric contractions induced by EM523 (30 μg/kg/hr) and porcine motilin (1 μg/kg/hr).

Fig. 7. The effect of intravenous dopamine and EM523 on gastrointestinal motility in a conscious dog in the fasting state. During dopamine (10 μg/kg/min, i.v.) infusion, gastrointestinal motility was suppressed and nausea was observed. EM523 at a dose of 30 μg/kg/hr stimulated gastrointestinal motility during dopamine infusion.
Fig. 8. The effect of intravenous infusion of EM523 (100 μg/kg/hr) and PGF2α (300 μg/kg/hr) on gastrointestinal motility in a dog the second day after laparotomy and the implantation of force transducers in the gastrointestinal tract. EM523 stimulated gastric contractions, and the strong contractions migrated along the intestine. PGF2α stimulated intestinal contractions, but the effect on gastric contractions was weak. During PGF2α infusion, retrograde migrating contractions were induced and the dog vomited.

Fig. 9. The effect of intravenous infusion of EM523 (100 μg/kg/hr) and PGF2α (300 μg/kg/hr) on gastrointestinal motility in a dog the third day after laparotomy and the implantation of force transducers in the gastrointestinal tract.
day after the operation, PGF\textsubscript{2a} at 300 µg/kg/hr stimulated jejunal and ileal motility, but the effect on gastric and duodenal motility was weak (Fig. 8B). During PGF\textsubscript{2a} infusion, vomiting was observed in two of the 3 dogs. The third day after the operation, PGF\textsubscript{2a} at a dose of 300 µg/kg/hr stimulated jejunal and ileal contractions, but not gastric or duodenal motility (Fig. 9B).

DISCUSSION

In this study, EM523 given via i.v. infusion induced strong migrating contractions starting in the stomach. The contractions were similar to the naturally occurring IMC in the fasting state and to those induced by motilin. On the other hand, PGF\textsubscript{2a} induced contractions in the stomach, intestine and colon almost simultaneously; and the pattern of contractions was different from that by EM523 or motilin. Although PGF\textsubscript{2a} induced relatively strong contractions in the jejunum and ileum, its effect on the stomach was weak. EM523 had 1/50 the potency of motilin and 6 times the potency of PGF\textsubscript{2a} for stimulation of jejunal motility, indicating that EM523 is a potent stimulator of intestinal as well as gastric motility.

EM523- and motilin-induced gastrointestinal contractions were completely inhibited by 0.1 mg/kg of atropine, and they were strongly inhibited by 3 mg/kg of hexamethonium, indicating that the cholinergic pathway is important in the exertion of their actions. As EM523 does not induce contractions in the isolated rat or guinea pig intestine, this agent is not an acetylcholine-receptor agonist (8). EM523 seems to stimulate the release of acetylcholine from the cholinergic nerve endings. Atropine at a dose of 0.1 mg/kg strongly inhibited duodenal contractions stimulated by PGF\textsubscript{2a}, but jejunal and ileal contractions were only partially inhibited by atropine. This suggests that the cholinergic pathway is partly involved in PGF\textsubscript{2a}-stimulated intestinal motility.

Ohtawa et al. (10) reported that EM523 increased the plasma level of immunoreactive motilin to 60% of the mean maximum value during spontaneous phase III contractions. They also showed that pretreatment with anticanine motilin serum inhibited EM523-induced contractions by 19%. Their findings indicate that endogenous motilin release is involved in EM523-induced gastrointestinal contractions. EM523 seems to exert its action mainly by acting on the motilin receptors in the nervous system, as EM523 is a motilin receptor agonist in humans and rabbits (9).

Itoh et al. (11) reported that 5HT\textsubscript{3} receptor antagonists inhibit the spontaneous and motilin-induced phase III contractions in the stomach. In this study, ICS-205-930 strongly inhibited EM523- as well as motilin-induced gastric contractions. 5HT\textsubscript{3} receptors are reported to be distributed in the area postrema (12–14), the enteric nervous system (15–17) and afferent vagus nerves (14, 17, 18) in ferrets, rats and guinea pigs. Boeckxstaens et al. (19) reported that stimulation of the 5HT\textsubscript{3} receptors resulted in acetylcholine-mediated contractions through cholinergic efferent nerves in the canine ileal myenteric plexus. The stimulation caused by EM523 and motilin may be transmitted through 5HT\textsubscript{3} receptors in the afferent vagus nerve or in the area postrema to the efferent pathway of the vagus nerve to stimulate endogenous acetylcholine release.

Gastrointestinal motility is under the control of the autonomic nervous system; the sympathetic nervous system inhibits and parasympathetic nervous system stimulates gastrointestinal motility. It is considered that impairment of this nervous control causes gastrointestinal ileus, which is often observed after surgery. Indeed, plasma catecholamine levels are elevated following surgery (20), and high serum levels of catecholamines are associated with inhibited motility (21). When motility was inhibited by dopamine infusion, similar to the condition under sympathetic nervous control, EM523 induced a strong migrating burst of contractions, whereas PGF\textsubscript{2a} mainly stimulated intestinal contractions, and its onset of action in the upper and lower gastrointestinal tract was almost simultaneous. In our preliminary study, EM523 did not inhibit apomorphine-induced vomiting in the dog. Thus, the contractions induced by EM523 during dopamine infusion are unlikely due to antagonism of dopamine-2 receptors. Dopamine is known to inhibit acetylcholine release from the nerve terminals and cause inhibition of gastrointestinal motility (22). EM523 seems to be able to overcome the inhibition of acetylcholine release caused by dopamine.

After laparotomy and the implantation of force transducers, gastrointestinal motility in the dog was strongly depressed for 2 or 3 days. One day after the operation, apparent contractions were not observed in the stomach or intestine; however, on the second day, slight contractions were observed, and the amplitude and the number of contractions were increased. Two or 3 days after the operation, irregular contractions were observed along the gastrointestinal tract. One day after the operation, the stomach and the intestine was insensitive to EM523 and PGF\textsubscript{2a}; only the high dose of EM523 induced contractions, and the amplitude was relatively low. The responsiveness of the stomach and intestine to EM523 and PGF\textsubscript{2a} recovered gradually day by day. Three days after the operation, relatively strong contractions in the stomach were induced by EM523, and the strong contractions migrated along the intestine. PGF\textsubscript{2a} stimulated the intestinal motility, especially in the ileum, but the effect on gastric and duodenal motility was weak. The results indicated that EM523 stimulates upper gastrointestinal
motility rather than lower intestinal motility, whereas PGFZa stimulates lower intestinal motility in the postoperative state.

In summary, EM523 induced strong and migrating contractions in the gastrointestinal tract of conscious dogs, and the cholinergic pathway and 5HT3 receptors are involved in its action. In contrast, PGFZa mainly stimulated intestinal motility, and the cholinergic pathway seems to be partly involved in the action. EM523 was active when the motility was inhibited by laparotomy or dopamine infusion, indicating that this agent may be useful for the treatment of gastrointestinal motor disorders such as postoperative ileus, intestinal pseudo-obstruction and diabetic gastroparesis.

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