ELSEVIER

European Journal of Pharmacology 395 (2000) 165–172

ejp

www.elsevier.nl/locate/ejphar

EM574, an erythromycin derivative, improves delayed gastric emptying of semi-solid meals in conscious dogs

Fumihiko Sako [a], Shogo Marui [b], Nobuhiro Inatomi [c,*], Zen Itoh [d], Satoshi Ōmura [e]

[a] *Pharmacology Laboratories II, Takeda Chemical Industries, Ltd., 2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka 532-8686, Japan*
[b] *Medicinal Chemistry Research Laboratories, Takeda Chemical Industries, Ltd., 2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka 532-8686, Japan*
[c] *Discovery Research Laboratories III, Takeda Chemical Industries, Ltd., 2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka 532-8686, Japan*
[d] *Gastrointestinal Research Laboratory, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi 371-0034, Japan*
[e] *The Kitasato Institute, Tokyo 108-0072, Japan*

Received 7 October 1999; received in revised form 23 February 2000; accepted 29 February 2000

Abstract

The gastroprokinetic effects of de(*N*-methyl)-*N*-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal (EM574), a non-peptide motilin receptor agonist, were investigated in conscious dogs in a normal state and with experimentally-induced gastroparesis. Gastric emptying of semi-solid meals was assessed indirectly from acetaminophen absorption with simultaneous recording of gastric antral motility. In the normal state, post-prandial intraduodenal administration of EM574 (30 mg/kg) stimulated antral motility and significantly enhanced gastric emptying as potently as did intravenous porcine motilin (0.003 mg/kg/h). Intraduodenal cisapride at 1 mg/kg denal cisapride at 1 mg/kg elicited antral contractions and tended to accelerate gastric emptying but at 3 mg/kg, gastric emptying was not enhanced despite a further increase in the motor index. In dogs with gastroparesis induced by intraduodenal oleic acid or intravenous dopamine, EM574 (0.03 mg/kg) increased antral motility and reversed the delayed gastric emptying completely. Cisapride (1 mg/kg) partially ameliorated the impaired emptying under these conditions. In atropinized dogs, no acceleration of gastric emptying by EM574 was observed. These results indicate that EM574 potently accelerates gastric emptying of caloric meals in dogs in a normal state and with experimentally-induced gastroparesis, and also suggest that the effect is mediated through stimulation of a cholinergic neural pathway.

Keywords: EM574; Motilin; Cisapride; Gastric emptying; (Dog)

1. Introduction

De(*N*-methyl)-*N*-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal (EM574), a non-peptide motilin receptor agonist in humans (Satoh et al., 1994) and rabbits (Sato et al., 1997), is a highly potent stimulant of gastro-intestinal motility in dogs and has no antibacterial activity (Tsuzuki et al., 1989; Inatomi et al., 1996). It is derived from erythromycin by chemical modification (Omura et al., 1987; Tsuzuki et al., 1989) based on the fact that erythromycin stimulates gastro-intestinal motility and mimics the effect of motilin in dogs (Itoh et al., 1984a, b, 1985) and humans (Tomomasa et al., 1986).

Recently, it has been reported that erythromycin improves the impaired gastric emptying in patients with severe diabetic gastroparesis (Janssens et al., 1990), post-vagotomy gastroparesis (Mozwecz et al., 1990; Xynos et al., 1992), idiopathic gastroparesis (Richards et al., 1993), primary anorexia nervosa (Stacher et al., 1993) and gastroparesis after cancer chemotherapy (Maliakkal et al., 1991). In addition, erythromycin accelerates gastric emptying in normal subjects (Annese et al., 1992). Thus, erythromycin derivatives with potent gastro-intestinal motor-stimulating activity but lacking antibacterial activity seem promising prokinetic agents.

At present, EM574 is under clinical study. It has been proven that this agent accelerates gastric emptying in healthy subjects with minimal side effects (Choi et al., 1998). In this study, in order to predict the clinical useful-

* Corresponding author. Tel.: +81-6-6300-6142; fax: +81-6-6300-6086.

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00185-0

ness of EM574, we examined the effects of the agent on normal and experimentally impaired gastric emptying in conscious dogs in comparison with the effects of cisapride, a 5-HT_4 receptor agonist (Craig and Clarke, 1990) used worldwide as a prokinetic agent.

2. Materials and methods

2.1. Preparation of animals

A total of 17 male beagles weighing 8–13 kg (Oriental Yeast, Tokyo, Japan) were used. The studies were done in accordance with the Guide for Care and Use of Laboratory Animals adopted by the Committee of Animal Care at Takeda Chemical Industries. The animals were put under anesthesia by intravenous injection of sodium pentobarbital (30 mg/kg), and force transducers (F-12 IS-60; Star Medical, Tokyo, Japan) were chronically implanted onto the serosa of the gastric antrum (3 cm proximal to the pylorus). Medical silicone tubes (Silastic tube; Dow Corning, Midland, MI, USA) were introduced into the superior vena cava through a branch of the external jugular vein and into the duodenum through an incision 6 cm distal to the pylorus as routes for administration of test materials. After the procedure, the lead wires of the transducers and the outer end of the silicone tube were protected by a jacket. The dogs were housed in individual cages and fed a dry-type dog meal (300 g) once a day. Drinking water was given freely. The following experiments were done starting at least 2 weeks after surgery.

2.2. Measurement of gastric motility

Gastric antral motility was continuously recorded on a polygraph (FWR3701; Graphtech, Tokyo, Japan) by connecting the lead wires of the transducers to the cables of the amplifier (PA-001; Star Medical). To measure motility quantitatively, signals from the amplifiers also were input to a signal processor (7T18; Nihon Denki-Sanei, Tokyo, Japan) every 100 ms. The area of contractions was calculated, expressed as a percentage, assuming that the maximum contractions of the interdigestive migrating complex lasted for 1 min, and was used as the motor index (Inatomi et al., 1989).

2.3. Measurement of gastric emptying

After 18 h of fasting, dogs received 10 ml/kg caloric semi-solid meals (OKUNOS-A; 14.5% carbohydrate, 5.1% protein, 2.8% lipid, 240 J/ml; Horika-foods, Niigata, Japan) thoroughly mixed with 30 mg/kg acetaminophen suspended in 0.2 ml/kg 0.5% methyl cellulose. Venous blood samples were withdrawn every 15 min. Plasma acetaminophen concentrations were determined by reversed-phase high-performance liquid chromatography according to the method of Adriaenssens and Prescott (1978) with minor modifications. *o*-Acetamidophenol was used as an internal standard.

2.4. Experimental protocols

2.4.1. Effects of EM574, motilin and cisapride on normal gastric emptying

The dogs ingested the test meal within 5 min. EM574 (0.003, 0.01 and 0.03 mg/kg), cisapride (0.3, 1 and 3 mg/kg) or isotonic saline (0.2 ml/kg) was administered intraduodenally immediately after the meal. Porcine motilin (0.001 and 0.003 mg/kg/h) or isotonic saline (1 ml/kg/h) was continuously infused intravenously for 30 min during the post-prandial period.

2.4.2. Effects of EM574 and cisapride on oleic acid-delayed gastric emptying

To impair gastric emptying, 10 ml of 48% oleic acid emulsion was instilled into the duodenum in 1 min immediately after the meal. EM574 (0.01 and 0.03 mg/kg), cisapride (1 mg/kg) or isotonic saline was administered intraduodenally 15 min after the meal.

2.4.3. Effects of EM574 and cisapride on dopamine-delayed gastric emptying

To delay gastric emptying, dopamine hydrochloride at 0.6 mg/kg/h was continuously infused intravenously during the 90-min post-prandial period. EM574 (0.01 and 0.03 mg/kg), cisapride (1 mg/kg) or isotonic saline was administered intraduodenally 1 min after the meal.

2.4.4. Effect of EM574 on gastric emptying in atropinized dogs

To elucidate the mechanism of the gastroprokinetic effect of EM574, atropine sulfate (0.1 mg/kg) was injected intravenously 1 min before the intraduodenal administration of EM574 (0.03 mg/kg).

2.4.5. Tolerance study

EM574 at 0.03 mg/kg, b.i.d. was administered intraduodenally for 14 days consecutively. Gastric emptying tests were done on days 1 and 15 in the morning after administration of EM574 (0.03 mg/kg) and on days 5 and 22 in the morning after the administration of isotonic saline.

2.5. Drugs and chemicals

EM574 was synthesized by Takeda Chemical Industries. The following drugs were used: cisapride (isolated from Risamol®; Yoshitomi, Osaka, Japan), porcine motilin

(Peptide Institute, Minou, Japan), oleic acid, dopamine hydrochloride and atropine sulfate (Wako, Osaka, Japan). EM574 was dissolved in absolute ethanol, to which lactobionic acid (75 mM) was added at a volume of $< 5\%$ of the final volume, and then diluted with isotonic saline. Cisapride was dissolved in 0.5% w/v tartaric acid solution. Porcine motilin, dopamine hydrochloride and atropine sulfate were dissolved in isotonic saline. Oleic acid was emulsified in milk with 4.8% polyethylene glycol 400.

2.6. Statistical analysis

Data are expressed as means $\pm$ S.E. The data were analyzed by paired *t*-test and repeated measures analysis of variance. Bonferroni's correction was used when multiple groups were compared by paired *t*-test. A P value of < 0.05 was considered statistically significant.

3. Results

3.1. Effects of EM574, motilin and cisapride on normal gastric emptying

Feeding the test meal caused slight contractions in the gastric antrum (Fig. 1). The intraduodenal administration of EM574 at 0.03 mg/kg after feeding induced marked antral contractions (Fig. 1). Gastric emptying, expressed as the elevation of the plasma acetaminophen concentration, was also accelerated with EM574 at 0.03 mg/kg (Fig. 2, top).The gastric emptying as well as the antral motor index was enhanced by EM574 in a dose-dependent manner; a significant effect was observed at 0.03 mg/kg (Fig. 3, top). Intravenous infusion of porcine motilin at 0.003 mg/kg/h during the 30-min post-prandial period stimulated antral contractile activity (Fig. 1) and accelerated

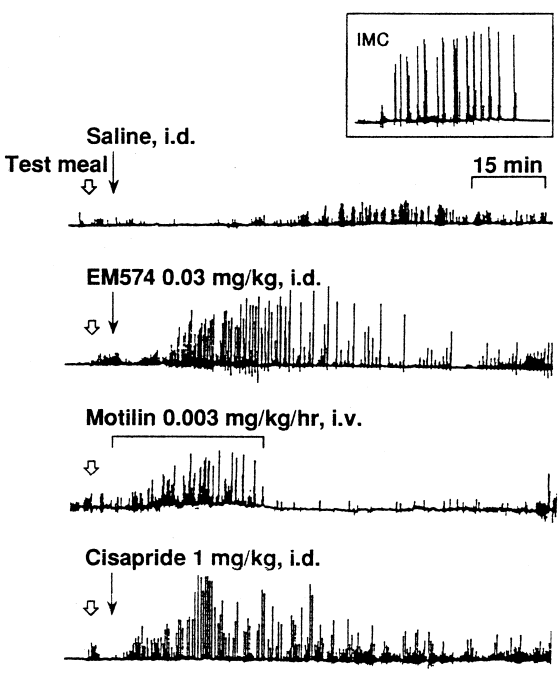

Fig. 1. Effects of EM574, porcine motilin and cisapride on gastric antral motility after a semi-solid meal. The meal was ingested within 5 min. EM574 and cisapride were administered intraduodenally immediately after feeding. Motilin was infused intravenously during the 30-min post-prandial period. A representative pattern of interdigestive migrating complex (IMC) is shown in the inset to indicate the magnitude of contractions.

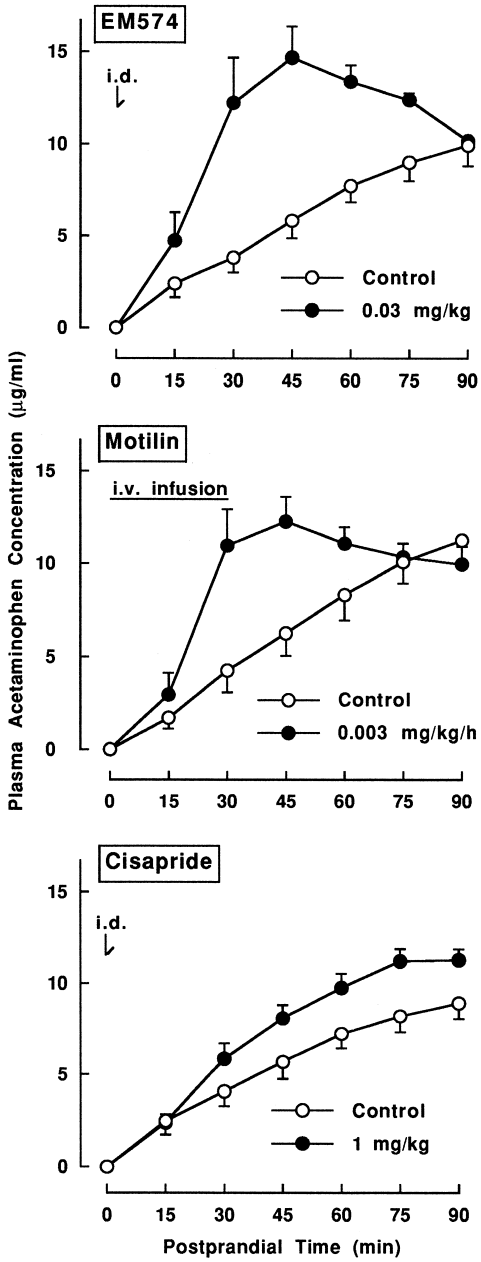

Fig. 2. Gastric emptying curves with intraduodenal EM574 (0.03 mg/kg; top), intravenous motilin (0.003 mg/kg/h; middle) and intraduodenal cisapride (1 mg/kg; bottom). Data are expressed as means $\pm$ S.E. for five dogs.

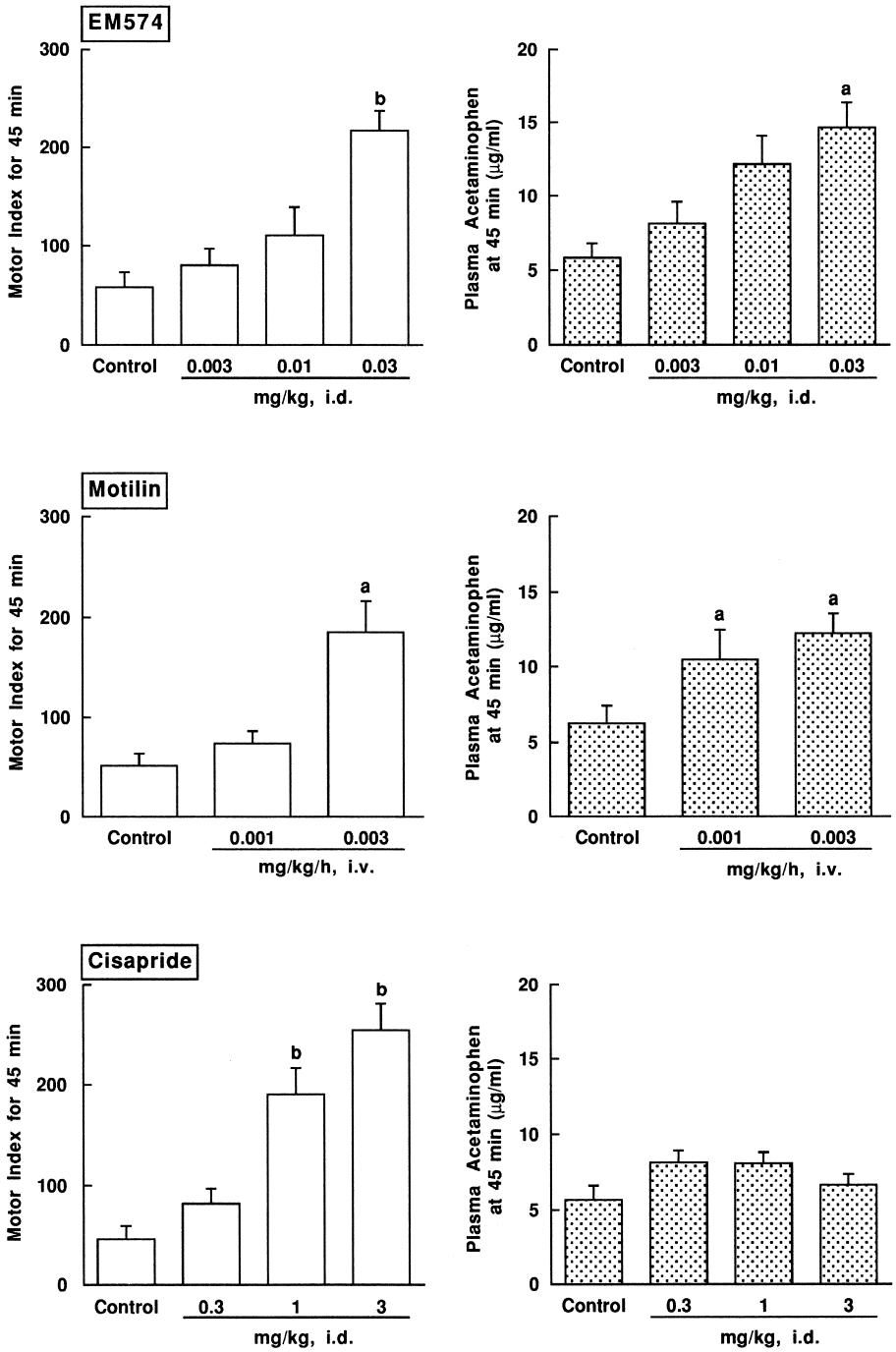

Fig. 3. Dose-related effects of EM574 (top), motilin (middle) and cisapride (bottom) on gastric motor index (left) and gastric emptying indicated as plasma acetaminophen concentration (right). EM574 and cisapride were administered intraduodenally immediately after the meal. Motilin was infused intravenously during the 30-min post-prandial period. Data are expressed as means ± S.E. for five dogs. [a]$P < 0.05$, [b]$P < 0.01$ compared to each control by paired *t*-test with Bonferroni's correction.

gastric emptying (Fig. 2, middle). The effect of motilin was dose-dependent; the dose of 0.003 mg/kg/h enhanced both the motor index and emptying significantly (Fig. 3, middle). Cisapride (1 mg/kg) given intraduodenally evoked marked antral contractions (Fig. 1) and slightly enhanced gastric emptying (Fig. 2, bottom). At 3 mg/kg, however, gastric emptying was not enhanced despite a further increase in the motor index (Fig. 3, bottom).

3.2. Effects of EM574 and cisapride on experimentally-induced gastroparesis

The instillation of oleic acid emulsion into the duodenum after the m motor index and delayed gastric emptying (Fig. 4 and Table 1). EM574 at 0.03 mg/kg given intraduodenally 15 min after feeding significantly increased the antral motor index and reversed delayed gastric

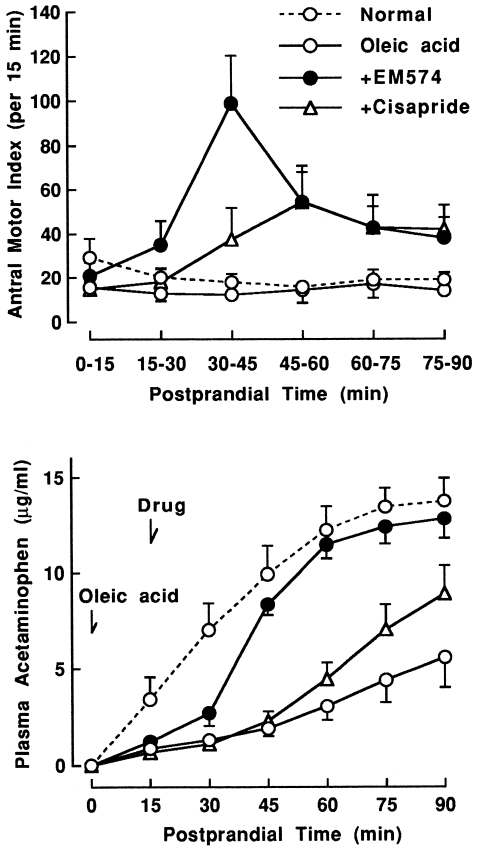

Fig. 4. Changes in gastric antral motor index (upper) and gastric emptying (lower) with EM574 or cisapride dosing in oleic acid-induced gastroparesis. Immediately after the meal, 10 ml of 48% oleic acid emulsion was instilled into the duodenum. EM574 (0.03 mg/kg) or cisapride (1 mg/kg) was administered intraduodenally 15 min after feeding. Data are expressed as means ± S.E. for six dogs.

Table 1
Effects of EM574 and cisapride on experimentally induced gastroparesis

	Antral motor index	Plasma acetaminophen (μg/ml)
Oleic acid-induced gastroparesis	(15–60 min)	(60 min)
Normal	53.6 ± 9.1[a]	12.2 ± 1.3
Oleic acid[b]	39.3 ± 10.2	3.1 ± 0.7
EM574[c] 0.01 mg/kg	70.0 ± 12.9	4.0 ± 1.2
EM574 0.03 mg/kg	188.1 ± 36.5[d]	11.4 ± 0.7[a]
Cisapride[c] 1 mg/kg	109.5 ± 30.7[d]	4.5 ± 0.8
Dopamine-induced gastroparesis	(0–45 min)	(45 min)
Normal	82.9 ± 13.2	7.3 ± 0.1[a]
Dopamine[e]	51.0 ± 8.4	3.3 ± 0.8
EM574[f] 0.01 mg/kg	84.4 ± 9.2	6.4 ± 1.5
EM574 0.03 mg/kg	241.0 ± 50.8	12.3 ± 1.1[a]
Cisapride[d] 1 mg/kg	98.7 ± 26.1	5.3 ± 1.6

[a] $P < 0.01$ compared to each gastroparesis control by paired *t*-test with or without Bonferroni's correction.

[b] Ten milliliters of 48% oleic acid was instilled into the duodenum after feeding.

[c] Drugs were administered intradoudenally 15 min after the meal.

[d] $P < 0.05$.

[e] Dopamine hydrochloride at 0.6 mg/kg/h was infused intravenously during postprandial period.

[f] Drugs were given intradoudenally 1 min after the meal.

emptying almost completely. Cisapride at 1 mg/kg induced significant antral contradictions and tended to ameliorate the delayed emptying (Fig. 4 and Table 1).

Post-prandial intravenous infusion of dopamine tended to suppress antral motility and impaired gastric emptying significantly (Fig. 5 and Table 1). EM574 (0.03 mg/kg) given intraduodenally 1 min after the meal tended to stimulate antral motility and reversed the delayed emptying significantly. EM574 (0.01 mg/kg) as well as cisapride (1 mg/kg) tended to induce moderate antral contractions and partially ameliorated the delayed emptying (Fig. 5 and Table 1).

3.3. Effect of EM574 on gastric emptying in atropinized dogs

Atropine pretreatment (0.1 mg/kg), which suppressed test meal-elicited antral contractions almost completely for 60 min, inhibited the increase in the antral motor index by EM574 only during the first 30 min (Fig. 6, upper). The accelerating effect of EM574 on gastric emptying was abolished by atropine (Fig. 6, lower). The plasma ac-

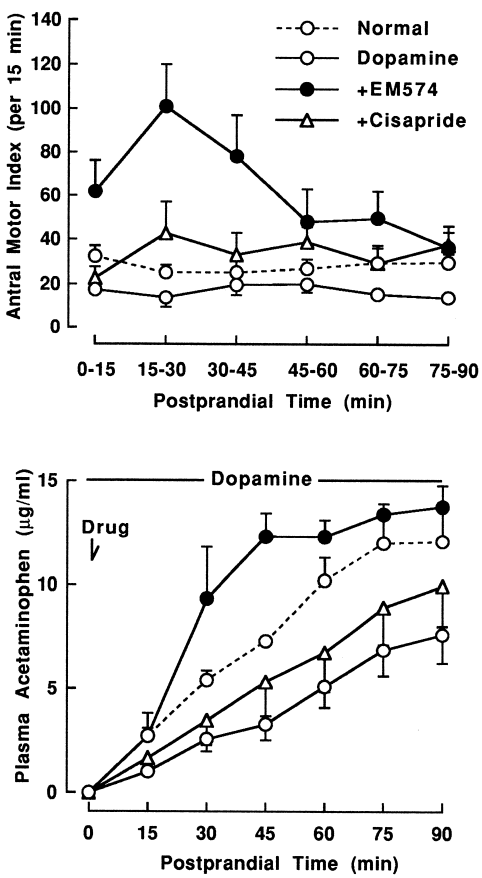

Fig. 5. Changes in gastric antral motor index (upper) and gastric emptying (lower) with EM574 or cisapride dosing in dopamine-induced gastroparesis. Dopamine hydrochloride (0.6 mg/kg/h) was intravenously infused during the 90-min post-prandial period. EM574 (0.03 mg/kg) or cisapride (1 mg/kg) was administered intraduodenally 1 min after the meal. Data are expressed as means ± S.E. for five dogs.

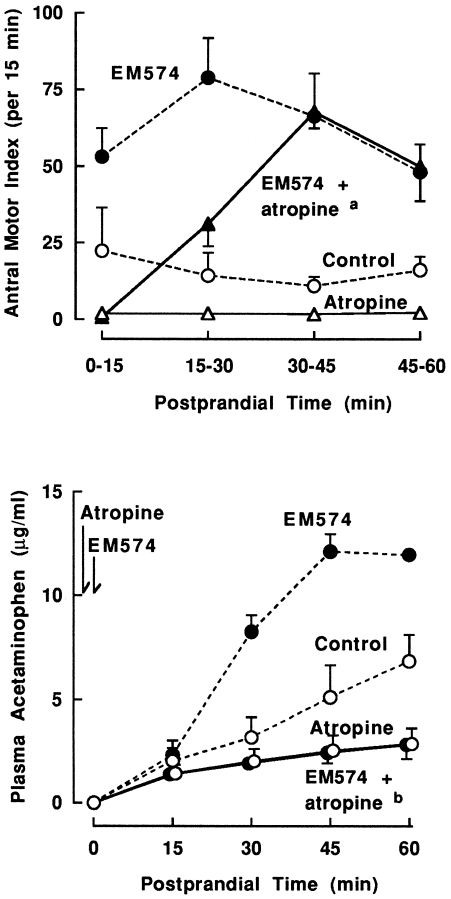

Fig. 6. Inhibition by atropine of the enhancing effects of EM574 on gastric antral motility (upper) and gastric emptying (lower). Atropine sulfate (0.1 mg/kg) was injected intravenously 1 min before intraduodenal administration of EM574 (0.03 mg/kg) or isotonic saline. Data are expressed as means ± S.E. for three dogs. [a] $P < 0.05$, [b] $P < 0.01$ compared to EM574 alone by repeated measures analysis of variance.

etaminophen concentrations in atropinized dogs treated with EM574 were as low as those in saline-treated atropinized dogs.

3.4. Tolerance study

Five days before (as pre-control) and 7 days after (as post-control) repeated treatment with EM574, the 45-min value for plasma acetaminophen was 8.9 ± 0.5 and 10.5 ± 1.1 μg/ml, respectively (not significant; $n = 5$). On the first EM574 (0.03 mg/kg) treatment, the plasma acetaminophen level was increased to 15.3 ± 2.0 μg/ml ($P < 0.05$ compared to pre-control). After repeated treatment with EM574 (0.03 mg/kg, b.i.d.) for 14 consecutive days, the 45-min level was 15.15. ± 2.8 μg/ml on dosing EM574 (0.03 mg/kg). This value was not significantly different from that of pre-control due to the disappearance of the enhancing effect of EM574 observed in one of five dogs. In the rest, the effect of EM574 was as almost same as or more prominent than that seen on day 1. Therefore, the mean acetaminophen values on days 1 and 15 were almost the same.

4. Discussion

To demonstrate the potential usefulness of any given prokinetic agent in gastro-intestinal disorders, the prokinetic activity indicated by enhanced contractile activity must be confirmed with a parallel demonstration of enhanced gastric emptying of caloric meals. We measured gastric antral motility and gastric emptying simultaneously, as the enhancement of gastric emptying by erythromycin is associated with strong antral contractions in humans (Sarna et al., 1991; Stacher et al., 1993).

We carried out a gastric emptying test by feeding the dogs caloric semi-solid meals containing acetaminophen, then measured the plasma acetaminophen concentrations according to Heading et al. (1973), who showed that the peak plasma acetaminophen concentration correlates significantly with the half-time of gastric emptying measured with radioisotopes in humans. We used a caloric, semi-solid meal, OKUNOS-A, as a test meal. Harasawa et al. (1979) reported the usefulness and validity of the combination of OKUNOS-A and acetaminophen as test meal for gastric emptying.

EM574 and motilin dose-dependently enhanced gastric antral motility and gastric emptying in normal dogs. While smooth muscular motilin receptors and the agonistic actions of erythromycin derivatives on them have been clarified in rabbits (Bormans et al., 1986; Sato et al., 1997), cats (Depoortere et al., 1993) and humans (Peeters et al., 1988; Satoh et al., 1994), no specific motilin binding sites have been detected in the canine alimentary tract (Peeters et al., 1988; Satoh et al., 1994), no specific motilin binding sites have been detected in the canine alimentary tract (Peeters et al., 1988; our unpublished data). However, we believe that EM574 as well as motilin elicits gastric contractions via putative neuronal motilin receptors in dogs, because the actions were abolished in the interdigestive state (Inatomi et al., 1996) and diminished in the digestive state (this study) by atropine treatment. This study indicated that the dose of EM574, reported as motilin, needed to stimulate antral motility in the digestive state was about 10 times higher than the dose needed in the interdigestive state (Inatomi et al., 1996). This may also support the identity of the sites of actions of motilin with those of EM574. Recently, the existence of neuronal motilin receptors in rabbits has been suggested by results of functional studies (Parkman et al., 1995; Van Assche et al., 1997) and of receptor binding studies (Depoortere and Peeters, 1997; Sato et al., 1997). Further investigations would be needed to reveal the precise mechanisms of the prokinetic actions of erythromycin derivatives as well as of motilin. In this study, cisapride did not enhance normal gastric emptying despite the increase in motility as reported previously

(Wulschke et al., 1986; Edwards et al., 1987). This might have been due to the transient increase in antropyloroduodenal resistance evoked by cisapride (Malbert et al., 1992; Fraser et al., 1993). The prominent enhancing effect of EM574 on gastric emptying could suggest that it induces more coordinated gastroduodenal contractions than does cisapride.

We used two gastroparesis models, induced by intraduodenal instillation of oleic acid and intravenous infusion of dopamine. Lipid or free fatty acid in the lumen of the intestine is a potent inhibitor of gastric emptying (Hunt and Knox, 1968). This inhibition is thought to be mediated mainly by cholecystokinin (Liddle et al., 1986; Kleibeuker et al., 1988), which relaxes the proximal stomach and elicits contractions of the pylorus (Yamagishi and Derbas, 1978). Dopamine acts as an inhibitory modulator of gastro-intestinal motility by reducing both tonic and phasic contractile activity and blocking antroduodenal coordination (Valezuela, 1976; Van Neuten, 1980). It suppresses the release of acetylcholine from the guinea pig stomach (Kusunoki et al. 1985). In both experimentally-induced gastroparesis models, EM574 (0.03 mg/kg) increased the antral motor index significantly and reversed the impaired gastric emptying almost completely. The effects of EM574 were more prominent than those of cisapride in both models. The effect of EM574 (0.01 mg/kg) on the delayed emptying seems to be less potent than its effect in the normal state. This may be a result of the inhibitory actions of oleic acid or dopamine described above.

In atropinized dogs, the acceleration of gastric emptying by EM574 was abolished, with transient inhibition of antral motility, although an atropine-resistant contractile component was observed from 30 min after EM574 administration. This suggests that EM574-induced antral contractions via a cholinergic neural pathway play a very important role in enhancement of gastric emptying. Shiba et al. (1995) have already reported that atropine-resistant contractions in the post-prandial stomach are evoked by EM523, another erythromycin derivative, an effect which is eliminated by coadministration of an NK_1 receptor antagonist and a 5-HT3 receptor antagonist in conscious dogs. The atropine-resistant component alone might be insufficient for antroduodenal coordination.

There is in vitro evidence, using rabbit gastric smooth muscle, that motilin receptor concentration may be down-regulated after continued stimulation with erythromycin (Depoortere et al., 1991). Therefore, a partial form of tolerance during long-term treatment with erythromycin has been proposed (Richards et al., 1993). We investigated the efficacy of EM574 on gastric emptying during 2-week administration of 0.03 mg/kg b.i.d., a dose at which EM574 was proven to have significant accelerating effects on normal and delayed gastric emptying. After the 2-week administration, only one of five dogs showed disappearance of the EM574-induced emptying enhancement. This result indicates the low frequency of tolerance during appropriate pharmacological dosing of EM574 in dogs. However, we could not exclude the possibility that higher doses and/or more prolonged administration of EM574 might induce tolerance. Further clinical investigation regarding the repeated dosing is required.

In conclusion, we have shown that EM574 exerts potent accelerating effects on normal and experimentally delayed gastric emptying of caloric meals in dogs, and suggest that gastric antral contractions stimulated by EM574 via acholinergic neural pathway play an important role in enhancing gastric emptying. The motilin receptor agonist, EM574, seems to be a promising new gastroprokinetic agent.

Acknowledgements

We wish to thank Mr. K. Matsushita for his technical assistance.

References

Adriaenssens, P.I., Prescott, L.F., 1978. High performance liquid chromatographic estimation of paracetamol metabolites in plasma. Br. J. Clin. Pharmacol. 6, 87–88.

Annese, V., Janssens, J., Vantrappen, G., Tack, J., Peeters, T.L., Willemse, P., Cutem, V., 1992. Erythromycin accelerates gastric emptying by inducing antral contractions and improved gastroduodenal coordination. Gastroenterology 102, 823–828.

Bormans, V., Peeters, T.L., Vantrappen, G., 1986. Motilin receptors in rabbit stomach and small intestine. Regul. Pept. 15, 143–153.

Choi, M.-G., Camilleri, M., Burton, D.D., Johnson, S., Edmonds, A., 1998. Dose-related effects of *N*-demthyl-*N*-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal on gastric emptying of solids in healthy human volunteers. J. Pharmacol. Exp. Ther. 285, 37–40.

Craig, D.A., Clarke, D.E., 1990. Pharmacological characterization of a neural receptor for 5-hydroxytryptamine in guinea pig ileum with properties similar to the 5-hydroxytryptamine-4 receptor. J. Pharmacol. Exp. Ther. 252, 1378–1386.

Depoortere, I., Peeters, T.L., 1997. Demonstration and characterization of motilin-binding sites in the rabbit cerebellum. Am. J. Physiol. 272, G994–G999.

Depoortere, I., Peeters, T.L., Vantrappen, G., 1991. Effect of erythromycin and of octreotide on motilin receptor density in the rabbit. Regul. Pept. 32, 85–94.

Depoortere, I., Peeters, T.L., Vantrappen, G., 1993. Distribution and characterization of motilin receptors in the cat. Peptides 14, 1153–1157.

Edwards, C.A., Holden, S., Brown, C., Read, N.W., 1987. Effect of cisapride on the gastrointestinal transit of a solid meal in normal human subjects. Gut 28, 13–16.

Fraser, R., Horowitz, M., Maddox, A., Dent, J., 1993. Dual effects of cisapride on gastric emptying and antropyloroduodenal motility. Am. J. Physiol. 264, G195–G201.

Harasawa, S., Tani, N., Suzuki, S., Miwa, M., Sakita, R., Nomiyama, T., Miwa, T., 1979. Gastric emptying in normal subjects and patients with peptic ulcer: a study using the acetaminophen method. Gastroenterol. Jpn. 14, 1–10.

Heading, R.C., Nimmo, J., Prescott, L.F., Tothill, P., 1973. The depen-

dence of paracetamol absorption on the rate of gastric emptying. Br. J. Pharmacol. 47, 415–421.

Hunt, J.N., Knox, M.T., 1968. A relation between the chain length of fatty acids and the slowing of gastric emptying. J. Physiol. (London) 194, 327–336.

Inatomi, N., Satoh, H., Maki, Y., Hashimoto, N., Itoth h, Z., Ōmura, S., 1989. An erythromycin derivative, EM-523, induced motilin-like gastrointestinal motility in dogs. J. Pharmacol. Exp. Ther. 251, 707–712.

Inatomi, N., Sato, F., Marui, S., Itoh, Z., Ōmura, S., 1996. Vagus-dependent and vagus-independent mechanism of action of the erythromycin derivative EM574 and motilin in dogs. Jpn. J. Pharmacol. 71, 29–38.

Itoh, Z., Nakaya, M., Suzuki, T., Arai, H., Wakabayashi, K., 1984a. Erythromycin mimics exogenous motilin in gastrointestinal contractile activity in the dog. Am. J. Physiol. 247, G688–G694.

Itoh, Z., Suzuki, T., Nakaya, M., Inoue, M., Mitsuhashi, S., 1984b. Gastrointestinal motor-stimulating activity of macrolide antibiotics and analysis of their side effects on the canine gut. Antimicrob. Agents Chemother. 26, 863–869.

Itoh, Z., Suzuki, T., Nakaya, M., Inoue, M., Arai, H., Wakabayashi, K., 1985. Structure–activity relation among macrolide antibiotics in initiation of interdigestive migrating contractions in the canine gastrointestinal tract. Am. J. Physiol. 248, G320–G325.

Janssens, J., Peeters, T.L., Vantrappen, G., Tack, J., Urbain, J.L., De Roo, M., Muls, E., Bouillon, R., 1990. Improvement of gastric emptying in diabetic gastroparesis by erythromycin. N. Engl. J. Med. 322, 1028–1031.

Kleibeuker, J.H., Beekhuis, H., Jansen, J.B.M., Pipers, D.A., Lambers, C.B.H.W., 1988. Cholecystokinin is a physiological hormonal mediator of fat-induced inhibition of gastric emptying in man. Eur. J. Clin. Invest. 18, 173–177.

Kusunoki, K., Taniyama, K., Tanaka, C., 1985. Dopamine regulation of [^{3}H]acetylcholine release from guinea pig stomach. J. Pharmacol. Exp. Ther. 234, 713–719.

Liddle, R.A., Morita, E.T., Conrad, C.K., Williams, J.A., 1986. Regulation of gastric emptying in humans by cholecystokinin. J. Clin. Invest. 77, 992–996.

Malbert, C.H., Serthelon, J.P., Dent, J., 1992. Changes in antroduodenal resistance induced by cisapride in conscious dogs. Am. J. Physiol. 263, G202–G208.

Maliakkal, B.J., Polodori, G., Gordon, C., Davis, L., Desai, T.K., 1991. Severe gastroparesis following cancer chemotherapy and prokinetic response to erythromycin. Gastroenterology 100, A466.

Mozwecz, H., Pavel, D., Pitrak, D., Orellana, P., Schlesinger, P.K., Layden, T.J., 1990. Erythromycin stearate as prokinetic agent in postvagotomy gastroparesis. Dig. Dis. Sci. 35, 902–905.

Omura, S., Tsuzuki, K., Sunazuka, T., Marui, S., Toyoda, H., Inatomi, N., Itoh, Z., 1987. Macrolides with gastrointestinal motor stimulating activity. J. Med. Chem. 30, 1941–1943.

Parkman, H.P., Pagano, A.P., Vozzelli, M.A., Ryan, J.P., 1995. Gastrokinetic effects of erythromycin: myogenic and neurogenic mechanisms of action in rabbit stomach. Am. J. Physiol. 269, G418–G426.

Peeters, T.L., Bormans, V., Vantrappen, G., 1988. Comparison of motilin binding to crude homogenates of human and canine gastrointestinal smooth muscle tissue. Regul. Pept. 23, 171–182.

Richards, R.D., Davenport, K.G., Hurm, K.D., McCallum, R.W., 1993. The treatment of idiopathic and diabetic gastroparesis with acute intravenous and chronic oral erythromycin. Am. J. Gastroenterol. 88, 203–207.

Sarna, S.K., Soergel, K.H., Koch, T.R., Stone, J.E., Wood, C.M., Ryan, R.P., Arndorfer, R.C., Cavanaugh, J.H., Nellans, H.N., Lee, M.B., 1991. Gastrointestinal motor effects of erythromycin in humans. Gastroenterology 101, 1488–1496.

Sato, F., Sekiguchi, M., Marui, S., Inatomi, N., Shino, A., Itoh, Z., Ōmura, S., 1997. EM574, an erythromycin derivative, is a motilin receptor agonist in the rabbit. Eur. J. Pharmacol. 322, 63–71.

Satoh, M., Sakai, T., Sano, I., Fujikura, K., Kayama, H., Oshima, K., Itoh, Z., Ōmura, S., 1994. EM-574, an erythromycin derivative, is a potent motilin receptor agonist in human gastric antrum. J. Pharmacol. Exp. Ther. 271, 574–579.

Shiba, Y., Mizumoto, A., Inatomi, N., Haga, N., Yamamoto, O., Itoh, Z., 1995. Stimulatory mechanism of EM523-induced contractions in postprandial stomach of conscious dogs. Gastroenterology 109, 1513–1521.

Stacher, G., Peeters, T.L., Bergmann, H., Wiesnagrotzki, S., Schneider, C., Granser-Vacariu, G.V., Gaupmann, G., Kugi, A., 1993. Erythromycin effects on gastric emptying, antral motility and plasma motilin and pancreatic polypeptide concentrations in anorexia nervosa. Gut 34, 166–172.

Tomomasa, T., Kuroume, T., Arai, H., Wakabayashi, K., Itoh, Z., 1986. Erythromycin induces migrating motor complex in human gastrointestinal tract. Dig. Dis. Sci. 31, 157–161.

Tsuzuki, K., Sunazuka, T., Marui, S., Toyoda, H., Ōmura, S., Inatomi, N., Itoh, Z., 1989. Motilides, macrolides with gastrointestinal motor stimulating activity: I. *O*-Substituted and tertiary *N*-substituted derivatives of 8,9-anhydroerythromycin A 6,9-hemiacetal. Chem. Pharm. Bull. 37, 2687–2700.

Valezuela, J.E., 1976. Dopamine as a possible neurotransmitter in gastric relaxation. Gastroenterology 71, 1019–1022.

Van Assche, G., Depoortere, I., Thijs, T., Janssens, J.J., Peeters, T.L., 1997. Concentration-dependent stimulation of cholinergic motor nerves or smooth muscle by [Nle^{13}]motilin in the isolated rabbit gastric antrum. Eur. J. Pharmacol. 337, 267–274.

Van Neuten, J.M., 1980. Is dopamine an inhibitory modulator of gastric motility?. Trends Pharmacol. Sci. 9, 233–235.

Wulschke, S., Ehrlein, H.J., Tsiamitas, C., 1986. The control mechanisms of gastric emptying are not overridden by motor stimulants. Am. J. Physiol. 251, G744–G751.

Xynos, E., Mantides, A., Papageorgiou, A., Fountos, A., Pechlivanides, G., Vassilakis, J.S., 1992. Erythromycin accelerates delayed gastric emptying of solids in patients after truncal vagotomy and pyloroplasty. Eur. J. Surg. 158, 407–411.

Yamagishi, T., Debas, H.T., 1978. Cholecystokinin inhibits gastric emptying by acting on both proximal stomach and pylorus. Am. J. Physiol. 234, E375–E378.