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Gastrointestinal Motor-Stimulating Activity of Macrolide Antibiotics and Analysis of Their Side Effects on the Canine Gut

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For clarification of the nature of the side effects of macrolide antibiotics on the gastrointestinal tract, the motor-stimulating activity of these agents was studied in unanesthetized dogs. The results showed that erythromycin and oleandomycin, the 14-membered macrolides with two side chain sugars combined at C3 and C5 in a glycosidic linkage in parallel, strongly stimulate gastrointestinal motor activity, an action accompanied by vomiting at large doses. On the other hand, leucomycin, acetylspiramycin, and tylosin, belonging to a 16-membered macrolide with two side chain sugars in series combined at C5 of the lactone ring, did not induce contractions of the gastrointestinal tract. Motor-stimulating activity by erythromycin and oleandomycin was greatly inhibited by atropine sulfate. These results point to structure-physiological activity relationships.

Erythromycin (EM), oleandomycin (OM), kitasamycin (= leucomycin [LM]), and spiramycin (SPM), macrolides with a giant lactone ring (16), have wide clinical use. The incidence of side effects produced by these antibiotics is low. Hepatic (4, 9, 10) and gastrointestinal disorders (1, 2, 11, 12) including anorexia, abdominal pain, nausea, vomiting, and diarrhea are most commonly seen. It has recently been reported that gastrointestinal side effects are more frequent with intravenous administration of EM (1, 2, 12) than with oral administration (11). However, the actual nature of the side effects induced by macrolides in the gastrointestinal tract has not been clearly understood. In the present study, we examined in dogs the gastrointestinal motor-stimulating activity of the macrolide antibiotics currently used in medical and veterinary clinics.

MATERIALS AND METHODS

Preparation of animals. Four mongrel dogs (two males and two females) weighing 10 to 15 kg were used in the present study. They were anesthetized by intravenous (i.v.) injection of pentobarbital sodium (25 mg/kg of body weight), after which the abdominal cavities were opened and, as described previously (8), force transducers (7) were sutured on the serosa of the gastrointestinal tract, positioned to record circular muscle contraction in the gastric body (opposite the splenic hilum), the gastric antrum 3 cm proximal to the pyloric ring, the mid-duodenum (opposite the opening of the pancreatic duct), and the upper jejunum (20 cm distal to the Lig. Treitz). Lead wires were brought from the abdominal cavity through a skin incision between the scapulae and fixed onto the adjacent skin with silk sutures. After closure of the abdominal wound, a Silastic tube was implanted into the superior vena cava through a branch of the external jugular vein. The outer end of the tube was fixed with silk onto the skin adjacent to the incision. This tube, filled with a heparinized physiological saline, was used for i.v. injection of test materials and withdrawal of blood samples for measurement of concentrations of antibiotic in plasma (14). After surgery, the dogs were placed in canvas jackets to protect the lead wires and the Silastic tube from being scratched by the dogs. The dogs were housed individually and fed with a dry-type Gaines meal (20 g/kg of body weight) once a day during the experimental period. Gastrointestinal motor activity was recorded on a polygraph (RM 45, Nihon Kohden Kogyo, Co., Ltd., Tokyo, Japan) by connecting cables from the amplifiers to the lead wires of the transducers under the protector.

Experimental procedures. Test materials for i.v. injection were dissolved in normal saline and administered by a 10-s bolus injection through the implanted jugular type; the tube was flushed with 5 ml of normal saline after each injection of test materials. Oral administration was done by inserting tablets into the esophagus. The motor-stimulating activity of an antibiotic was tested three times at three different doses in each of the dogs. Motor-stimulating activity was quantified by using an integrator connected to the amplifier to measure the gastric motor activity in the area from the base line to the contractile wave from the injection time to 10 min after injection in each of the four dogs. The motor-stimulating effects of the antibiotics on the gastrointestinal tract are shown in actual recordings.

Measurement of macrolide concentrations in plasma. The concentrations of the macrolides in serum were determined by a paper disk technique (14), using *Micrococcus luteus* ATCC 9341 as the test organism. The procedure was as follows. Ten milliliters of the agar medium inoculated with 1% of an overnight culture of *M. luteus* ATCC 9341 was poured onto each plate. After the inoculated agar solidified, a paper disk (8 mm in diameter, Toyo Seisakusho Co., Tokyo, Japan) of the standard solution or sample was placed onto each plate. The plates were kept at 4°C for 1 h for preliminary diffusion and then were incubated at 37°C. Samples were compared with a standard diluted in serum by determining the zone diameter after 18 h of incubation. All controls and samples were run in triplicate paper disk. The determination limit of this assay was 0.05 μg of macrolide per ml of serum.

Test materials. Macrolides examined by i.v. injection in the present study included EM lactobionate (Erythrocin-i.v., Abbott Laboratories, North Chicago, Ill.), OM phosphate (Matromycin, Taito-Pfizer, Tokyo, Japan), kitasamycin tartrate (Leucomycin, Toyo Jyozo, Tokyo, Japan), tylosin tartrate (Tylocine, Eli Lilly-Shionogi Seiyaku, Osaka, Japan) and acetylspiramycin (Acetylspiramycin, Kyowa Hakko, Tokyo, Japan). The structures of these agents are shown in

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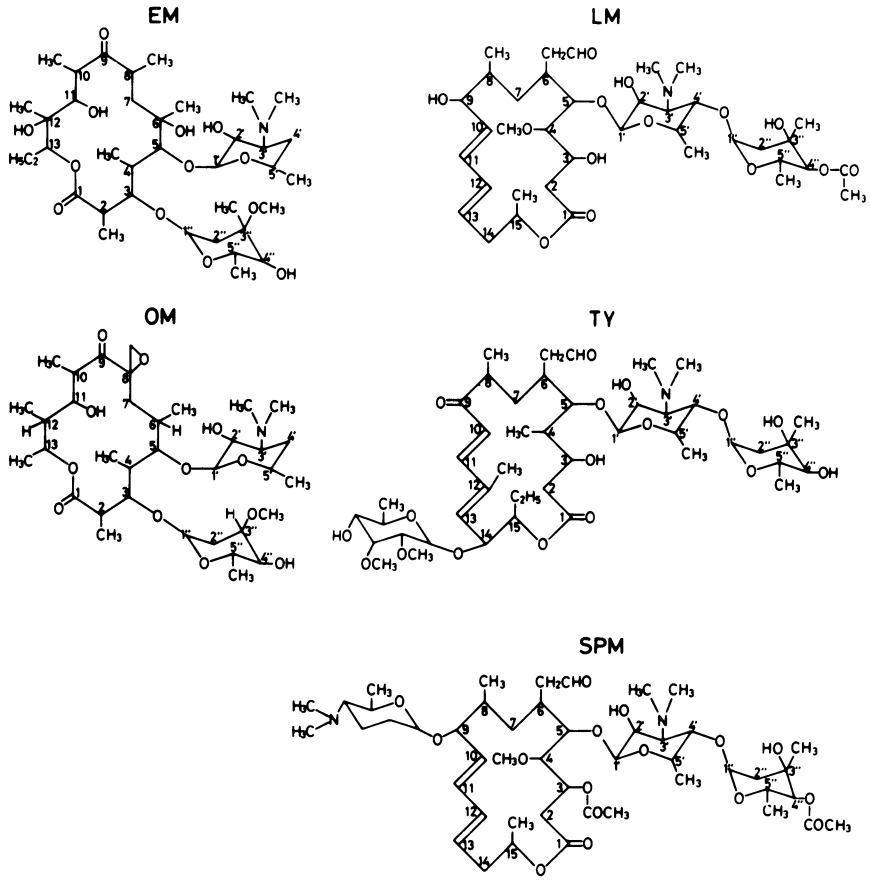

FIG. 1. Chemical structures of the 14- and 16-membered macrolides. The 14-membered macrolide antibiotics, Em and OM, have two side chain sugars in parallel at C3 and C5. The 16-membered macrolide antibiotics have two double bonds in the lactone ring and two side chain sugars combined in series at C5 of the lactone ring. Tylocin (Ty) and acetylspiramycin (SPM) are combined with another side chain sugar at C9 and C14, respectively.

Fig. 1. For injection, they were dissolved in distilled water and diluted with normal saline to the required concentration and injected i.v. in 10 s during the quiescent period in gastric motor activity in the interdigestive state. EM stearate (Erythrocin Tablet; Abbott) and kitasamycin (Leucomycin Tabs., Toyo Jyozo) were used for oral administration. Three different doses were examined, but no doses greater than 30 mg/kg of body weight were tested even if an antibiotic had no activity. Atropine sulfate was used as a pharmacological blocker.

Analysis of data. Quantitative gastric motor-stimulating activity was expressed as the mean of 12 observations (3 observations in each of the four dogs) ± standard error of the mean for each dose of each antibiotic, and statistical significance was calculated by Student's *t* test. *P* values less than 0.05 were considered significantly different between paired data.

RESULTS

Control contractile pattern of the gut in the interdigestive state. Figure 2 shows the changes in contractile activity of the gastric body, the gastric antrum, the mid-duodenum, and the upper jejunum in an unanesthetized dog over a period of 8 h. The interdigestive migrating contractions (IMC) in this particular dog occurred in the gastric body and antrum and the duodenum simultaneously at constant intervals (6), continued for a mean time of 26.3 ± 2.4 min, and stopped spontaneously as reported previously by us (8) and others (3, 5, 15). Toward the end of the period of the gastric IMC, the upper jejunum started strong contractions; the IMC in the stomach and the duodenum migrated to the upper jejunum in this way. During the period between the neighboring IMCs, there were no contractions in the stomach and the upper small intestine; in the stomach, the quiescence lasted for an average of 98.4 ± 13.6 min in four dogs as previously reported by us (6, 7) and others (3, 5, 15). While the dogs were healthy, the above-mentioned regular contractile pattern was constantly observed during the interdigestive state. Therefore, motor-stimulating activity was studied during the quiescent period of the interdigestive state. Intravenous injections of 10 ml of normal saline and oral administration of placebo (lactose) tablets during the quiescence of the interdigestive state did not affect gastrointestinal motor activity (Fig. 2, arrows).

Effect of i.v. injection of EM on gastrointestinal motor activity. (i) General contractile response. Figure 3 shows changes evoked by i.v. injection of EM (3 mg/kg of body weight) in contractile activity of the gastrointestinal tract in an unanesthetized dog over a period of 120 min. Strong contractions were induced in the stomach, duodenum, and jejunum, lasted for ca. 10 min, and then gradually declined. The tonicity of the gastric body increased rapidly to its maximum and did not return to the basal level for ca. 10 min. The contractile response had not stopped completely at 110

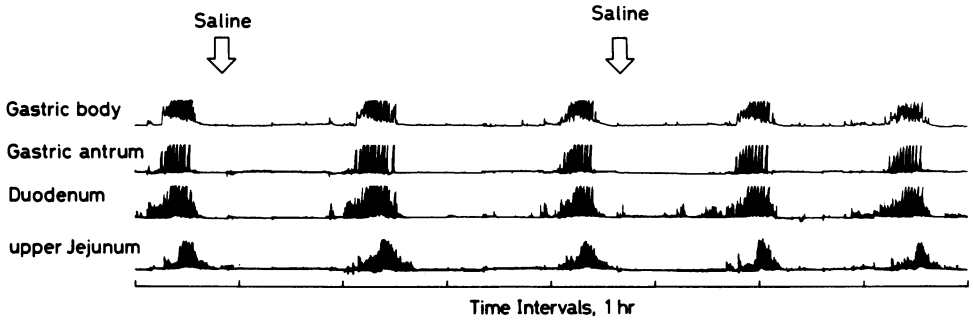

FIG. 2. Changes over 8 h in contractile activity in the gastric body, gastric antrum, duodenum, and upper jejunum during the interdigestive state in a dog. The characteristic feature of the interdigestive motor pattern is the regular occurrence of a group of strong contractions in the stomach and duodenum at constant intervals, with migration of these contractions to the upper jejunum. Arrows indicate i.v. bolus injections of normal saline, which did not influence contractile activity in the four transducer sites. Motor-stimulating activity of test materials was evaluated under this conditionsbp.

min after injection. The contractile force in the stomach was strong and similar to that of the gastric IMC, which is the strongest of all contractions that persist for 24 h. EM-induced contractions of the duodenum and jejunum sometimes exceeded the natural IMC of the duodenum and jejunum. The interdigestive regular motor pattern was greatly disrupted by an i.v. injection of EM at doses larger than 1.0 mg/kg of body weight and did not return again on the experimental day. EM injection was without effect on the gastrointestinal tract on the following days.

(ii) Detailed contractile pattern. Figure 4 shows detailed changes in the initial contractile response to an i.v. injection of 1.0 mg of EM per kg. The gastric body responded quickly, and its tonicity rose to a level similar to the maximum of the natural IMC. The frequency of tonic contractions is difficult to determine, but the number of tonic peaks was significantly larger than those of the natural IMC. The contractile response of the gastric antrum was a little slower than that at the gastric body, but the contractile force was also as strong as the natural IMC. Contractions in the duodenum, though not coordinated with those in the gastric antrum in the initial period, became coordinated with the gastric contractions within 4 to 5 min of injection. The upper jejunum responded immediately, but the maximum contractions were observed within 4 to 8 min of injection. The frequency of contractions at four sites was slightly increased during the immediate postinjection period but soon returned to the normal control level.

(iii) Dose-response relationship. In the present study, contractions in the gastric antrum induced by i.v. administration of EM and OM were analyzed quantitatively by measuring the area encompassing the contractile waves and base line. The results are shown in Fig. 5. Quantitative motor activity induced by three different doses of EM and OM was found to be significantly dose-related in the gastric antrum (Fig. 5). The regression line slopes for EM and OM are almost identical.

Effect of i.v. injection of other macrolide antibiotics on gastrointestinal motor activity. (i) OM. OM-induced gastrointestinal contractions are shown in Fig. 6. Strong contractions of the stomach and small intestine, similar to those produced by EM, were induced by i.v. injection of 27 mg of OM per kg. Characteristics of the contractile pattern induced by OM were identical in frequency and contractile force to those induced by EM (Fig. 6). The dose-response relationship of OM was identical to that of EM (Fig. 5), though the quantitative motor-stimulating activity induced by OM was much weaker than that evoked by EM.

(ii) LM. At a dose of 5 mg/kg, LM did not induce contractions in the gastrointestinal tract. Therefore, a dose

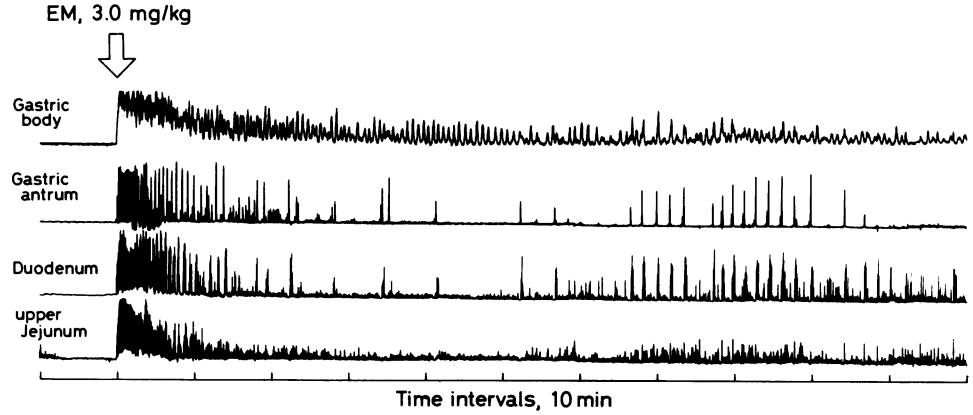

FIG. 3. Changes over 2 h in gastrointestinal motor activity in response to i.v. bolus injection of a dose of 3.0 mg of EM per kg. Very strong contractions were induced immediately in all four transducer sites and gradually declined. In the gastric body, strong slow phasic contractions continued for more than 2 h, but major motor response in the gastric antrum, duodenum, and upper jejunum terminated 20 to 30 min after injection. In this case, the dog did not vomit.

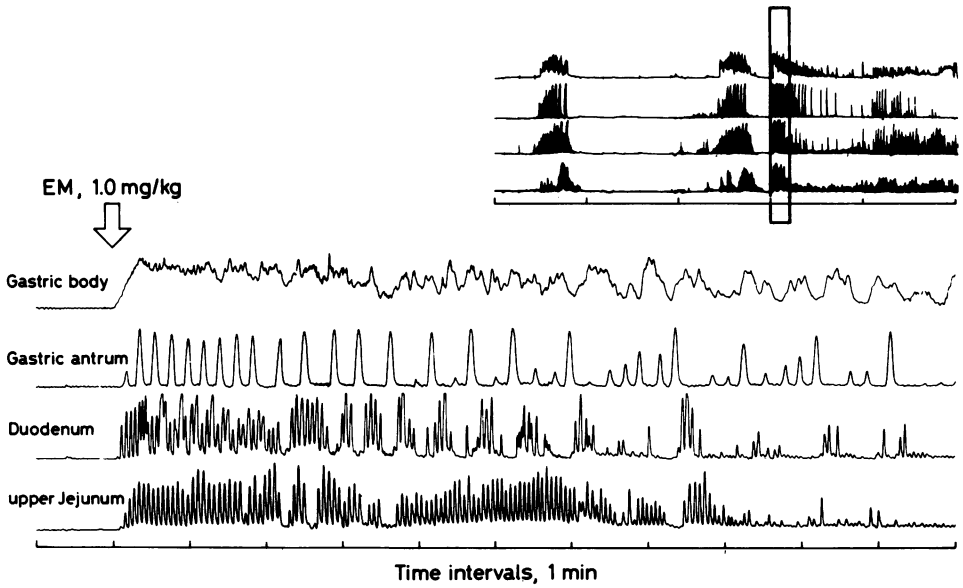

FIG. 4. Detailed 12-min contractile changes in the stomach, duodenum, and jejunum of a dog in response to a dose of 1.0 mg of EM per kg. Contractile response occurred immediately after injection of EM, but contractile response in the gastric antrum was always delayed for ca. 10 s. The initial response during the first 5 min was not coordinated between the neighboring transducer sites. However, the contractions became gradually coordinated, as shown in the right half of this figure. Vomiting always occurred during the initial period when contractions were not coordinated. The inset indicates the same record surrounded by a square and its immediately previous and subsequent changes taken at a slow paper speed for 5 h.

of 25 mg/kg (ca. 8 times the maximum dose of EM) was tested; the results are shown in Fig. 7. Even at this dose level, LM did not affect contractile activity of the stomach, duodenum, or jejunum. Administration of LM did not influence the IMC cycle.

(iii) SPM and tylosin. Neither SPM nor tylosin at doses of 25 mg/kg induced contractions in the gastrointestinal tract of the dog.

Effect of oral administration of EM and LM on gastrointestinal motor activity. Figure 8 shows the effect of oral administration of EM (3 and 10 mg/kg) on gastrointestinal contractile activity and on the concentrations of EM in plasma. Fifty minutes after oral administration, a group of strong contractions were evoked, and the increase in the plasma EM concentration coincided well with the increase in gastrointestinal motor activity (Fig. 8). It was found that the occurrence of contractions was closely related to the increase in the plasma EM concentration. Mean peak values after doses of 3 and 10 mg/kg were 5.2 ± 0.3 and 8.2 ± 0.4 μg of EM per ml of plasma; 1.0-mg/kg doses did not evoke contractions or give measurable plasma concentrations. Oral administration of LM in doses up to 3 mg/kg did not induce contractions of the gastrointestinal tract, even though plasma LM concentrations of 5.8 μg/ml were attained with oral doses of 30 mg/kg.

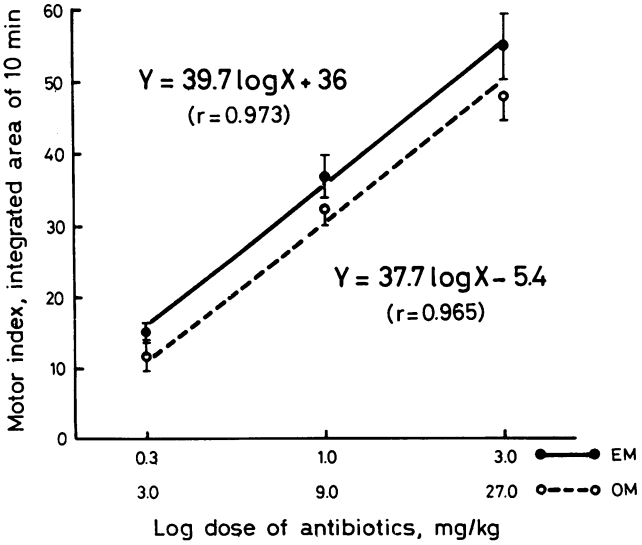

FIG. 5. Motor index, 10-min integrated area between contractile wave and base line as a function of log dose of EM and OM obtained from three observations in each of three dogs.

Blockade by atropine. Atropine sulfate (at a single bolus dose of 0.5 mg/kg and continuous i.v. infusion of 0.05 mg/kg per h) strongly inhibited contractions—induced by 5.0- and 10.0-mg/kg doses of EM—in the stomach, the duodenum, and the jejunum in a dog (Fig. 9). OM-induced contractions were also inhibited by atropine sulfate.

Side effects other than those on motor activity. EM and OM sometimes induced lip licking, nausea, and vomiting (a foamy brown viscous fluid) when the doses tested induced strong and long-lasting contractions (Fig. 6). However, the dogs ate their meals vigorously 3 to 4 h after the experiment even if they vomited in response to EM or OM. Other significant symptoms such as restlessness, tachypnea, tachycardia, salivation, etc., were not observed with EM or OM. During the experiments, the dogs were generally quiet and slept curled up in the experimental cage. Urination and defecation were sometimes evoked by large doses of EM, but no other symptoms were observed. LM, SPM, and tylosin did not affect appetite on the experimental day, nor did they evoke other untoward reactions.

DISCUSSION

The results of the present study showed that during the interdigestive state in a dog, a single i.v. injection of EM or OM induced strong contractions of the gastrointestinal tract. Contractile response to these drugs was dose-dependent.

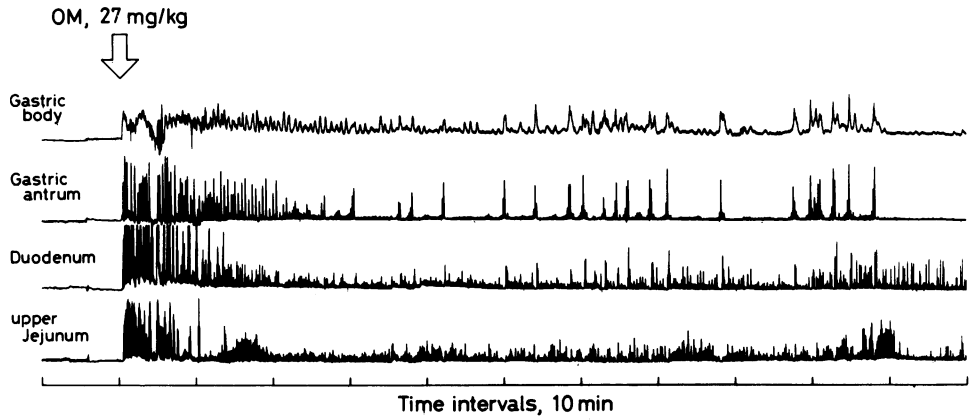

FIG. 6. Changes over 2 h in contractile responses of the gastrointestinal tract after a dose of 27 mg of OM per kg. Characteristics of contractile response were quite similar to those with EM (Fig. 3), but the motor-stimulating activity of OM was ca. one-tenth that of EM.

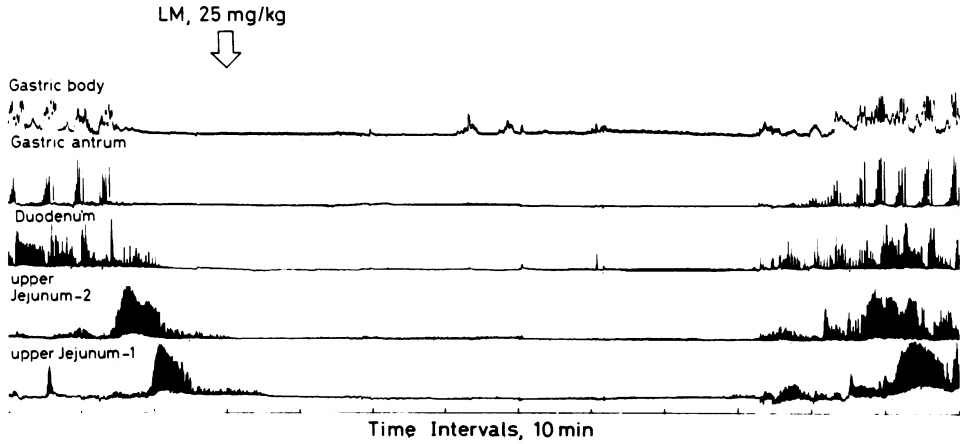

FIG. 7. A dose of 25 mg of LM per kg did not stimulate gastrointestinal motor activity, and the interdigestive contractile pattern was not affected by LM injection. In fact, the next cycle is seen to occur 115 min after the termination of the IMC in which LM was given.

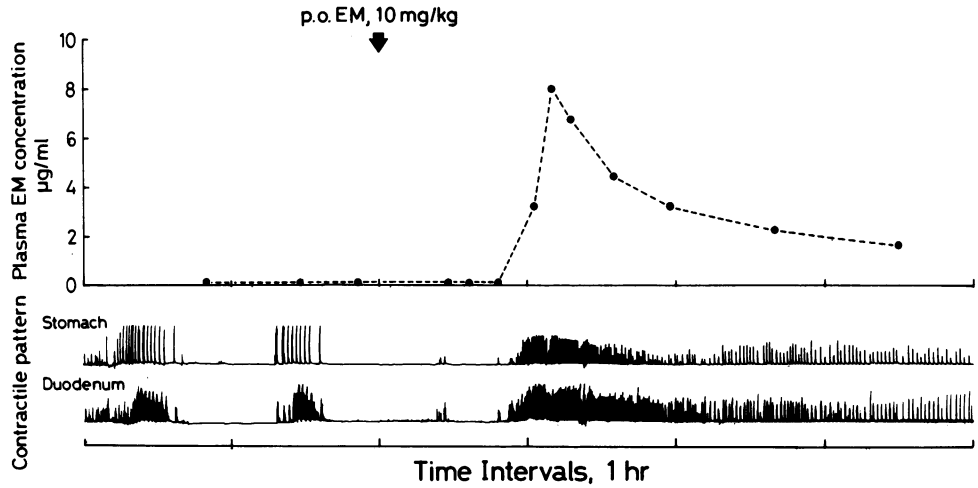

FIG. 8. Effect of oral administration of 10 mg of EM per kg on the plasma EM concentration (upper panel) and gastroduodenal motor activity (lower panel) in a dog. In association with the increase in plasma EM concentration, gastroduodenal contractile activity was increased. Contractile responses in the stomach and duodenum to EM were different from those of the interdigestive migrating contractions seen before EM administration. Gastroduodenal contractile activity remained increased as long as the plasma EM concentration was elevated.

The characteristic feature of the contractions was simultaneous and immediate occurrence of strong contractions in the gastric body and antrum, duodenum, and jejunum. The force of these contractions in the gastrointestional tract sometimes exceeded the maximum force of the IMC, the strongest contractions observed in the natural state (8).

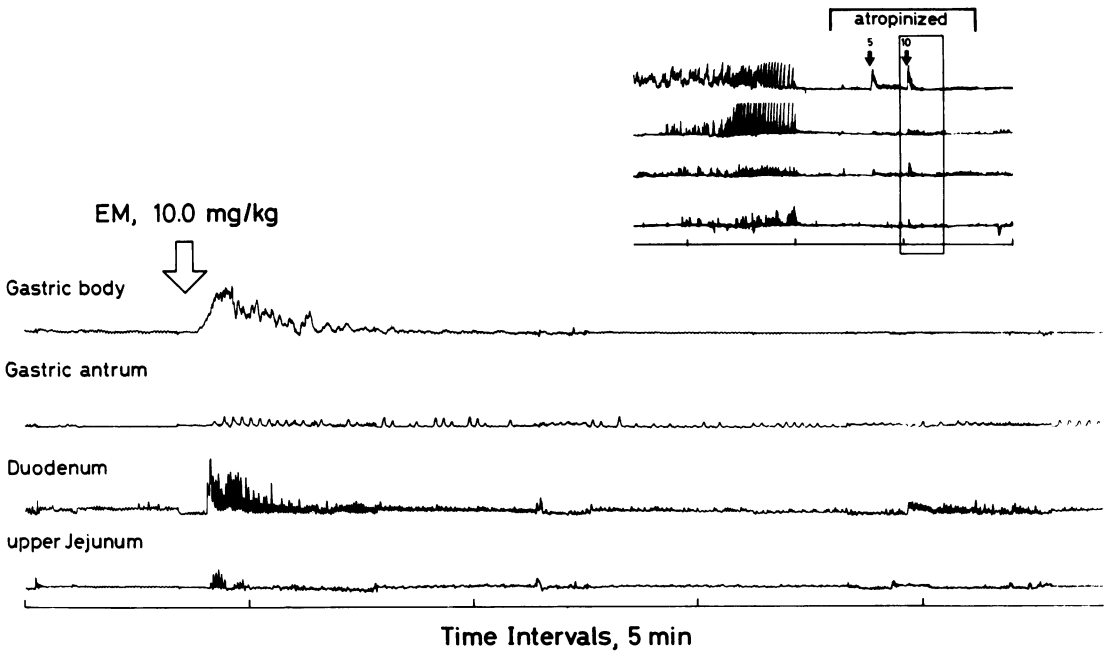

FIG. 9. Inhibition of the effect of 10.0 mg of EM per kg on gastrointestinal motor activity by pretreatment of the animal with atropine sulfate in a single i.v. bolus of 0.5 mg/kg and a continuous i.v. infusion of 0.01 mg/kg per h. The inset indicates the same record surrounded by a square and its immediately previous and subsequent changes taken at a slow paper speed for 3.5 h. The 5 and 10 in the inset indicate single bolus injections of 5 and 10 mg respectively, of EM per kg.

During the initial motor response within 5 min of injection, contractions between the neighboring organs were not coordinated; they became so later. Vomiting occurred during the initial period of motor response. Therefore, slow i.v. infusion may minimize the motor-stimulating activity of these drugs.

Oral administration of EM also evoked strong contractions in the gastrointestinal tract, the dimensions of which were related to the plasma concentration. Contractions induced by oral EM were not similar to the contractions induced by i.v. EM in the contractile pattern; however, the contractile force and frequency of the contractions induced by oral EM were quite similar to those of the contractions induced by i.v. EM. The diversity of the contractile pattern is probably related to differences in the rate of increase in the plasma EM concentration after i.v. and oral administration. Oral administration of LM did not stimulate gastrointestinal motor activity; this was similar to the results of i.v. administration.

It was found that the motor-stimulating activity of EM and OM was greatly inhibited by pretreatment of the animals with atropine. This suggests involvement of a cholinergic pathway in the action of EM or OM.

The results of this study suggested that there was a distinct relationship between chemical structure and gastrointestinal motor-stimulating activity. EM and OM have 14-membered lactone rings to which a dimethylamino sugar and a neutral sugar are attached at C3 and C5 in a parallel glycosidic linkage (Fig. 1). The three other macrolides, which lack the capacity to stimulate gastrointestinal contractions, carry 16-membered lactone rings with two double bonds plus dimethylamino sugar and neutral sugar substitutes in serial glycosidic linkage at C5 of the lactone ring. It is not known why the 16-membered macrolide antibiotics lack gastrointestinal motor-stimulating activity, but the difference in glycosidic linkage of the two side chain sugars may give rise to the results of the present experiment, because the comparison of cubic molecular models in the two groups strongly suggests that a great difference between the two chemical configurations exists in the glycosidic linkage of the two side chain sugars to the lactone ring. Moreover, it is of great interest to note that the 16-membered macrolide antibiotics scarcely induce macrolide resistance in *Staphylococcus aureus* (13). This points to an important area for future development of new macrolides.

In conclusion, the macrolide antibiotics of a 14-membered lactone ring with two side chain sugars in parallel have strong motor-stimulating activity in the gastrointestinal tract and, in consequence, are likely to induce side effects on the gastrointestinal tract if they are given in large doses. If there is no difference in antibacterial activity between 14- and 16-membered macrolides, the preferable chemical conformation of the macrolide antibiotics would be a macrolide ring with two side chain sugars in a glycosidic linkage in series.

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