EFFECT OF NEW NALIDIXIC ACID ANALOGUES ON GASTROINTESTINAL CONTRACTILE ACTIVITY IN CONSCIOUS DOGS : DETECTION OF SIDE-EFFECTS ON THE GASTROINTESTINAL TRACT

ZEN ITOH[1] and SUSUMU MITSUHASHI[2]
Laboratories for Gastroenterology, College of Medical Technology, Gunma University[1], Maebashi and Episome Institute[2], Seta-gun, Gunma

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The effects of intravenous injection of three new nalidixic acid analogues on gastrointestinal contractile activity in conscious dogs were studied to find possible side-effects on the gastrointestinal tract.

It was found that OPC-7241, 9-Fluoro-5-methyl-8-(4-methyl-1-piperazinyl)-6,7-dihydro-1-oxo-1 H,5 H-benzo(ij)quinolizine-2-carboxylic acid, induced retrograde migrating contractions followed by nausea and vomiting in doses of 20 and 40 mg/kg of body weight. The incidence of nausea and vomiting was 100% at a dose of 40 mg/kg, while that of nausea was 67% and of vomiting 27% at 20 mg/kg. It was concluded that OPC-7241 may have reverse effects on the gastrointestinal tract when given to humans.

INTRODUCTION

The induction of side effects is a serious problem in developing new drugs. Among them, adverse effects due to subjective sensations, such as nausea or abdominal discomfort are especially difficult to detect in animal experiments. In many cases, these adverse effects are detected only after phase studies in human have been completed.

We have, therefore, investigated whether side effects of this kind are detectable or not in experimental animals by monitoring gastrointestinal motor activity in unrestrained conscious dogs. In the present report, three different new analogues of nalidixic acid antibacterial agents were studied for their motor stimulating activity in the gastrointestinal tract as well as side effects.

MATERIALS AND METHODS

Preparation of animals. Five healthy adult mongrel dogs were used in the present study. They were anesthetized by intravenous injection of pentobarbital sodium (30 mg/kg of body wt) and force transducers developed in our laboratory were sutured onto the serosal surface of the gastrointestinal tract from the gastric body to the ileum[1]. Each transducer was sutured in the direction of circular muscle contraction. The lead wires of the transducers were taken out from the abdominal cavity through stab wounds made on both sides of the abdominal wall and brought out from a skin incision made between the scapulae through subcutaneous tunnels.

A Silastic tube was introduced into the superior vena cava through the external jugular vein and the outer end of this tube was fixed onto the neck skin adjacent to the skin incision and used as a route for intravenous injection.

Experimental procedures. The dogs were housed in individual experimental cages during the experiments and fed once a day a Gaines meal and given water ad libitum. The lead wires and the Silastic tube were protected from the dog's scratching by means of a jacket protector. Gastrointestinal contractile activity was monitored by connecting cable leads from amplifiers to the lead wires of the implanted force transducers and contractile activity was recorded on a polygraph. Experiments were carried out during the quiescent period of the interdigestive state usually 10-15 min after the termination of the interdigestive migrating contractions (IMC) in the stomach. Test materials were

dissolved in 10 ml of normal saline and injected intravenously through the implanted Silastic tube in about 10 seconds. As a control study, apomorphine was injected subcutaneously in order to induce nausea and vomiting.

The same experiments were repeated 3 times for each dose of the three drugs. The occurrence of side effects other than those on the gastrointestinal tract was assessed by watching the dogs to see whether they exhibited unusual reactions to the injected drug, such as retching, lip licking, urination or defecation, etc.

Test materials. Three analogues of new nalidixic acid were tested in the present study. These agents are reported to be highly active *in vitro* and *in vivo* against gram-negative and gram-positive bacteria.

1. DL-8280 (Ofloxacin)[2] :
   9-Fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2, 3-dihydro-7 H-pyrido-(1, 2, 3-de)1, 4-benzoxazine-6-carboxylic acid.
2. AM-715 (Norfloxacin)[3] :
   1-Ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.
3. OPC-7241[4] :
   9-Fluoro-5-methyl-8-(4-methyl-1-piperazinyl)-6, 7-dihydro-1-oxo-1 H, 5 H-benzo (ij) quinolizine-2-carboxylic acid.

Apomorphine, a gift from Janssen Pharmaceutica, Beeres, Belgium, was used as an emetic.

These drugs were dissolved in normal saline and injected intravenously or subcutaneously as a 10 sec bolus dose.

RESULTS

Interdigestive contractile pattern of the gastrointestinal tract. Figure 1 shows 8-hr changes in gastrointestinal contractile activity before and after feeding in a conscious dog. As can be seen in this figure, the gastrointestinal contractile pattern is quite different before and after feeding ; after feeding gastrointestinal motor activity is continuous and homogenous, whereas before feeding it is cyclic and intermittent. During the interdigestive state, a group of strong contractions occurred in the stomach at constant intervals (mean interval was 123±6.1 min), stopped abruptly and were followed by a long quiescence. However, the contractions in the stomach migrated along the small intestine at a constant velocity in a caudad direction. The characteristic type of contraction seen only during the fasted state is now called "interdigestive migrating contractions (IMC)"[5,6]. Figure 2 demonstrates detailed changes in interdigestive motor activity in the gastrointestinal tract. In the present study, test materials were given at 10-15 min after the termination of the IMC in the stomach.

Induction of nausea and vomiting by apomorphine. Figure 3 shows 12-min changes in gastrointestinal contractile activity when apomorphine

Fig. 1 Gastrointestinal contractile pattern before and after feeding in a conscious dog. During the interdigestive state, a group of contractions occurring in the stomach at constant intervals migrated along the small intestine at a constant velocity. Upon feeding, the contractile pattern abruptly changed to the digestive pattern, which is characterized by continuous regular contractions throughout the gastrointestinal tract.

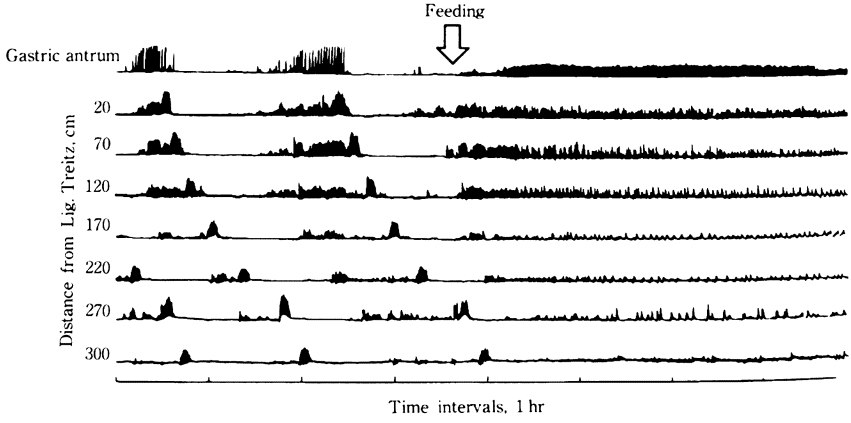


Fig. 2 Detail changes in contractile activity in the gastrointestinal tract during the interdigestive tract. A group of strong contractions which occurred in the stomach and the duodenum stopped abruptly and migrated along the small intestine.

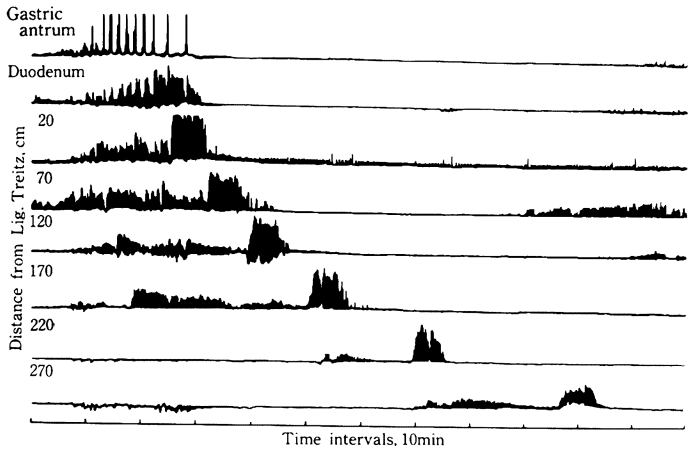
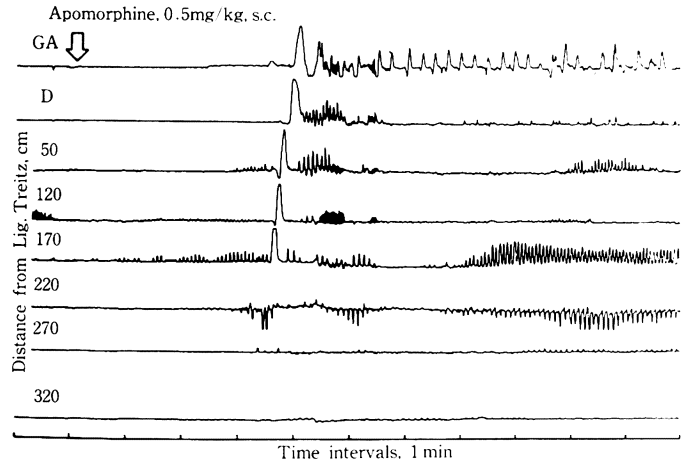


Fig. 3 Effect of apomorphine (0.5 mg/kg of body wt) on gastrointestinal contractile activity in a conscious dog. A strong contraction was induced in the small intestine at 170 cm distal to the ligament of Treitz and the contraction migrated in an orad (retrograde) direction. After the contraction reached the gastric antrum, vomiting occurred.



(0.5 mg/kg) was injected subcutaneously during a quiescent period of the interdigestive state in a conscious dog. A series of strong contractions originating in the small intestine are seen to migrate in an oral (retrograde) direction and vomiting was always observed after the retrograde migrating contraction reached the gastric antrum, as indicated by two arrows. These motor events were followed by irregular contractions in the gastrointestinal tract thereafter. The strong retrograde migrating contractions are particularly characterized only by nausea and vomiting. On the other hand, smaller doses of apomorphine did not always induce nausea or vomiting, but retrograde migrating contractions could be induced as shown in Figure 4. In this case, the dose of apomorphine was reduced to 0.05 mg/kg and the dog did not exhibit any noticeable symptoms.

With this dose, the incidence of nausea was less than 25% in each of the 5 dogs. The decrease in

tonicity in the gastric body for about one min may indicate an entry of the intestinal contents propelled back into the stomach by the strong retrograde migrating contractions. The initiation of retrograde migrating contractions always originated at the mid-intestine, 170 cm distal to the ligament of Treitz, although the dose of apomorphine was different[7]. The velocity of the retrograde migrating contractions from the site of origination to the gastric antrum was not significantly different among the 5 dogs, the mean velocity being 6.2± 2.1 cm/sec.

Bioassay for side-effects of three antibacterial agents on the gastrointestinal contractile activity. Figure 5 shows the effect of ofloxacin (40 mg/kg) on gastrointestinal contractile activity

Fig. 4 Effect of subcutaneous injection of apomorphine (0.05 mg/kg) on gastrointestinal contractile activity in a conscious dog. A strong contraction was induced at a similar site in the small intestine to the case in Fig. 3 and migrated in a retrograde direction. However, nausea and vomiting did not occur in this case.

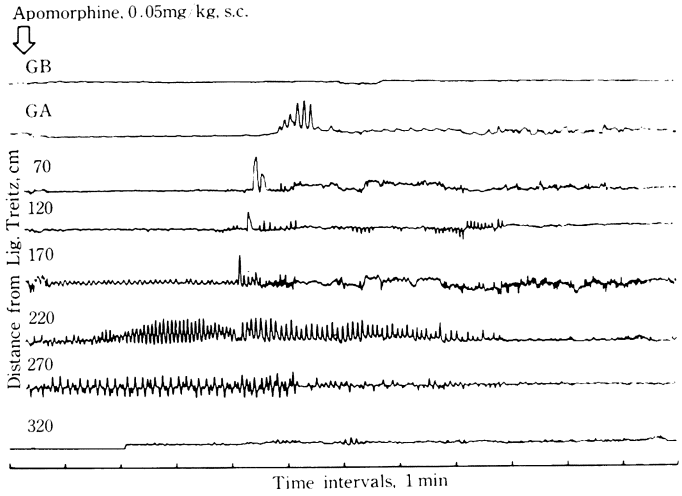
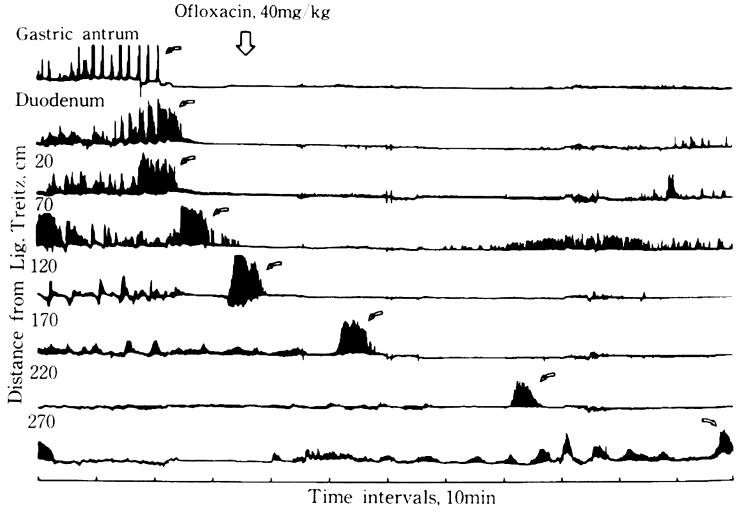


Fig. 5 Effect of intravenous injection of ofloxacin (40 mg/kg) on gastrointestinal contractile activity in a conscious dog. Arrows indicate the normal migration of the interdigestive migrating contractions. No noticeable contractile response was observed.



n a conscious dog. Arrows indicate caudal migration of the interdigestive migrating contractions. n the present study, the antibiotic was given 10 iin after the termination of the IMC in the omach. As seen in this figure, ofloxacin did not iduce any noticeable contraction in the gastrointestinal tract, nor did it disrupt the caudal migration the IMC along the small intestine. The cycle of regular occurrence of IMC in the stomach was not affected by intravenous injection of this antibacterial agent in any of the five dogs. In Figure 6, the effect of intravenous injection of norfloxacin (40 mg/kg) on gastrointestinal contractile activity is shown. It was found that norfloxacin also had no significant effect on gastrointestinal contractile activity. The caudad migration of the IMC along

Fig. 6 Effect of intravenous injection of norfloxacin (40 mg/kg) on gastrointestinal contractile activity in a conscious dog. AM-715 did not induce any significant contractions nor did it interrupt the migration of the interdigestive migrating contractions as indicated by arrows. In this case, the cycle of regular occurrence of the interdigestive migrating contractions in the stomach was not affected ; initiation of the next cycle indicated by arrows on the right-hand side is seen.

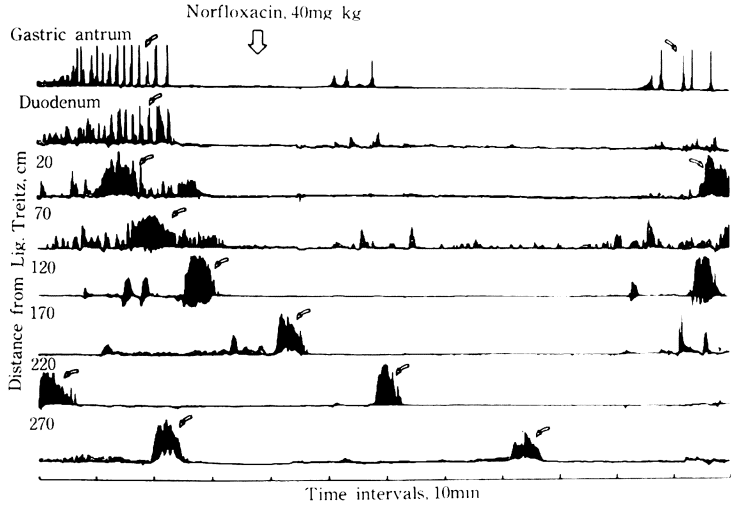


Fig. 7 Effect of intravenous injection of OPC-7241 (20 mg/kg) on contractile activity in the gastrointestinal tract in a conscious dog. A series of retrograde migrating contractions indicated by arrows are seen to be induced 17 min after the injection. In this case the dog did not vomit.

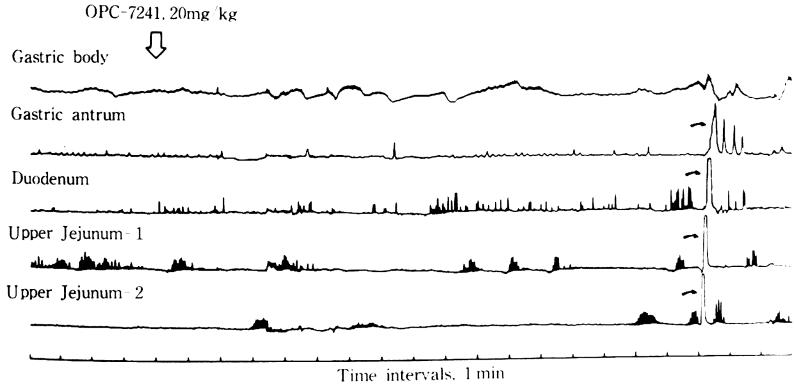


Table 1 Incidence of retrograde migrating contractions, nausea, vomiting and defecation in conscious dogs

Drugs	Dose, mg/kg	Retrograde migrating contractions	Nausea	Vomiting	Defecation
Ofloxacin	20	0*	0	0	0
〃	40	0	0	0	13
Norfloxacin	20	0	0	0	0
〃	40	0	0	0	6
OPC-7241	20	100	67	27	20
〃	40	100	100	100	33

* Values are per cent of incidence obtained from 3 experiments in each of 5 dogs.

the small intestine was not affected by intravenous injection of norfloxacin. As indicated by arrows in the right-hand side of this figure, the next cycle of IMC is seen to have occurred at the regular interval. Figure 7 shows the effect of an intravenous injection of OPC-7241 (20 mg/kg) on contractile activity in the gastrointestinal tract in a dog. As seen on the right-hand side of this figure, typical strong retrograde migrating contractions are induced 17 min after the intravenous bolus injection of this antibacterial agent, but the dog did not vomit in this particular case. However, when the dose was doubled, to 40 mg/kg, the intravenous injection of OPC-7241 always induced nausea and vomiting accompanied by strong retrograde migrating contractions. In Table 1, the incidence of retrograde migrating contractions with or without nausea, vomiting and defecation induced by the three antibacterial agents is listed.

DISCUSSION

In the present study, OPC-7241 was found to induce nausea and vomiting accompanied by retrograde migrating contractions. It is reported that the incidence of nausea and vomiting in the cat[8,9] and dog[7] is closely related to the incidence of retrograde migrating contractions in the gastrointestinal tract. The induction of retrograde migrating contractions is not always followed by nausea and vomiting, but when doses were increased, the incidence of nausea and vomiting was significantly increased ; in this case when the dose was increased to 40 mg/kg, the incidence reached 100%. This was also true in the case of apomorphine ; the dose of apomorphine at 0.05 mg induced nausea only in 25% of the total experiments, although retrograde migrating contractions were always induced.

Neither apomorphine nor OPC-7241 induced any noticeable contractions in the gastrointestinal tract before retrograde migrating contractions were induced ; in other words, the retrograde migrating contractions are the first response to these agents. However, it is also known that there is another type of contractile response in the gastrointestinal tract when nausea and vomiting are induced by some antibiotics. As we reported previously[10], erythromycin (EM) and oleandomycin (OM) initiate very strong contractions in the gastrointestinal tract at the same time. Under these physiological conditions, although contractions are strong and continuous, they are usually well coordinated between neighboring sites. However, in the case of EM or OM, the induced contractions are very strong and do not coordinate well between two sites ; for instance, the gastric antrum and the duodenum strongly contract simultaneously. These uncoordinated contractions are considered to cause retrograde migrating contractions in the gastrointestinal tract[10]

On the other hand, it is considered that nausea and vomiting are induced through the chemoreceptor trigger zone (CTZ)[11]. If a substance stimulates the CTZ directly, nausea and vomiting are induced.

This happens with apomorphine. However, when a part of the peritoneum was stimulated, for instance, the stimuli on the peritoneum are considered to be conveyed to the CTZ and then nausea and vomiting are induced. In the case of OPC-7241, since no noticeable contractions were seen before retrograde migrating contractions, we assume that the effect was delivered to the CTZ directly, just as in the case of apomorphine.

It is difficult to tell the structure-activity relationship among these three drugs used in the present study. The chemical structures of ofloxa-

cin and OPC-7241 are more similar to each other than to that of norfloxacin. In spite of this, the incidence of side effects on the gastrointestinal tract was quite different in ofloxacin and OPC-7241.

Therefore, it is impossible to interpret the difference from the viewpoint of their chemical structure.

In conclusion, the results of this study indicate that OPC-7241 may induce side effects in the gastrointestinal function when given to human in view of its action in inducing retrograde migrating contractions in the gastrointestinal tract in conscious dogs.

References

1) ITOH, Z.; R. HONDA, S. TAKEUCHI, I. AIZAWA & R. TAKAYANAGI: An extraluminal force transducer for recording contractile activity of the gastrointestinal smooth muscle in conscious dogs. Its construction and implantation. Gastroent. Jpn. 12: 275～283, 1977
2) SATO, K.; Y. MATSUURA, M. INOUE, T. UNE, Y. OSADA, H. OGAWA & S. MITSUHASHI: *In vitro* and *in vivo* activity of DL-8280, a new oxazine derivative. Antimicrob. Agents Chemother. 22: 548～553, 1982
3) HIRAI, K.; A. ITO, Y. ABE, A. SUGUE, T. IRIKURA, M. INOUE & S. MITSUHASHI: Comparative activities of AM-715 and pipemidic and nalidixic acids against experimentally induced systemic and urinary tract infections. Antimicrob. Agents Chemother. 19: 188～189, 1981
4) MORITA, J.; K. WATANABE & T. KOMANO: Mechanism of action of new synthetic nalidixic acid-related antibiotics: Inhibition of DNA supercoiling catalyzed by DNA gyrase. Agric. Biol. Chem. 48: 663～668, 1984
5) SZURSZEWSKI, J. H.: A migrating electric complex of the canine small intestine. Am. J. Physiol. 217: 1757～1763, 1969
6) ITOH, Z.; S. TAKEUCHI, I. AIZAWA & R. TAKAYANAGI: Characteristic motor activity of the gastrointestinal tract in fasted conscious dogs measured by implanted force transducers. Am. J. Dig. Dis. 23: 229～238, 1978
7) AIZAWA, I.; K. NEGISHI, T. SUZUKI & Z. ITOH: Gastrointestinal contractile activity associated with vomiting in the dog. In: Gastrointestinal Motility, edited by C. ROMAN, MTP Press, Lancaster, 1984. pp. 159～165.
8) WEISBRODT, N. W. & J. CHRISTENSEN: Electrical activity of the cat duodenum in fasting and vomiting. Gastroenterology 63: 1004～1010, 1972
9) STEWART, J. J.; T. F. BURKS & N. W. WEISBRODT: Intestinal myoelectric activity after activation of central emetic mechanism. Am. J. Physiol. 233: E 131～137, 1977
10) ITOH, Z.; T. SUZUKI, M. NAKAYA, M. INOUE & S. MITSUHASHI: Gastrointestinal motor-stimulating activity of macrolide antibiotics and analysis of their side effects on the canine gut. Antimicrob. Agents Chemother. 26: 863～869, 1984
11) WANG, S. C. & H. L. BORISON: A new concept of organization of the central emetic mechanism: Recent studies on the sites of action of apomorphine, copper sulfate and cardiac glycosides. Gastroenterology 22: 1～12, 1952

犬の胃腸収縮に及ぼすピリドンカルボン酸系誘導体の影響：胃腸障害の発現について

伊藤　漸[1]・三橋　進[2]

群馬大学医療技術短期大学部[1]

エピゾーム研究所[2]

新ピリドンカルボン酸系合成抗菌剤であるオフロキサシン（ofloxacin），ノルフロキサシン（norfloxacin）および OPC-7241 を，犬に静脈内投与したところ，三者の間で胃腸障害発現に著明な相違が観察された。

OPC-7241 [9-Fluoro-5-methyl-8-(4-methyl-1-piperazinyl)-6, 7-dihydro-1-oxo-1 H, 5 H-benzo (ij) quinolizine-2-carboxylic acid] は，20 mg/kg および 40 mg/kg 静脈内投与で，悪心および嘔吐を伴う腸管の逆行性収縮を発現した。悪心および嘔吐の発現率は，40 mg/kg 投与で 100%，20 mg/kg 投与時では，悪心 67%，嘔吐 27% であった。

一方，オフロキサシンおよびノルフロキサシン投与で，胃腸障害は全く観察されなかった。

以上の結果より，我々の犬胃腸運動測定モデルは，前臨床評価の一環として有用であると思われる。