Effect of Novel Motilide ABT-229 versus Erythromycin and Cisapride on Gastric Emptying in Dogs

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ABSTRACT

ABT-229 (8,9-anhydro-4'-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin B-6,9-hemiacetal), a synthetic derivative of erythromycin (ERY) with no antibiotic activity, has been shown to bind to motilin receptors and stimulate contractile activity of the antrum and small intestine. The objective of this study was to determine the effect of ABT-229 on canine gastric emptying (GE) and contractile activity of the antrum and duodenum in response to a solid meal. Six beagles were used to determine GE of a solid meal and contractile activity of the antrum and duodenum in response to either vehicle, ABT-229 (0.17, 0.83, 2.5, or 5.0 μg/kg/min), ERY (33.3 μg/kg/min), or cisapride (CIS) (10 μg/kg/min). Lag (t_{lag}), half-emptying (t_{1/2}), and complete emptying (t_{full}) times were determined. Contractile data were analyzed for motility index and gastroduodenal coordination. Compared with vehicle, ABT-229 dose dependently accelerated GE, t_{lag} was decreased at the two highest doses, t_{1/2} was decreased compared with vehicle at the three highest doses, and t_{full} was decreased at all doses compared with vehicle. ERY also decreased t_{1/2} and t_{full}, whereas CIS decreased all GE parameters. The slopes of the linear phase of GE curves for all drugs and doses were greater than those for vehicle. ABT-229 dose dependently increased the motility index as well as gastroduodenal coordination. ABT-229 (two highest doses) and CIS accelerated GE of a solid meal by decreasing the lag phase and increasing the rate of GE, whereas ERY only increased the rate of GE. The data suggest that ABT-229 is 7- to 40-fold more potent than ERY in accelerating GE.

Gastric emptying (GE) is delayed in diseases such as diabetes mellitus (Horowitz et al., 1986, 1987; Brogna et al., 1989; Janssens et al., 1990; Schmid et al., 1991; Peeters et al., 1992) and functional dyspepsia (Corinaldesi et al., 1987; Davis et al., 1988). Gastric emptying also is delayed in as many as 50% of patients after truncal vagotomy, antrectomy, and Roux-Y gastrojejunostomy (Hocking et al., 1981; Vogel et al., 1983; Carlson et al., 1991). The common factor in these conditions is upper gastrointestinal motor dysfunction. Therefore, a prokinetic drug with a primary site of action in the upper gut (stomach, and first half of the small intestine) might provide benefit in treatment of these disorders.

Motilin, a 22-amino acid peptide, has been shown to accelerate GE in normal subjects (Christofides et al., 1979, 1981) and in patients with diabetic gastroparesis (Schmid et al., 1991; Peeters et al., 1992) when given as an i.v. infusion. Motilin is thought to accelerate GE by increasing the force of postprandial antral contractions and by promoting coordination between antral and duodenal motor activity (Annese et al., 1992). Erythromycin (ERY), a 14-membered macrolide with antibiotic activity, binds to motilin receptors in the antrum and duodenum (Peeters et al., 1989). It also stimulates contractile activity similar to motilin (Annese et al., 1992). Additionally, ERY accelerated GE in normal subjects and in diabetic gastroparetic patients to the same extent as motilin (Janssens et al., 1990).

ABT-229 (8,9-anhydro-4'-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin B-6,9-hemiacetal) is a more potent synthetic derivative of ERY with no antibiotic activity (Lartey et al., 1995, Faghih et al., 1998). As with ERY, ABT-229 is thought to stimulate contractile activity through activation of motilin receptors (Clark et al., 1999). Previously, ABT-229 has been reported to stimulate contractile activity of the antrum and small intestine in fasted conscious dogs (Faghih et al., 1998). The objective of this study was to determine the effect of ABT-229 on GE of a solid meal as well as postprandial motor activity of the antrum and duodenum, and to compare them with the effects of cisapride (CIS) and ERY.

ABBREVIATIONS: GE, gastric emptying; ERY, erythromycin; ABT-229, 8,9-anhydro-4'-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin B-6,9-hemiacetal; CIS, cisapride; PEG, polyethylene glycol.
Materials and Methods

Experiments were conducted on six conscious beagle dogs, weighing 9.5 to 11.3 kg and trained to stand in a sling. The procedures used in this study were approved by the Institutional Animal Care and Use Committee of Abbott Laboratories, Abbott Park, IL.

Surgical Preparation. After an overnight fast, dogs were initially anesthetized with thiopental, 20 mg/kg i.v., and prepared for surgery in accordance with standard procedures. Isoflurane (1–1.5%) delivered via a semiclosed system was used during the surgical procedure as a general anesthetic. Through a midventral laparotomy, a silicon catheter (3.2-mm o.d. × 1.6-mm i.d.) was placed intraluminally with its tip 2 cm distal to the pylorus. A stainless steel collection cannula was placed 20 cm distal to the pylorus. Additionally, two strain gage force transducers (RB Products, Stillwater, MN) were sutured to the serosal surface of the antrum 2 and 5 cm proximal to the pylorus, and four transducers were placed on the duodenum 2, 6, 10, and 14 cm distal to the pylorus. The cather was tunneled s.c. to the midscapular region and connected to an s.c. access port (Access Technologies, Skokie, IL). The abdominal incision was closed in two layers. An access port catheter also was inserted into the external jugular vein. At least 2 weeks were allowed for recovery from surgery. Experiments were initiated only after the animals were consuming a normal diet.

Recording and Analysis of Contractile Activity. Contractile activity was recorded with a Grass polygraph (model 7) equipped with 7P1 low-level d.c. preamplifiers and 7DA driver amplifiers. The signals were simultaneously digitized at 10 Hz into computer files for identification of individual contractions and determination of the area under each contraction. Each record was analyzed from time of feeding until 90% of the meal had emptied; the data are expressed as the average motility index (area/minute) during that time. The area of each contraction at each site was standardized to the mean area of the 10 largest contractions during phase III activity at that site. This was done to account for differences between sensitivities of transducers at different sites. Additionally, the records were inspected visually for phenomena the computer program might not recognize, such as gastroduodenal coordination. Gastroduodenal coordination was defined as a contraction or group of contractions that originated in the antrum, while the duodenum was quiescent, and then propagated aborad into the duodenum within 10 s, migrating through the duodenum at a constant velocity.

Experimental Protocol. After an overnight fast, dogs were placed in a sling for GE studies. Beginning 30 min before the animals were fed, a solution containing a nonabsorbable marker (polyethylene glycol 4000 [PEG]) was perfused at 0.5 ml/min through the duodenal catheter and continued for the remainder of the experiment. Simultaneously, an i.v. infusion of either vehicle, ABT-229 (0.17, 0.83, 2.5, or 5.0 μg/kg/min), CIS (10 μg/kg/min), or ERY (33.3 μg/kg/min) was initiated and continued for 30 min at a volume rate of 0.24 ml/min. At the end of the drug infusion, the dogs were fed 175 g of commercial dog food (Alpo Prime Cuts) mixed thoroughly with 7P1 low-level d.c. preamplifiers and 7DA driver amplifiers. The activity was recorded with a Grass polygraph (model 7) equipped with 7P1 low-level d.c. preamplifiers and 7DA driver amplifiers. The equations used to determine GE were previously derived and reported by Orihata and Sarna (1994a,b). Mean flow rate (FRln) of the liquid fraction of the chyme for each sample (n) was calculated as follows:

\[ FR_{ln} = (\frac{[\text{PEG}_{n}]}{[\text{PEG}_{1n}]}) \cdot PR \]

where \([\text{PEG}_{n}]\) and \([\text{PEG}_{1n}]\) are the concentrations of PEG in the perfusion solution and nth sample of the liquid phase, respectively, and PR is the perfusion rate in milliliters per minute.

The mean flow rate for the solids (FRsn) of each sample (n) was determined as follows:

\[ FR_{sn} = FR_{ln} \cdot (\frac{V_{ln}}{V_{na}}) \]

where the amount of solid meal that passes the cannula for each sample is derived as follows:

\[ SME_{n} = FR_{sn} \cdot [\text{Cr}_2\text{O}_3]_{n} \cdot t_{n} \]

where SME is solid meal emptied for interval n, \([\text{Cr}_2\text{O}_3]_{n}\) is the concentration of \([\text{Cr}_2\text{O}_3]_{n}\) for the nth sample, and \(t_{n}\) is the duration of the nth sample interval.

The percentage of the total meal passing the cannula during each sample interval was calculated as follows:

\[ \%SME_{n} = \frac{\sum_{n=1}^{m} SME_{n}}{\sum_{n=1}^{m} SME_{n}} \]

where \(m\) is the total number of samples.

The GE curve is constructed as the cumulative addition of the \%SME at each time point (7).

\[ \%SME_{F} = \frac{\sum_{n=1}^{m} SME_{n}}{\sum_{n=1}^{m} SME_{n}} \]

Data Analysis. The beginning of the meal defines zero time. GE occurs in three phases: lag phase, linear phase, and postlinear phase. The lag phase was defined as the time from the beginning of the meal until 5% of the meal was emptied. The half-emptying time \((t_{1/2})\) was defined as the time postprandially when 50% of the meal was emptied. The total GE time \((t_{cul})\) was defined as the time when 90% of the meal was emptied. The slope of the linear phase of each GE curve (GE rate) was calculated with a linear regression model for the points between the end of the lag phase and the 90% \((t_{cul})\) emptied point (Camilleri et al., 1989; Iwanaga et al., 1998). The goodness of fit of the linear regression was determined from the square of the correlation coefficient \(r^2\).

All data are expressed as either the mean ± S.E. or the median with 25 to 75 percentiles. One-way ANOVA with repeated measures was used to determine whether there was a difference between mean values for parametric data. For nonparametric data the Friedman ANOVA with repeated measures was used to determine whether there was a difference between median values. When a difference was found, the Student-Newman-Keuls post hoc test was used to determine which means or medians were different. A P value of ≤.05 was considered to indicate a significant difference.

Drugs. ABT-229 lactobionate and ERY lactobionate were synthesized by Abbott Laboratories. CIS was synthesized by R. Faghiih (Abbott Laboratories). All doses refer to dose equivalents of compound free base. ABT-229 and ERY were dissolved in sterile water for injection (Abbott Laboratories) and CIS was dissolved in 1% lactic acid and adjusted to pH 3 to 4. Dosing solutions were prepared fresh for each experiment.

Results

GE. ABT-229 dose dependently accelerated GE compared with vehicle (Fig. 1). CIS and ERY also accelerated GE at the
Fig. 1. Cumulative mean GE curves of a solid meal in response to ABT-229, ERY, and CIS. All compounds significantly increase the slope compared with vehicle.

Fig. 2. Effect of ABT-229, ERY, and CIS on the duration of the GE lag phase ($t_{\text{lag}}$), $t_{1/2}$, and $t_{\text{full}}$. $n = 6$; $^*P < .05$ compared with control; $^\dagger P < .05$ compared with 2.5; $^\S P < .05$ compared with 5.0.
doses tested (Fig. 1). ABT-229 at the two highest doses significantly decreased the lag phase, as did CIS compared with vehicle (Fig. 2). Additionally, there was a significant difference in $t_{lag}$ between the 0.17 and 0.83 $\mu$g/kg/min doses of ABT-229, as well as ERY and the two highest doses of ABT-229 (Fig. 2). ABT-229 at the three highest doses, in addition to ERY and CIS, significantly decrease $t_{lag}$ compared with vehicle (Fig. 2). Compared with vehicle, $t_{lag}$ was significantly decreased by all doses of ABT-229, as well as by ERY and CIS (Fig. 2). The GE rate during the linear phase was significantly increased by all doses of ABT-229, as well as by ERY and CIS compared with vehicle (Table 1); however, there was no difference in GE rate between doses of ABT-229 and/or between CIS and ERY. In all experiments, the regression coefficient for the linear phase was >0.9.

**Postprandial Contractile Activity.** Two types of coordinated gastroduodenal contractile activity were observed (Figs. 3 and 4). At the two highest doses of ABT-229 and with CIS, a contractile pattern was induced that was characterized by a high amplitude (equal to the maximum amplitude observed during phase III activity) propagated antral contraction with quiescence in the duodenum. A migrating cluster of contractions in the duodenum then followed the antral contraction. This type of coordinated activity occurred during the first 60 min after the meal (Fig. 3). The frequency of this type of coordinated contractile pattern was significantly increased compared with vehicle and with the two lowest doses of ABT-229 and ERY (Table 2).

The second type of coordinated antral duodenal activity (Fig. 4) was characterized by a propagated antral contraction of normal postprandial amplitude (15–20% of maximal phase III activity amplitude) with quiescence in the duodenum in at least the first two recording sites. The antral contraction was followed by a propagated duodenal contraction. ABT-229 caused a dose-dependent increase in this low-amplitude, coordinated activity, with the increase compared with vehicle becoming significant at 0.17 $\mu$g/kg/min and higher doses. ERY and CIS also significantly increased this type of coordinated activity at the doses tested (Table 3).

There was also a dose-dependent increase in the motility index of both the antrum and duodenum with ABT-229, which became significant at doses of 0.83 $\mu$g/kg/min and higher (Fig. 5). Additionally, both CIS and ERY significantly increased the postprandial motility index (Fig. 5).

**Discussion**

The rate of GE is a function of the difference in pressures between the stomach and duodenum and of resistance to flow across the gastroduodenal junction (Kelly, 1981). Therefore, a compound that stimulates antral contractions, while the duodenum is quiescence, would be expected to increase flow across the gastroduodenal junction. Additionally, if propagated duodenal contractions occur after the antral contraction, the chyme would be carried away, reducing resistance to flow across the gastroduodenal junction when the next antral contraction occurs. This type of contractile activity is classified as gastroduodenal coordination (Kelly, 1981). In contrast, if duodenal contractions occur at the same time as the

![Fig. 3. Recording illustrating high-amplitude, coordinated gastroduodenal contractile activity. This type of activity was seen in the first 60 min postprandially at the two highest doses of ABT-229 and with CIS. Note the quiescence in the duodenum during the antral contraction. Arrows indicate coordinated contractile activity. A, antrum and D, duodenum and the number after A and D indicates the distances of the transducers from the pylorus.](image)

![Fig. 4. Recording illustrating low-amplitude, coordinated gastroduodenal contractile activity. This type of activity was recorded throughout the postprandial period with all doses of ABT-229, as well as with CIS and ERY. Note the quiescence in the duodenum during the antral contraction. Arrows indicate coordinated contractile activity. A, antrum and D, duodenum and the number after A and D indicates the distances of the transducers from the pylorus.](image)

**TABLE 1**

GE rates during the linear phase of GE

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Slope</th>
<th>$\mu$g/kg/min</th>
<th>%/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABT-229</td>
<td>Vehicle</td>
<td>18.4 ± 0.9</td>
<td>0.17</td>
<td>31.8 ± 2.4*</td>
</tr>
<tr>
<td>ERY</td>
<td>33.3</td>
<td>35.8 ± 2.2*</td>
<td>10</td>
<td>28.5 ± 4.0*</td>
</tr>
</tbody>
</table>

* $P < .05$ compared with vehicle ($n = 6$).

**TABLE 2**

Frequency of high-amplitude, coordinated gastroduodenal contractile activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Median 25–75 Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABT-229</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0*</td>
<td>0–0</td>
</tr>
<tr>
<td>0.17</td>
<td>0*</td>
<td>0–0</td>
</tr>
<tr>
<td>0.83</td>
<td>0*</td>
<td>0–0.5</td>
</tr>
<tr>
<td>2.5</td>
<td>11.5**##</td>
<td>7.0–18.0</td>
</tr>
<tr>
<td>5.0</td>
<td>23.0**</td>
<td>15.0–29.0</td>
</tr>
<tr>
<td>ERY</td>
<td>33.3</td>
<td>0*</td>
</tr>
<tr>
<td>CIS</td>
<td>10</td>
<td>6.0***</td>
</tr>
</tbody>
</table>

* $P < .05$ compared with ABT-229 at 2.5 and 5.0 $\mu$g/kg/min; and, ** $P < .05$ compared with control, ERY, and ABT-229 at 0.17 and 0.83 $\mu$g/kg/min; *** $P < .05$ compared with ABT-229 at 5.0 $\mu$g/kg/min ($n = 6$).
antral contraction, the pressure gradient across the gastroduodenal junction would be decreased and there would be less flow. All three compounds examined in this study induced gastroduodenal coordination.

ERY has previously been shown to be a gastrokinetic agent in both animals (Lin et al., 1994) and humans (Annese et al., 1992; Tack et al., 1992). ERY is thought to exercise its gastrokinetic effects by increasing the motility of the antrum (Annese et al., 1992; Tack et al., 1992), increasing proximal gastric tone (Bruley DesVarannes et al., 1995), and increasing the coordination between antral and duodenal contractions (Annese et al., 1992; Tack et al., 1992). At the dose of ERY used in this study, we also observed an increase in antral motility and gastroduodenal coordination.

This study shows that ABT-229, a synthetic derivative of ERY without antibiotic activity (Lartey et al., 1995; Faghih et al., 1998), dose dependently accelerated gastric emptying of a solid meal by decreasing the lag phase and increasing the rate of GE during the linear phase. Additionally, ABT-229 increased postprandial contractile activity and gastroduodenal coordination in the dog. Depending on the parameter examined, ABT-229 appears to be ~7- to 40-fold more potent than ERY in this regard.

GE may be accelerated by at least two mechanisms with ABT-229. First, at the two highest doses of ABT-229, the lag phase was significantly decreased, probably as a result of high-amplitude, coordinated gastroduodenal contractions observed in the early postprandial period. It is also likely that ERY would have stimulated high-amplitude, coordinated activity in the dog if given at a higher dose than in this study. In humans, ERY has been reported to stimulate high-amplitude, coordinated gastroduodenal contractile activity (Annese et al., 1992) and in dogs to decrease the lag phase at higher doses than were used in this study (Lin et al., 1994). CIS also had been reported to decrease the lag phase, which is probably a result of the induction of high-amplitude, coordinated gastroduodenal contractile activity (Schuurkes, 1990). Second, GE may be accelerated through both the stimulation of low-amplitude, coordinated gastroduodenal contractile activity and an increase in motility index observed throughout the linear phase of GE. These factors are probably the cause of increased GE rate and appear to be shared by ABT-229, ERY, and CIS.

There is a third possible mechanism by which ABT-229 may enhance GE rate. Previously, ERY has been reported to increase postprandial proximal gastric tone and pressure in humans (Bruley DesVarannes et al., 1995). We did not, however, measure gastric tone in this study, so it remains to be determined if increased GE rate in dog is triggered by a rise in proximal gastric tone.

In conclusion, we have shown that ABT-229 dose dependently accelerates GE by increasing postprandial gastroduodenal coordination and by increasing the motility index. Furthermore, ABT-229 is ~7- to 40-fold more potent than ERY in this regard.

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