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

VERNE E. COWLES, HUGH N. NELLANS, TERESE R. SEIFERT, LESLIE M. BESECKE, JASON A. SEGRETI, KURT M. MOHNING, RAMIN FAGHIH, MARLEEN H. VERLINDEN, and CRAIG D. WEGNER

Pharmaceutical Products Division, Department of Integrative Pharmacology and Gastroenterology Venture, Abbott Laboratories, Abbott Park, Illinois

Accepted for publication February 2, 2000

This paper is available online at http://www.jpet.org

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 the effect of ABT-229 on canine gastric emptying (GE) and contractile activity. 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. Compared with vehicle, ABT-229 dose dependently accelerated GE, tlag was decreased at the two highest doses, t1/2 was decreased compared with vehicle at the three highest doses, and tfull was decreased at all doses compared with vehicle. ERY also decreased t1/2 and tfull, whereas CIS decreased all GE parameters. 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.

Received for publication November 15, 1999.

1 This work was presented in part at Digestive Disease Week in conjunction with the 100th annual meeting of the American Gastroenterological Association, May 16–19, Orlando, FL.

2 Current address: Depomed, Inc., 1360 O’Brien Dr., Menlo Park, CA 94025.

3 Current address: Pharmacia Corporation, 4901 Searle Pkwy., Rm. J322A, Skokie, IL 60077.

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 catheter 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 125 mg of chromium oxide (Cr₂O₃), a solid-phase marker. After feeding, chyme samples were collected every 5 min for the first 30 min to identify the lag phase of the GE curve. After this point, samples were collected at 40 and 50 min and then at 20-min intervals until solid food particles were no longer present.

Sample Analysis and Calculation of GE. The samples were centrifuged at 2000 rpm for 20 min, and the volumes of the supernatant (Vₙ) and the solid pellet (Vsₙ) were measured. One milliliter of the supernatant (PEGₙ) and perfusion solution (PEGₜₙ) were used to determine the PEG concentrations by the method of Malawer and Powell (1967). The concentration of Cr₂O₃ was measured from the sediment of each sample by the method of Bolin et al. (1952).

The equations used to determine GE were previously derived and reported by Orihata and Sarna (1994a,b). Mean flow rate (FRₙ) of the liquid fraction of the chyme for each sample (n) was calculated as follows:

\[ FRₙ = (\text{[PEGₙ]} / \text{[PEGₜₙ]}) \cdot PR \]

where [PEGₙ] and [PEGₜₙ] 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 (FRₙ) of each sample (n) was determined as follows:

\[ FRₙ = FRₙ \cdot (Vₙ / Vₕ) \]

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

\[ \text{SME}ₙ = FRₙ \cdot \text{[Cr₂O₃]}ₙ \cdot tₙ \]

where SMEₙ is solid meal emptied for interval n, [Cr₂O₃]ₙ is the concentration of Cr₂O₃ for the nth sample, and tₙ 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:

\[ \% \text{SME}ₙ = \frac{\text{SME}ₙ}{\sum \text{SME}ₙ} \]

where m is the total number of samples.

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

\[ \% \text{SME}ₜ = \frac{\sum \text{SME}ₙ}{\sum \text{SME}ₙ} \]

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_{total}) 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_{total}) 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 (r²).

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-Krus post hoc test was used to determine which means or medians were different. ANOVA with repeated measures was used to determine whether there was a difference between mean values for each experiment.

Drugs. ABT-229 lactobionate and ERY lactobionate were synthesized by Abbott Laboratories. CIS was synthesized by R. Faghih (Abbott Laboratories). All doses refer to dose equivalents of compound free base. ABT-229 and ERY were dissolved in sterile water (Abbott Laboratories). All doses refer to dose equivalents of compound free base. ABT-229 and ERY were dissolved in sterile water (Abbott Laboratories). All doses refer to dose equivalents of compound free base.

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 ($t_{\text{lag}}$), $t_{\frac{1}{2}}$, and $t_{\text{full}}$. $n = 6$; *$P < .05$ compared with control; †$P < .05$ compared with 2.5; §$P < .05$ 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 (Fig. 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

**TABLE 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Slope</th>
<th>µg/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></td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>10</td>
<td>28.5 ± 4.0*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < .05 compared with vehicle ($n = 6$).
antral contraction, the pressure gradient across the gas-
troduodenal junction would be decreased and there would be
less flow. All three compounds examined in this study in-
duced 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 gas-
trokinetic 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 increas-
ing the coordination between antral and duodenal contrac-
tions (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 gastroduode-
nal coordination in the dog. Depending on the parameter
examined, ABT-229 appears to be ~7- to 40-fold more potent
than ERY in the conscious dog.

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 ob-
served in the early postprandial period. It is also likely that
ERY would have stimulated high-amplitude, coordinated ac-
tivity in the dog if given at a higher dose than in this study.
In humans, ERY has been reported to stimulate high-ampli-
tude, coordinated gastroduodenal contractile activity (An-
nese 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, coor-
dinated gastroduodenal contractile activity (Schuurkes,
1990). Second, GE may be accelerated through both the stim-
ulation of low-amplitude, coordinated gastroduodenal con-
tractile activity and an increase in motility index observed
throughout the linear phase of GE. These factors are proba-
bolically 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, how-
ever, measure gastric tone in this study, so it remains to be
determined if increased GE rate in dogs is triggered by a rise
in proximal gastric tone.

In conclusion, we have shown that ABT-229 dose depen-
dently accelerates GE by increasing postprandial gastroduo-
denal coordination and by increasing the motility index. Fur-
thermore, ABT-229 is ~7- to 40-fold more potent than ERY in
this regard.

### References

Cutsem E (1992) Erythromycin accelerates gastric emptying by inducing antral
contractions and improved gastroduodenal coordination. *Gastroenterology*

Bolin DW, King RP and Klosterman EW (1952) A simplified method for the deter-
mination of chromic oxide (Cr₂O₃) when used as an index substance. *Science*
(Wash DC) 116:634–635.

V and Onorato S (1989) Cisapride and gastric emptying of a solid meal in dyspeptic
patients with neuropathy and in healthy volunteers. *Eur J Clin Pharmacol*
35:411–413.

Camilleri M, Colemont LJ, Phillips SF, Brown ML, Thomford GM, Chapman N and
Zinzmeister AR (1989) Human gastric emptying and colonic filling of solids char-

Carlson RG, Hocking MP, Courting-KR, Sinsky CA and Vogel SB (1991) Eryth-
TABLE 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Mean ± S.E.</th>
<th>Vehicle</th>
<th>0.17</th>
<th>0.83</th>
<th>2.5</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABT-229</td>
<td>27 ± 10</td>
<td>52 ± 19*</td>
<td>74 ± 16*</td>
<td>95 ± 20*</td>
<td>103 ± 12*++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERY</td>
<td>33.3</td>
<td>71 ± 7*</td>
<td>87 ± 4*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>10</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P ≤ .05 compared with control; **P ≤ .05 compared with ABT-229 at 0.17
μg/kg/min (n = 6).

Fig. 5. Effect of ABT-229, ERY, and CIS on the motility index of the antrum and duodenum. *P < .05 compared with vehicle.


Send reprint requests to: Dr. Verne Cowles, Depomed, Inc., 1380 O’Brien Dr., Menlo Park, CA 94025. E-mail: vcowles@depomedinc.com