Dose-Related Effects of N-Demethyl-N-Isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal on Gastric Emptying of Solids in Healthy Human Volunteers

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ABSTRACT

The purpose of our study was to evaluate the effects of a new nonantibiotic motilide derived from erythromycin, EM574, on gastric emptying and to evaluate its safety. Thirty healthy volunteers received one of five oral doses of EM574 (5, 10, 20, 30 mg and placebo) in a randomized, double-blind, five-period, cross-over design; each dosing period was separated by 1-wk washout. Gastric emptying was measured by 13C-octanoic acid breath test. A total of 10, 20, 30 mg of EM574 significantly accelerated both lag phase and gastric half-emptying time (P < .001) compared to placebo. The 5-mg dose of EM574 also significantly shortened the gastric half-emptying time (P < .05). Mean gastric half-emptying times were 173, 158, 147, 149 min with EM574 5, 10, 20, 30 mg, respectively (placebo, mean 189 min). EM574 accelerated gastric emptying in a dose-related manner (P < .001 for linear trend, P < .05 for quadratic trend). However, the 30-mg dose did not accelerate gastric emptying more than the 20-mg dose. EM574 was well tolerated; 7 of 56 participants receiving the 20- or 30-mg dose developed nausea, and only 2 of 28 receiving the 30-mg dose experienced vomiting. EM574 accelerates gastric emptying in a dose-dependent manner with minimal side effects after a single administration of up to 20 mg. EM574 shows promise for treatment of patients with impaired gastric emptying.

The potential use of erythromycin as a prokinetic agent was first reported in 1990 (Janssens et al., 1990). In patients with gastroparesis, a low dose of erythromycin (up to 3 mg/kg) is effective when administered intravenously (Ramirez et al., 1994; Janssens et al., 1990). However, chronic treatment with oral erythromycin was not as efficacious as intravenous erythromycin (Ramirez et al., 1994; Janssens et al., 1990; Kao et al., 1995; Fiorucci et al., 1994; Desautels et al., 1995; Richards et al., 1993). There are limited data on the dose-related effects of erythromycin on acceleration of gastric emptying (Mathis and Malbert, 1995; Desautels et al., 1995).

The clinical use of erythromycin as a prokinetic agent in patients with gastric stasis is limited by the lack of long-term effectiveness of oral erythromycin (Ramirez et al., 1994; Fiorucci et al., 1994), and by the concern about the potential for emergence of resistant bacterial strains during long-term use (Tanis et al., 1993). These limitations led to the development of nonantibiotic derivatives for use as prokinetics. One of them, EM574 is a newer erythromycin derivative that was shown to have no significant antibiotic activity. EM574 stimulates gastric and intestinal motility both in vivo and in vitro (Satoh et al., 1994; Funabashi et al., 1996). EM574 directly stimulates smooth muscle cell contraction by acting on motilin receptors in the human gastric antrum in vitro (Satoh et al., 1994).

The aims of our study were to assess the effect of single administration of four oral doses of EM574 and placebo on the gastric emptying of solids in healthy human volunteers, and to evaluate the adverse effects of EM574 after single oral administration.

Methods

Subjects

Thirty healthy volunteers (20 males, 10 females; mean age 30 yr, range 19–46 yr) were recruited by public advertisement and received one of five oral doses (placebo 0, 5, 10, 20 and 30 mg of EM574; TAP Holdings Inc., Deerfield, IL) on each study day. The study design was a randomized, double-blind, five-period cross-over, single center trial. There was a 1-wk washout period between study days. None of the healthy participants had a history of, or current gastrointestinal symptoms suggesting gastric stasis, or gastroesophageal reflux before study initiation. In addition, no subject had a history of any previous significant abdominal surgery (except for...
appendectomy or cholecystectomy). All subjects signed informed consent to participate in the study which was approved by the Mayo Clinic’s Institutional Review Board. Females of childbearing potential had a negative pregnancy test before participation.

Study Procedures

All participants were admitted to the Biostudies Unit at Mayo Clinic 1 day before each study day. A medical history and physical examination were performed at the screening visit. After an overnight fast, placebo or a single dose of EM574 (one of four doses: 5, 10, 20, 30 mg) was administered orally 30 min before ingestion of the test meal on each study day. The test meal consisted of two egg whites and one yolk coated with 100 mg of [13C]-octanoic acid (Aldrich Co., Milwaukee, WI). The egg meal was placed on a slice of whole wheat bread and ingested with a glass of skim milk, for a total calorie value of 240 kcal and nutrient composition of 35% protein, 40% carbohydrate, 25% fat and 2.6 g of fiber.

Breath samples were obtained at baseline (before the meal) and every 15 min for 6 hr after meals. Each breath sample was collected in a 3-liter plastic bag and a 25-ml aliquot of the breath sample was stored in a sterile vacutainer. [13CO2] was measured using a gas chromatograph/isotope ratio mass spectrometer (Delta S, Finnigan Mat, Bremen, Germany) (Choi et al., 1997; Balagopal et al., 1996). In this method, carbon dioxide is first purified in a gas chromatograph in the measurement of its isotope ratio mass spectrometer.

Adverse Events

All adverse events that occurred during the course of the study were reported in detail on a standard case report form. Severity of the adverse events were rated according to the following definitions: Mild: the adverse event was transient and easily tolerated by the subjects; Moderate: the adverse event caused the subject discomfort and interrupted the normal activities of the subject; Severe: the adverse event caused considerable interference with the normal activities of the subject or was potentially incapacitating or life-threatening. Screening blood profiles of hematology, chemistry and urine analysis were obtained before the start of the study and after the final dosing period.

Data Analysis

Summarizing [13C]-octanoic acid breath test values. [13CO2] in the breath samples was determined by isotope ratio mass spectrometry and expressed as a percentage of [13CO2] recovery per hour. The percentages of [13CO2] cumulative values over 6 hr were fit using a model given by the formula as described previously in the literature (Gooss et al., 1993; Choi et al., 1997): y = m(1 - e^{-kt}) + , where y is the cumulative percentage of [13CO2] excretion in breath at time t (hr) and m, k and are estimated parameters. In this model, m represents the total cumulative [13C] recovery when time is infinite. The duration of the lag phase was defined as the time when 10% of the test meal was emptied from the stomach. The duration of the lag phase and the gastric half-emptying time (t1/2) were derived after estimating k and .

Statistical Analysis

Gastric emptying data for the placebo, 5-, 10-, 20- and 30-mg oral doses of EM574 were compared using a crossover analysis of variance model that included effects for dose, period, sequence and subject within sequence. The repeated statement with the polynomial transformation was used to obtain the P values for linear and quadratic trends of the dose response curve. Adverse effects data were displayed and abnormal laboratory variables were identified. The frequency of adverse events was tabulated by body system. All statistical analyses were performed using SAS version 6.11 (SAS Institute Inc., 1989). All tests were two-sided and a significance level of .05 was used.

Results

Three volunteers withdrew during the 5-wk study; none of the withdrawals were related to adverse events. All other participants were able to complete the studies.

Effect of EM574 on gastric emptying. Although 27 participants completed five study periods, data of 4 subjects were excluded because of sampling errors. Thus, the data from 23 of 30 participants (15 males, 8 females, mean age 29 yr, range 18–45 yr) were available for analysis of gastric emptying. Both lag phase and gastric half-emptying time were significantly shorter after oral administration of 10, 20, 30 mg of EM574 (P < .001) when compared to placebo (fig. 1). The 5-mg dose of EM574 also significantly shortened the gastric half emptying time. Increasing doses of EM574 accelerated gastric emptying in a dose-related manner (fig. 1, P < .001 for a linear trend; P < .05 for a quadratic trend). However, the highest (30 mg) oral dose of EM574 tested was not associated with faster gastric emptying compared to the 20-mg dose. The gastric emptying data of two participants who vomited after receiving the 30-mg dose were excluded from the analysis because vomiting likely influenced the intragastric content of [13C]-octanoic acid.

Adverse events of EM574. Few drug-related adverse events were reported after the 5-, 10- and 20-mg doses; however, 11 of the 28 subjects had at least one adverse event after the 30-mg dose. Two participants experienced abdominal pain after receiving 20 mg of EM574 and four participants experienced abdominal pain after receiving 30 mg (table 1). In addition, 2 of the 28 participants experienced vomiting and abdominal cramps after taking the 30-mg dose. However, these side effects were easily tolerated by the subjects. The three subjects who withdrew from the study prior to the final (fifth) study did not withdraw because of adverse effects. The other 27 participants completed the studies.

Standard laboratory evaluations undertaken following the last administration of study medication showed no clinically significant hematological or biochemical changes from baseline; values outside the normal range were not regarded as clinically significant.

Fig. 1. The effect of oral EM574 on the duration of lag phase and gastric half-emptying time, shown as mean and S.E.M. Note EM574 significantly accelerated gastric emptying in a dose-dependent manner up to 20 mg (*P < .05, **P < .001 compared to placebo).
TABLE 1

Drug-related adverse events observed during the study

<table>
<thead>
<tr>
<th>Body System</th>
<th>Placebo (N = 28)</th>
<th>EM574 5 mg  (N = 29)</th>
<th>EM574 10 mg (N = 29)</th>
<th>EM574 20 mg (N = 28)</th>
<th>EM574 30 mg (N = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any event</td>
<td>1 (3.6%)</td>
<td>0</td>
<td>3 (10.3%)</td>
<td>2 (7.1%)</td>
<td>11 (39.3%)</td>
</tr>
<tr>
<td>Body as a whole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>0</td>
<td>1 (3.4%)</td>
<td>2 (7.1%)</td>
<td>4 (14.3%)</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>0</td>
<td>1 (3.4%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Digestive system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eructation</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Increased appetite</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>0</td>
<td>1 (3.4%)</td>
<td>2 (7.1%)</td>
<td>5 (17.9%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (7.1%)</td>
</tr>
<tr>
<td>Nervous system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>1 (3.6%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Skin and appendages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweating</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3.6%)</td>
</tr>
<tr>
<td>Special senses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal vision</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3.6%)</td>
</tr>
<tr>
<td>Amblyopia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3.6%)</td>
</tr>
</tbody>
</table>

* N. Number of participants in each treatment group.

A subject’s most severe adverse event was used in this analysis. A subject who reported two or more different costart terms that are in the same body system was counted only once in the body system total. The severity of all adverse events was mild except for indicated events (* one person experienced a moderately severe adverse event. (Two of five people experienced moderately severe adverse events).

Discussion

The aim of this study was to assess the effects of various dose levels of EM574 on gastric emptying in humans in vivo and to evaluate its adverse effects after a single oral administration. Oral EM574 accelerated gastric emptying of solids in healthy volunteers in a dose-dependent manner; however, the highest dose (30 mg) used did not accelerate gastric emptying more than the 20-mg dose. Both 10 and 20 mg of EM574 significantly accelerated gastric emptying without inducing significant adverse effects. Thus, these dosages are excellent candidates for use in clinical trials in patients with disorders of gastric motility and emptying. However, further studies in large numbers of participants and with repeated dosing over several weeks or months are still needed to assess the safety of this agent.

Our data demonstrated that both the duration of lag phase and gastric half-emptying time were accelerated by a single oral dose of EM574 without significant adverse effects in healthy volunteers except for two participants who developed vomiting with the 30-mg dose and three subjects who dropped out before the fifth study. It is worth emphasizing that the gastric emptying parameters calculated from the \(^{13}C\)-octanoic acid breath test include the time required for absorption, metabolism and excretion of the substrate. If one assumes this mean value for the absorption and metabolism of octanoate and excretion of CO\(_2\) also applied to all the participants in the present study, the 5-, 10-, 20-, 30-mg doses of EM574 would have respectively shortened the gastric half-emptying time by 11, 22, 30 and 29% relative to placebo.

The 10-, 20-, 30-mg doses of EM574 significantly shortened the lag phase and the gastric half-emptying time. This finding confirms the prokinetic effect of erythromycin-like compounds on gastric emptying of solids. Intravenous erythromycin results in dumping of solid meals and a loss of gastric sieving (Lin et al., 1994).

The mechanism of action of EM574 is not fully understood. The human stomach responds to relatively low concentrations of EM574 or its biologically active metabolites (Funabashi et al., 1996) because the 5-mg oral dose stimulated gastric emptying. Erythromycin analogs (e.g., EM523) stimulate myenteric cholinergic neurons (Ohtawa et al., 1993) and directly stimulates motilin receptors on human antral smooth muscle cells in vitro (Satoh et al., 1994). Increase in amplitude of gastric contractions occurred after oral administration of erythromycin (Mathis and Malbert, 1995); this effect peaked at 60 min and lasted 180 minutes; the timing of this effect suggests an effect of a metabolite reaching the stomach via the circulation. In contrast, the increase in amplitude of duodenal contractions commenced 10 min after administration of erythromycin, was maximal within 20 min, and lasted for 40 min. This rapid onset has been interpreted as evidence that oral erythromycin has a direct local action on motilin receptors located on duodenal muscle (Mathis and Malbert, 1995). Inatomi et al. (1996) recently showed that high dose EM574 stimulates nonvagal, intrinsic cholinergic neurons whereas lower doses stimulate vagal cholinergic pathways.

The highest EM574 dose (30 mg) was no more effective than the 20-mg dose in accelerating gastric emptying, and two subjects reported vomiting after receiving 30 mg EM574. This minor toxicity is also consistent with previous observations with erythromycin. Higher doses of i.v. erythromycin also increase contractile activity, and disrupt the migrating motor complex (Zara et al., 1985), and induce retrograde giant contractions in association with retching and vomiting in the dog (Holle et al., 1992; Otterson and Sarna, 1990). It has been postulated that high doses of erythromycin nonspecifically inhibit membrane cation permeability abolishing the stimulatory effect of erythromycin (Minocha and Galligan, 1991; Lees and Percy, 1981; Peeters, 1993). Moreover, high
doses of oral erythromycin increase the amplitude of gastric contractions less than medium doses of the drug (Mathis and Malbert, 1995). A partial form of tachyphylaxis has been attributed to erythromycin (Richards et al., 1993), and the effect of repeated administration of EM574 needs to be elucidated.

This study used a relatively new technique to measure gastric emptying of solids; the technique has been thoroughly characterized in recent years (Ghoos et al., 1993; Choi et al., 1997; Choi et al., 1998). Previous studies showed that $^{13}$C-octanoic acid breath test could be useful in intraindividual comparisons of gastric emptying because the coefficient of variation on replicate studies was relatively low (mean of 14%). We suggested that it would be ideal for pharmacodynamic studies in large numbers of participants in view of its safety, noninvasiveness and excellent reproducibility within individuals (Choi et al., 1997). In the current study, $^{13}$C-octanoate acid breath tests were easily performed simultaneously in 30 healthy volunteers at a Biostudies Unit on five separate occasions. Our study also confirmed the simplicity of the $^{13}$C octanoic acid breath test that does not require any special equipment at the study site, in contrast to the alternative standard method that requires a scintigraphic camera to measure radioisotope emptying from the stomach. However, some supervision of the technical staff is needed to ensure satisfactory sample collection. Regrettably one technician involved in this study erroneously transferred expired air samples from the 3-liter bag to storage tubes (vacuators) over a period of 1 hr in four participants whose data were therefore lost.

In summary, the dose-dependent acceleration of gastric emptying by EM574 suggests that this may be effective as a gastrokinetic agent. Motilides may prove useful either alone or in combination with other prokinetic agents. Previous clinical trials showed that oral motilides such as erythromycin improved gastric emptying in neuropathic (Janssens et al., 1990; Kao et al., 1995; Catnach et al., 1993; Richards et al., 1993) and myopathic upper gut motility disorders (Fi-orucci et al., 1994), as well as in idiopathic gastroparesis (Richards et al., 1993). The effectiveness of oral macrolides in accelerating gastric emptying in healthy humans after single administration contrasts with the lack of significant effects of oral metoclopramide in enhancing gastric emptying of solids. Another oral prokinetic agent, the benzamide 5HT4 cisapride (Camilleri et al., 1986, Lazzaroni et al., 1987, Duan et al., 1995), has been previously reported to accelerate gastric emptying of solids. EM574, however, is the only agent that induces dose-related acceleration of gastric emptying in healthy participants. EM574, therefore, shows promise as a gastrokinetic agent and further studies are warranted using multiple dosing in patients with gastric stasis.

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References


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