Comparison of selective M₃ and nonselective muscarinic receptor antagonists on gastrointestinal transit and bowel habits in humans

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Abstract

Bharucha AE, Ravi K, Zinsmeister AR. Comparison of selective M₃ and nonselective muscarinic receptor antagonists on gastrointestinal transit and bowel habits in humans. Am J Physiol Gastrointest Liver Physiol 299: G215–G219, 2010. First published April 15, 2010; doi:10.1152/ajpgi.00072.2010.—Although in vitro studies show that muscarinic M₃ receptors primarily mediate the effects of acetylcholine on gastrointestinal contractility, the muscarinic receptor subtypes regulating gastrointestinal motor activity and transit in humans in vivo are unclear. We hypothesized that muscarinic M₃-specific but not nonspecific receptor antagonists would delay gastrointestinal and colonic transit in transit. In this parallel-group study, gastric emptying, small intestinal transit, and colonic transit were assessed by scintigraphy on days 4–6 in 72 healthy subjects (49 women) who received placebo (n = 16), the M₃ antagonist darifenacin ER (7.5 mg (n = 20) or 15 mg daily (n = 17)), or the nonspecific antagonist tolterodine [4 mg daily (n = 19)] for 6 days. Bowel habits were recorded by daily diaries. Both doses of darifenacin substantially delayed [P < 0.01 vs. placebo (for both doses), P < 0.01 vs. tolterodine (for 15 mg)] small intestinal transit, i.e., colonic filling at 6 h (placebo [39.6 ± 6.4%], 7.5 mg ER [34.4 ± 6.1%], 15 mg ER [20.4 ± 6.3%]). Darifenacin (15 mg) also delayed (P < 0.01 vs. placebo and tolterodine) half-time for ascending colonic emptying [placebo (12.0 ± 1.5 h), 7.5 mg (18.6 ± 1.9 h), 15 mg (22.9 ± 2.6 h)] and colonic transit (geometric center) at 24 [placebo (2.8 ± 0.2), 7.5 mg (2.4 ± 0.2), 15 mg (1.9 ± 0.2)] but not 48 h. Darifenacin did not affect gastric emptying and tolterodine did not affect bowel habits or gastrointestinal transit. With muscarinic antagonists used at clinically approved doses, these findings demonstrate that muscarinic M₃ receptors regulate small intestinal and colonic transit in humans; colonic effects are more pronounced in the right than left colon. A doses that affect small and large intestinal transit, M₃ antagonists do not affect gastric emptying in humans. The efficacy of darifenacin in diarrhea-predominant irritable bowel syndrome should be evaluated.

Cholinergic; motility; colonic; small intestine; irritable bowel syndrome

The effects of acetylcholine, which is the primary excitatory neurotransmitter in both the urinary bladder and the gastrointestinal tract, are mediated by M₁–5 muscarinic receptors. Although M₂ receptors outnumber M₃ receptors by a ratio of 3:1 and 4:1 in bladder and gastrointestinal tract smooth muscle, respectively, in vitro studies suggest that M₃ receptors are primarily responsible for mediating the excitatory effects of acetylcholine in both organs (1, 17). Muscarinic M₁ receptors mediate slow excitatory postsynaptic transmission in the myenteric plexus. In addition, presynaptic M₁ receptors in the guinea pig and M₂–M₄ receptors in the mouse small intestine inhibit acetylcholine release from nerve terminals (14, 20, 24, 30). Thus the net gastrointestinal effects of nonspecific muscarinic antagonists on gastrointestinal motor activity likely reflect a balance between excitatory and inhibitory effects. However, although muscarinic antagonists are widely used to treat gastrointestinal symptoms (e.g., abdominal cramping and diarrhea) and an overactive bladder, the relative contribution of muscarinic receptor subtypes to normal gastrointestinal motor functions in humans has not been studied. This question is important since the magnitude of antimuscarinic effects and muscarinic receptor selectivity for various muscarinic antagonists are organ and species specific (2, 8).

In clinical trials of patients with an overactive bladder, the incidence of constipation is higher with M₃ selective muscarinic antagonists, for example trospium chloride (10.9 vs. 5.8% for placebo) (26) and darifenacin [14.8% (7.5 mg daily) and 21.3% (15 mg daily) vs. 6.2% (placebo)] (9) than with nonspecific antagonists, e.g., tolterodine (7%) vs. placebo (4%) (33). These observations suggest that M₃-selective muscarinic antagonists have more pronounced effects than nonspecific antagonists on gastrointestinal motor activity in humans. Thus our hypothesis was that a muscarinic M₃-selective but not the muscarinic nonspecific receptor antagonist tolterodine would delay gastrointestinal and colonic transit in healthy subjects.

Methods

Healthy subjects. Seventy-two healthy volunteers aged 19 to 58 yr old (mean age, 35 yr; 24 men and 48 women) were recruited by public advertisement. None had an underlying illnesses or previous gastrointestinal surgery (other than appendectomy or cholecystectomy) or used medications other than oral contraceptives and thyroid hormone replacement therapy. Functional gastrointestinal disorders, anxiety, and depression were excluded by validated screening questionnaires, a clinical interview, and a physical examination (5, 36). Seventy subjects completed the study. Of the two remaining subjects, one had medication-related anticholinergic side effects, and logistical constraints precluded participation in another subject.

Drug. Subjects were randomly assigned, stratified by sex and age (i.e., < and ≥ 50 yr), to placebo ([n = 16], darifenacin 7.5 mg extended release (ER) (n = 20), darifenacin 15 mg ER (n = 17), or tolterodine 4 mg long acting (n = 19), administered once daily for 6 days. Tolterodine is a competitive nonspecific muscarinic receptor antagonist whereas darifenacin is an M₃-selective receptor antagonist. These doses are approved by the Food and Drug Administration for treating urinary symptoms. Medication compliance was assessed both by the return of an empty pill bottle at the conclusion of the study and by recording the time the medication was taken in the bowel diary.

After oral administration, both tolterodine and darifenacin are extensively metabolized, highly bound to plasma proteins, and extensively metabolized by CYP2D6 in the liver. Tolterodine is initially metabolized to the pharmacologically active 5-hydroxymethyl metabolite, whose antimuscarinic effects are similar to those of tolterodine.

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and small bowel transit were measured by a 99mTc-labeled egg meal. Transit were assessed by established and validated scintigraphic tech-
tiques on days 4–6 after starting medication (11). Gastric emptying and small bowel transit were measured by a 99mTc-labeled egg meal. Colonic transit was measured by 111In-labeled charcoal pellets within a capsule coated by methacrylate. Gastric emptying was summarized as the half-time for gastric emptying. Colonic filling (i.e., the proportion of 99mTc reaching the colon) at 6 h was used to measure orocecal transit (i.e., a surrogate for small bowel transit). Colonic filling is expressed by measuring the proportion of total 99mTc counts at 6 h, corrected for decay and tissue attenuation, which are in the colon, typically in the cecum and ascending colon. Overall colonic transit was summarized as the colonic geometric center (GC) at 4, 24, and 48 h. The GC represents the average of counts in different colonic regions (ascending, transverse, descending, and rectosigmoid colon) and stool, weighted by factors of 1 to 5, respectively, at these time points. Therefore, a higher GC represents faster colonic transit. Ascending colonic emptying was summarized by the half-time (t_{half}) calculated by linear interpolation of values on the ascending colonic emptying curve. For 6 days before and 6 days after beginning medication, subjects also recorded stool form (Bristol stool scale), and ease of defecation (1 being manual disimpaction to 7 being incontinence of stool) for every bowel movement in a bowel diary.

Statistical analysis. The primary end point was colonic transit as measured by the GC at 24 h (GC_{24}). Secondary end points included the t_{half} for gastric emptying, the colonic filling at 6 h, which is a measure of small intestinal transit, GC of colonic transit at 48 h (GC_{48}), and effects on bowel habits (i.e., stool frequency, form, ease of passage, and incomplete evacuation). Data were analyzed by an analysis of covariance incorporating sex as a covariate along with the main effect for treatment group. Analysis of posttreatment bowel diaries incorporated baseline symptom scores as covariates. Since the interaction term (sex by treatment) was not significant, the reported results are based on the main effects models. To accommodate an intent-to-treat analysis, subjects with missing data on any end point had their missing values imputed by using the overall mean in subjects with data for that end point. A corresponding adjustment in the residual error degrees of freedom was made by subtracting one degree of freedom for each missing value imputed for a given end point. The analysis incorporated Bonferroni corrections for three comparisons with placebo (i.e., 2 doses of darifenacin and 1 dose of tolterodine) and two comparisons among drugs (i.e., tolterodine vs. each of 2 doses of darifenacin).

The effect of darifenacin on colonic transit in humans is unknown. Therefore, the sample size was estimated from the pooled distribution of colonic transit (i.e., GC_{24}) in healthy subjects in previous studies wherein the mean GC_{24} was 2.75 and the SD was 1.0. With a sample size of 18 subjects per group, this study had 80% power (with a two-sample t-test and two-sided alpha level of 0.05) to identify a difference of 1.0 unit in the GC_{24} between any two drugs. Differences greater than one unit are considered to be clinically relevant (16, 34).

RESULTS

Subject characteristics. Seventy of 72 subjects completed the study. Data were imputed for two subjects (both darifenacin 7.5 mg) who dropped out prior to the transit study. Consistent with the stratified randomization, the age and sex were evenly distributed among groups (Table 1). The body mass index (BMI) was also not significantly different among groups.

Effects on gastrointestinal transit. Neither tolterodine nor darifenacin had significant effects on gastric emptying (Table 2) compared with placebo, both doses of darifenacin delayed small bowel transit (P < 0.01); the higher dose also delayed (P = 0.0001) ascending colonic emptying and colonic transit at 24 h (GC_{24}) but not at 48 h (GC_{48}). The higher dose of darifenacin (15 mg) also delayed (P = 0.003) small bowel and colonic transit (GC_{24}) and the ascending colonic emptying t_{half} vs. tolterodine (Table 2). In contrast, tolterodine did not significantly affect gastric emptying or small intestinal or colonic transit (Table 2, Fig. 1).

The effects on gastrointestinal and colonic transit parameters were correlated. The t_{half} for ascending colonic emptying was inversely correlated with colonic filling at 6 h (r = −0.46, P < 0.0001), GC_{24} (r = −0.81, P < 0.0001), and GC_{48} (r = −0.53, P < 0.0001), implying that a longer ascending colonic emptying time was associated with less colonic filling at 6 h (i.e., slower small intestinal transit) and with slower colonic transit at 24 and 48 h.

Gastric emptying and colonic transit were slower (P < 0.01) in women. The effects of sex were not modified by treatment, i.e., the sex-by-treatment interaction was not significant. Thus the t_{half} for gastric emptying was 133 ± 4 (means ± SE) min in women and 111 ± 6 min in men. The GC_{48} for colonic transit was lower, reflecting slower colonic transit, in women (i.e., 3.8 ± 0.1) than in men (i.e., 4.0 ± 0.2).

Effects on bowel habits. The Bristol stool form scale score was lower (P < 0.01), reflecting harder stools, in subjects who

### Table 1. Demographic characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Tolterodine</th>
<th>Darifenacin (7.5 mg)</th>
<th>Darifenacin (15 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>16</td>
<td>19</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Age</td>
<td>32.8 ± 1.7</td>
<td>34.9 ± 2.4</td>
<td>35.8 ± 2.3</td>
<td>34.1 ± 2.7</td>
</tr>
<tr>
<td>Women</td>
<td>10</td>
<td>13</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.1 ± 1</td>
<td>26 ± 1</td>
<td>28.1 ± 1</td>
<td>26.7 ± 0.9</td>
</tr>
</tbody>
</table>

All values represent observed mean [95% confidence interval (CI)].

(12, 32). Most (~93%) Caucasian subjects have the cytochrome P-450 enzyme CYP2D6 and are characterized as extensive metabolizers.

### Table 2. Effects of drugs on gastrointestinal transit

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Tolterodine</th>
<th>Darifenacin (7.5 mg)</th>
<th>Darifenacin (15 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric emptying, t_{half}, min</td>
<td>125.5 ± 6.7</td>
<td>127 ± 6.4</td>
<td>115.4 ± 6.4</td>
<td>120.4 ± 6.7</td>
</tr>
<tr>
<td>Colonic filling at 6 h, %</td>
<td>59.6 ± 6.4</td>
<td>47.1 ± 6</td>
<td>34.4 ± 6.1†</td>
<td>20.4 ± 6.3‡†</td>
</tr>
<tr>
<td>Colonic transit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending colonic emptying, t_{half}, h</td>
<td>12.0 ± 1.5</td>
<td>14.0 ± 1.0</td>
<td>18.6 ± 1.9*</td>
<td>22.9 ± 2.6††</td>
</tr>
<tr>
<td>GC_{24}</td>
<td>2.8 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>1.9 ± 0.2‡†</td>
</tr>
<tr>
<td>GC_{48}</td>
<td>3.8 ± 0.2</td>
<td>4 ± 0.2</td>
<td>3.6 ± 0.2</td>
<td>3.4 ± 0.2</td>
</tr>
</tbody>
</table>

All values represent least squares means (95% CI), adjusted for gender and BMI. GC_{24} and GC_{48}, geometric center of colonic transit at 24 and 48 h, respectively. *P = 0.02; †P < 0.01 vs. placebo; ‡P < 0.01 vs. tolterodine.
received darifenacin 15 mg (2.8 ± 0.2) than those who received tolterodine (3.6 ± 0.2) (Table 3). Otherwise, stool frequency, ease of stool passage, and the sense of incomplete evacuation were not significantly different among groups.

The BMI was associated with the GC24 (r = 0.26, P = 0.03) and GC48 for colonic transit (r = 0.32, P = 0.008). Moreover, stool frequency (r = 0.34, P = 0.004), stool form (r = 0.37, P = 0.002), and ease of passage (r = 0.28, P = 0.02) were all associated with GC24. However, the sense of incomplete evacuation was not associated with colonic transit.

**DISCUSSION**

Although muscarinic antagonists have been demonstrated to inhibit contractility and are often used to treat abdominal pain and diarrhea, their effects on gastrointestinal transit in humans are poorly understood. This is the first controlled study to compare the effects of nonspecific and relatively selective M3 receptor antagonists on gastrointestinal transit in humans. A lower dose of darifenacin delayed small intestinal transit whereas a higher dose also delayed ascending colonic emptying and colonic transit, demonstrating that muscarinic M3 receptors regulate small intestinal and colonic transit in humans. Darifenacin delayed GC24 and ascending colonic emptying but not GC48, suggesting a more pronounced effect on right than left colonic motor functions. In contrast to darifenacin, tolterodine, which has a comparable affinity for M3 receptors, delays orocecal and colonic transit in healthy subjects. Both doses of darifenacin delayed small intestinal and colonic transit in healthy subjects. The higher dose delayed small intestinal and colonic transit as measured by the GC at 24 h (GC48) compared with placebo and tolterodine. *P < 0.01 vs. placebo, ‡P < 0.01 vs. tolterodine.

**Fig. 1.** Effect of darifenacin and tolterodine on small intestinal and colonic transit in healthy subjects. Both doses of darifenacin delayed small intestinal transit [i.e., colonic filling at 6 h (CF6)] relative to placebo. The higher dose delayed small intestinal and colonic transit as measured by the GC at 24 h (GC48) compared with placebo and tolterodine. †P < 0.01 vs. placebo, ††P < 0.01 vs. tolterodine.

Table 3. **Effects of drugs on bowel habits**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Tolterodine</th>
<th>Darifenacin (7.5 mg)</th>
<th>Darifenacin (15 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of bowel movements/day</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Bristol stool form score</td>
<td>3.1 ± 0.2</td>
<td>3.6 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>2.8 ± 0.2‡</td>
</tr>
<tr>
<td>Ease of passage</td>
<td>3.9 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Incomplete evacuation*</td>
<td>10 ± 4</td>
<td>7 ± 4</td>
<td>3 ± 4</td>
<td>17 ± 4</td>
</tr>
</tbody>
</table>

All values represent least squares mean (95% CI), adjusted for gender and BMI. *Proportion (%) of bowel movements with incomplete evacuation. ‡P < 0.01 vs. tolterodine.
The effects of darifenacin (15 mg) on intestinal and colonic transit may be clinically relevant. Darifenacin also significantly reduced stool consistency compared with tolterodine; stool characteristics (i.e., consistency, frequency, and ease of passage) were significantly correlated with colonic transit as shown previously (3). The magnitude by which darifenacin delayed small intestinal and colonic transit is, respectively, comparable to and lower than the effect of codeine (30 mg po qid) on these parameters (16). In contrast to codeine, both doses of darifenacin delayed ascending colonic emptying compared with placebo. Current concepts, based on limited data, suggest that patients with chronic constipation who have delayed ascending and transverse colonic emptying also have delayed overall colonic transit (29). However, larger studies are necessary to evaluate whether a subset of patients with chronic constipation have an isolated delay in right colonic emptying. The effects of darifenacin on gastrointestinal transit are germane since urinary urgency is associated with functional constipation (4). From a therapeutic perspective, the effects of darifenacin on symptoms and gastrointestinal transit in irritable bowel syndrome (IBS), which can be associated with rapid transit, are worthy of further study (7). The darifenacin-induced delay in intestinal and colonic transit in this study is more pronounced than the effects of alosetron in patients with diarrhea-predominant IBS (31). Indeed, zamifenacin, which is also a relatively selective M3 antagonist, reduced the postprandial colonic contractile response in IBS (19).

In summary, these findings, using muscarinic antagonists at clinically approved doses, demonstrate that muscarinic M3 receptors mediate the excitatory effects of acetylcholine on small intestinal and colonic transit in humans. These effects are potentially clinically significant and more pronounced on the right than the left colon. At doses that affect small and large intestinal transit, M3 antagonists do not affect gastric emptying in humans. Further studies evaluating the effects of darifenacin on gastrointestinal and colonic transit in patients with urinary urgency and in diarrhea-predominant IBS are necessary.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


