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

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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 nonselective receptor antagonists would delay gastrointestinal and colonic transit in humans. 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 or n = 20) or 15 mg daily (n = 17)), or the nonselective 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%], mean ± SE), 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. At 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.

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–M₃ 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 effectively absorbed, 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.
and small bowel transit were measured by a $^{99m}$Tc-labeled egg meal. The transit was assessed by established and validated scintigraphic techniques on days 4–6 after starting medication (11). Gastric emptying and small bowel transit were measured by a $^{99m}$Tc-labeled charcoal pellets within a capsule coated by methacrylate. Gastric emptying was summarized as the proportion of stomach contents emptied at 2 and 4 h and by the half-time for gastric emptying. Colonic filling (i.e., the proportion of $^{99m}$Tc reaching the colon) at 6 h was used to measure orocecal transit (i.e., the sex-by-treatment interaction was not significant. Thus 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 ($P = 0.001$) 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 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 $t_{\text{half}}$ 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 $t_{\text{half}}$ 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>
<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, $t_{\text{half}}$, 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, $t_{\text{half}}$, 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>4.2 ± 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 oclonic 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 all five muscarinic receptors in the urinary bladder, did not delay gastrointestinal or colonic transit, confirming a previous study (3).

The observed effects of darifenacin are consistent with in vitro and in vivo studies in animals, suggesting that muscarinic receptor antagonists inhibit peristalsis and that, among muscarinic receptors, the M3 subtype is primarily responsible for regulating gastrointestinal motility (15). There are three potential explanations for why darifenacin but not tolterodine delayed small intestinal and colonic transit. First, darifenacin is a more potent competitive antagonist than tolterodine at M3 receptors (15). Second, tolterodine may offset the effects of antagonizing excitatory receptors by also blocking the inhibitory effects of muscarinic stimulation on motility, either by blocking presynaptic muscarinic receptors that inhibit acetylcholine release, which are of the M1, M2, or M4 variety (14, 20, 30), or by blocking M1 receptors on nonadrenergic-noncholinergic inhibitory pathways (13, 27). In the human colon, M3 receptors are located presynaptically on nerve fibers, suggesting they autoregulate acetylcholine release (18). Indeed, the M1 antagonist pirenzepine facilitated peristalsis in the guinea pig small intestine, perhaps by withdrawing tonic M1 receptor-mediated inhibition of acetylcholine release from circular muscle (14, 28). Third, tolterodine binds relatively selectively to muscarinic receptors in the urinary bladder (23). Perhaps this also explains why atropine, which is also a nonspecific muscarinic antagonist but in contrast to tolterodine binds to colonic muscarinic receptors, delays orocecal and colonic transit in humans and dogs, respectively (6, 10). Atropine also has a higher affinity for M3 than for M2 receptors (15).

In the stomach, M3 receptors are located not only on gastric smooth muscle but also increase pacemaker frequency via effects on interstitial cells of Cajal in the murine gastric fundus and antrum (21). Atropine reduced antral motor activity and delayed gastric emptying in humans (25, 35). In contrast, neither tolterodine nor darifenacin delayed gastric emptying, suggesting perhaps that compensatory mechanisms preserve gastric emptying despite antagonism of M3 receptors, as exemplified by the observation that contractile responses to carbachol but not gastric emptying were inhibited in muscarinic M2 and separately M3 knockout mice (22).

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**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, zanamifen, 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


