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Effects of a 5-HT₃ antagonist, ondansetron, on fasting and postprandial small bowel water content assessed by magnetic resonance imaging

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For Peer Review

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Effects of a 5-HT₃ antagonist, ondansetron, on fasting and postprandial small bowel water content assessed by magnetic resonance imaging.

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RUNNING HEAD: 5-HT₃ antagonist and small bowel water

Key words: ondansetron, MRI, colon, volume, gastric emptying

SUMMARY

Background

5-HT₃ antagonists have been shown to be effective in relieving symptoms of IBS-D. Using a recently validated MRI method we have demonstrated reduced fasting small bowel water content (SBWC) in IBS-D associated with accelerated small bowel transit. We hypothesized that slowing of transit with ondansetron would lead to an increase in SBWC by inhibiting fasting motility.

Aim

To assess the effects of ondansetron compared with placebo in healthy volunteers on SBWC and motility in two different groups of subjects, one studied using MRI and one using manometry.

Methods

Healthy volunteers were given either a placebo or ondansetron on the day prior to and on the study day. n=16 underwent baseline fasting and postprandial MRI scans for 270 min. In a second study, a separate group of n=18 volunteers were intubated and overnight MMC recorded. Baseline MRI scans were carried out after the tube was removed.

Results

Fasting SBWC was markedly increased by ondansetron ($P < 0.0007$). Ondansetron reduced the overall antroduodenal Motility Index ($P < 0.04$). The subjects who were intubated had significantly lower fasting SBWC ($P < 0.0002$) compared to the group of subjects who were not intubated.

Conclusions

5-HT₃ receptor antagonism increased fasting small bowel water. This was associated with reduced fasting antroduodenal Motility Index which may explain the clinical benefit of such

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INTRODUCTION

Serotonin (5-hydroxytryptamine [5-HT]) is a key signaling molecule in gastrointestinal physiology, acting via a range of different 5-HT receptors to stimulate secretion, sensation and motility (1-5). Ondansetron, a 5-HT₃ receptor antagonist inhibits the nausea and vomiting associated with chemotherapy. An important side effect in such patients is constipation. This has been exploited therapeutically in IBS-D patients in whom 5HT₃ receptor antagonists improve symptoms, reducing stool frequency, urgency and abdominal discomfort while increasing stool consistency (6). Other 5HT₃ receptor antagonists granisetron (7) and alosetron inhibit the colonic response to feeding and ondansetron inhibits the excessive postprandial increase in colonic tone characteristic of carcinoid syndrome (8). Although ondansetron slows colonic transit (9, 10) its effect on human small bowel motility and secretion is unclear.

Activation of 5-HT₃ receptors stimulate anion secretion (11) and motility, an effect blocked by 5-HT₃ receptor antagonists, but the relative importance of these effects in ondansetron's anti-diarrheal actions in man is uncertain. One key weakness in studies to date has been a lack of data on the effect on intestinal secretions, largely due to technical difficulties associated with the intestinal perfusion studies needed to answer such questions, which inhibit their use in patients.

We have recently validated a novel non invasive magnetic resonance imaging (MRI) technique for assessing small bowel water content (SBWC) (12, 13) which is highly acceptable to patients. Postprandially the SBWC depends on the balance between delivery of fluid via gastric emptying and the removal of fluid by either propulsion into the colon or absorption. By contrast, in the fasting state fluid flow is intermittent, with low basal levels interrupted periodically by increased flow driven by the MMC (14, 15) which propels residual fluid distally, ultimately into the colon.

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3 Our aim was to further understand the mode of action of ondansetron which our clinical
4 experience over the last 4 years suggests is highly beneficial to some IBS-D patients
5 (unpublished). We hypothesized that slowing of fasting small bowel transit with ondansetron
6 would lead to an increase in fasting small bowel fluid. We therefore performed a randomized,
7 double-blind, placebo-controlled, 2-way cross-over trial of ondansetron versus placebo to
8 assess its effect on the fasting and post prandial small bowel water content. Since our results
9 showed an increase in fasting small bowel water content we then performed a second RCT to
10 examine the effect of ondansetron on fasting small intestinal motility to see whether the change
11 in small bowel water was due to an inhibition of fasting motility.
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25 **MATERIALS AND METHODS**

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30 We carried out two separate randomized, double-blind, placebo-controlled, 2-way cross-
31 over studies in healthy volunteers. These studies were approved by the Local Research Ethics
32 Committee and by the Institution's R&D department. All volunteers gave informed written
33 consent before participating and the studies were carried out to Good Clinical Practice
34 standards. Web-based, computerized, block randomizations were provided by the local Clinical
35 Trials Support Unit and the codes were broken only after locking the databases. The drug packs
36 containing drug or matched placebo were manufactured according to Good Manufacturing
37 Practice.
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47 The inclusion criteria for the volunteers were: male or female, healthy volunteers, 18 to
48 65 years old, of normal body mass index. Exclusion criteria were pregnancy and breastfeeding,
49 any history of gastrointestinal illness, use of medication known to affect gastrointestinal transit,
50 such as opiates and anti-cholinergics, and unsuitability for MRI scanning (such as having any
51 metal implants or pacemakers). The subjects were asked to abstain from any medication likely
52 to alter gut motility for 24 hours (inhaler medications for asthma and the oral contraceptive pill
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3 were allowed), alcohol for 24 hours, caffeine for 18 hours and strenuous exercise for 24 hours
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5 prior to the study days. They were also asked to have consumed no food or drink since the
6
7 previous evening dinner (except for a small cup of water on wakening). They were asked to fill
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9 an eligibility questionnaire on the morning of each study day.
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12 13 14 **Study 1: MRI**

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18 Eighteen healthy volunteers were recruited for this study. Two subjects (1 male and 1
19
20 female) were excluded, since one did not comply with the fasting criteria (as shown by
21
22 presenting with a full stomach at the baseline MRI scans) and the other suffered from diarrhea
23
24 before the dosing period (as shown by the stool diaries). Hence, 16 subjects completed the
25
26 entire study. They were 8 females and 8 males (age 20.7 ± 0.2 y, body mass index 22.8 ± 0.6
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28 kg/m^2). Each volunteer was enrolled for a 2-day study period on 2 separate occasions,
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30 approximately one week apart.
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34 The volunteers received either 8 mg ondansetron t.d.s. or identical looking placebo on
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36 day 1. On day 2 they attended at the MRI unit at 8am after an overnight fast. Here they received
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38 a further dose of either 16 mg ondansetron or placebo followed by 50 ml water and one hour
39
40 later they underwent a set of fasting MRI scans (as shown schematically in the top diagram of
41
42 Figure 1).
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44
45 After their baseline MRI scans the subjects ate a standard 362 kcal rice pudding meal
46
47 comprising of 220 g of creamed rice pudding (J Sainsbury's, London, UK), 34 g of seedless
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49 raspberry jam (Robertson's, Addlestone, Surrey, UK) and 15 g of coarse wheat bran, eaten with
50
51 a glass of 100 ml of orange juice from concentrate (J Sainsbury's, London, UK) as previously
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53 used (13, 16). The meal provided 71.4 g carbohydrate and 4.38 g fat.
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56 After the meal, the subjects underwent serial imaging at 45 min intervals until $t=270$ min
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58 while subjective feelings of the volunteers' fullness, hunger, appetite, nausea, abdominal pain
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3 and bloating were assessed using self-assessment visual analogue symptoms scales. They
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5 also completed a Bristol Stool Diary (17) for one week beginning 4 days before scanning on
6
7 each occasion.
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10 The MRI scanning was carried out on a 1.5 T Philips Achieva scanner. Each volunteer
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12 was positioned supine in the scanner with a 4-element, parallel imaging body coil wrapped
13
14 around the abdomen. After acquiring a scout scan to locate the position of the abdominal
15
16 organs, a range of MRI scans were taken. A balanced gradient echo (balanced turbo field echo,
17
18 BTFE) sequence with echo time TE=1.19 ms and repetition time TR=2.4 ms) was used to
19
20 assess gastric emptying. This comprised 20 transverse images with an in-plane resolution of
21
22 1.56 mm x 1.56 mm and slice thickness of 10 mm, with no gap between slices. A single shot,
23
24 fast spin echo sequence (similar to that used for MR cholangiopancreatography (MRCP)) with
25
26 effective TE=320 ms was used to assess small bowel water content (12). This comprised 24
27
28 coronal images with in-plane resolution interpolated to 0.78 mm x 0.78 mm and a slice thickness
29
30 of 7 mm, with no gap between slices (acquired voxel size = 1.56 x 2.83 x 7 mm³). A dual
31
32 gradient echo imaging sequence (dual-echo fast field echo, FFE) with TE1=2.3 ms, TE2=4.6 ms
33
34 and TR=158 ms was used to visualize the anatomy. This comprised 24 coronal plane and 45
35
36 transverse images with in-plane resolution 1.76 mm x 1.76 mm and a slice thickness of 7 mm,
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38 with no gap between slices. Each image set was acquired on a single expiration breath-hold,
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40 the duration of which varied between 13 s and 24 s depending on the sequence. Including set-
41
42 up and imaging the volunteers spent approximately 6 min inside the magnet for every time
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44 point, spending the rest of the time sitting upright in an adjacent room. Figure 2 shows relevant
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46 examples of these MRI images.
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51 52 53 **Study 2: manometry** 54 55 56 57 58 59 60

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3 Twenty-five healthy volunteers were recruited for this study. Seven of these withdrew: 5
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5 due to discomfort with the intubation procedure on the first occasion, 1 due to equipment failure,
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7 and 1 due to changes in their commitments. Hence, 18 subjects completed the entire study.
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9 They were 8 female and 10 male (age 20.4 ± 0.7 y, body mass index 22.7 ± 0.6 kg/m²). Each
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11 volunteer was enrolled for a 3-day study period on 2 separate occasions, approximately one
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13 week apart.
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16 The subjects received either 8 mg ondansetron t.d.s. or identical looking placebo on
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18 days 1 and 2. At 12 noon on day 2 the volunteers attended at the study unit having eaten
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20 nothing since breakfast, which was eaten before 8am. Here, they were intubated in the seated
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22 position with a calibrated gastrointestinal catheter. The catheter used was a 6-channel, solid-
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24 state gastrointestinal transducer catheter with a steel weighted tip (Gaeltec, Dunvegan, Isle of
25
26 Skye, Scotland) and an outer diameter of 3.5 mm. Recording ports were positioned at 5cm,
27
28 10cm, 20cm, 22.5cm, 25cm and 27.5cm from the tip of the catheter. A local anesthetic spray
29
30 (Xylocaine spray, AstraZeneca) was administered, and they were allowed to sip water to aid the
31
32 passage of the catheter. The catheter was allowed to pass to the duodenum by normal
33
34 propagating activity (subjects were asked to recline on their right side to assist this), and its
35
36 position was confirmed by observing appropriate contractile activity (gastric or duodenal).
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38 Adjustments could then be made as necessary to the position of the catheter. Once correctly
39
40 positioned the tube was taped to the nose and the proximal end connected to a Flexilog 3000
41
42 ambulatory recorder (Oakfield Instruments Ltd, Eynsham, Oxon, UK). Subjects were then
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44 allowed to return home with a standard meal consisting of 450g of chicken lasagne (J.
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46 Sainsbury's, London, UK), 42g of wholegrain crackers (J. Sainsbury's, London, UK) and 40g of
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48 mature cheddar (Cathedral City, Dairy Crest Ltd, Shropshire, UK), with a total caloric content of
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50 809 kcal. They were instructed to consume their meal before 7pm. This meal was chosen to
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52 ensure complete digestion prior to going to bed, and volunteers were then asked to refrain from
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3 eating until the following morning, allowing the overnight fasting MMC frequency to be recorded.

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5 They were allowed to drink a small cup of water on waking.

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8 The subjects attended at the MRI unit at 7.30am on day 3, and the catheter was
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10 withdrawn and the recordings downloaded. They then received a final dose of 16 mg
11 ondansetron or placebo followed by 50 ml of water. One hour after dosing, a fasting set of MRI
12 scans was performed as described above for Study 1 and shown schematically in the bottom
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17 diagram of Figure 1.

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19 . Volunteers also completed a Bristol Stool Diary for 2 days before and 2 days during
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21 ondansetron or placebo treatment.
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25 **Data analysis**

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29 Measurements of the volume of the gastric contents were carried out on the axial
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31 images by manually tracing a region of interest around the meal within the stomach on each
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33 image slice using Analyze6 software (Biomedical Imaging Resource, Mayo Foundation,
34
35 Rochester, MN) and summing across the slices to determine the total volume at the different
36
37 time points. The surrounding organs and gastrointestinal gas were easily discriminated and
38
39 excluded from the region of interest. The time for half emptying was calculated from the plots of
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42 volume against time.
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45 The small bowel water content images were analyzed as previously described (12).
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47 Briefly, regions of interest were drawn manually around the small bowel on each of the MRCP
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49 images excluding regions such as the stomach, gall bladder and blood vessels using in-house
50
51 software on an IDL platform (IDL 5.4, Research Systems Inc, Boulder, CO). The software then
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53 calculated the number of voxels (a 3-dimensional data set) in each of the regions of interest with
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55 an intensity above a threshold calibrated using the corresponding subject's cerebrospinal fluid
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57 (12).
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3 Antroduodenal motility data was downloaded from the Flexilog 3000 recorders into the
4 Flexisoft III Data Display and Analysis software, Version 2, 6.0 (Sandhill Scientific - UK Ltd,
5 Eynsham, Oxon, UK) for an initial assessment and then exported into dedicated antroduodenal
6 analysis software (MMS – Medical Measurement Systems BV, Enschede, The Netherlands).
7 Motion artifacts (such as simultaneous pressure rises occurring at all transducers with similar
8 and identical duration) were eliminated and contractions were identified in all channels. A
9 contraction was deemed valid if it exceeded the baseline threshold of 10 mmHg. MMCs were
10 identified as periods of regular contractions exceeding the 10mmHg threshold at the maximum
11 frequency. Phase 3 contractions lasting less than 2 minutes, or with an amplitude of less than
12 10mmHg were excluded. A number of channel markers were used allowing assessment from
13 23.00 hours until removal of the catheter of the number of the fasting MMC phase 3 contractions
14 together with overall motility as assessed by the AUC (mmHg.s) and Motility Index ($LN((number$
15 $of\ contraction * Sum\ contraction\ amplitude)+1)$) for the total recording period.
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33 **Power and statistical analysis**

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38 The primary endpoint was the effect of ondansetron on fasting SBWC. Based on
39 previous data (13) we could calculate that to be able to detect a one standard deviation
40 increase in fasting SBWC with a power of 90% we would need 13 paired studies using $\alpha=0.05$.
41 We chose to study 18 to allow for dropouts or incomplete data.
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46 Statistical analysis was carried out using Prism 4 (GraphPad Software Inc.). Normality of
47 the data was tested using Shapiro-Wilk's test. Paired comparisons were performed using two-
48 tailed Student's t test for normal, and two-tailed Wilcoxon's test for non-normal data.
49 Comparisons between groups were performed using two tailed unpaired t test or Mann-
50 Whitney's U test respectively. 2-way analysis of variance was used to assess the significance of
51 differences over time and for this analysis non-normal data was Log transformed. Post hoc
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3 assessments were then performed using Bonferroni correction. The data are expressed as
4 mean (SEM) when normally distributed and as median (interquartile range) when not normally
5 distributed.
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10 11 **RESULTS**

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16 Ondansetron was well tolerated with no adverse events on both studies. All subjects in
17 Study 1 tolerated the test meal and serial MRI scanning procedures without difficulty. High
18 quality images as shown in Figure 2 were obtained in all subjects and no images were rejected
19 during the gastric emptying and small bowel water analysis. The procedures for the manometry
20 Study 2 were more demanding. From the initial 25 subjects recruited, dropouts, equipment
21 failures and overnight changes in the positioning of the tube reduced the available data to 18
22 subjects for the fasting MRI imaging, 17 subjects for the antral traces and 14 for the duodenal
23 ones.
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33 34 35 **Study 1: MRI**

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40 Figure 3 shows the fasting baseline ($t=-45$ min) small bowel water content individual data
41 points. This was significantly higher for ondansetron, median(IQR) 227(194-324) ml than for
42 placebo 167 (103-209) ml, $P < 0.0007$.
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47 The time courses of SBWC are shown in Figure 4. These showed a triphasic pattern
48 which matches well the one observed previously with the same meal in healthy subjects (13).
49 Postprandially, despite the stomach emptying half of its contents into the small bowel over the
50 first 90 minutes, SBWC fell significantly to median 17(9-34) ml at 90 min for placebo ($P < 0.001$)
51 and to 14(6-52) ml for ondansetron ($P < 0.001$). There was then a rise during a second phase
52 between 90 and 180 min during which there were no significant differences between drug and
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3 placebo. SBWC reached a plateau in a third phase between 180 and 270 min towards the end
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5 of which the two curves diverged though this difference was not statistically significant when
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7 corrected for multiple comparisons.
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10 There was no overall effect of ondansetron on gastric emptying (2-way ANOVA for effect
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12 of drug $P < 0.2$). The time to half empty the gastric contents ($T_{1/2}$) was the same between
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14 ondansetron (89 ± 6 min) and placebo (88 ± 5 min), $P < 0.9$. Only a small effect of ondansetron
15
16 was detected on the area under the gastric emptying curve, which was smaller for the
17
18 ondansetron (43753 ± 1895 ml min) than for the placebo (47313 ± 2343), $P < 0.05$.
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21 The symptoms scores for fullness, hunger, appetite, abdominal pain, nausea and
22
23 bloating showed no significant differences between ondansetron and Placebo. Daily stool
24
25 frequency and average stool consistency on the 2 days prior to the scanning day were not
26
27 significantly different between ondansetron and placebo. There was no effect of this short period
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29 of dosing on mean daily stool frequency during the 2 days post-dosing which was 1.5 ± 0.2 bowel
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31 movements on placebo and 1.6 ± 0.2 on ondansetron ($P < 0.7$). There was no significant
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33 difference in average stool consistency as measured from the Bristol Stool Form score which
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35 was 3.1 ± 0.3 on placebo and 3.8 ± 0.3 on ondansetron ($P < 0.1$).
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42 **Study 2: manometry**

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46 Ondansetron reduced all the antroduodenal motility parameters investigated, though
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48 only the difference in the Motility Index reached statistical significance (Table 1).
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51 These subjects were also scanned fasted immediately after removal of the
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53 nasoduodenal tube. A weak (Spearman's $r = -0.29$) negative correlation between fasting small
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55 bowel water content and Motility Index was observed though this was not significant ($P < 0.1$). In
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3 contrast with Study 1 though, here no difference in fasting small bowel water content between
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5 ondansetron (67 ± 12 ml) and placebo (74 ± 11 ml) was found ($P < 0.6$).

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7 However comparing the fasting small bowel water content of the 2 studies (Figure 5) shows that
8
9 irrespective of the study arm, the small bowel water content after overnight intubation 55(35-97)
10
11 ml was substantially lower than for the group that was not intubated 203(153-279) ml, Mann
12
13 Whitney's $P < 0.0001$. The average Global Well Being scores from the Bristol Stool
14
15 Questionnaires were also significantly lower for the intubated group (6.6 ± 0.4) than for the non
16
17 intubated group (8.4 ± 0.4), $P < 0.0002$ indicating that the intubation procedure was stressful.
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20 21 22 23 DISCUSSION

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27 Our main finding that ondansetron significantly increased fasting small bowel water
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29 content in a group of healthy volunteers could be either due to an increase in small bowel
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31 secretion or a reduction in fasting small bowel propulsive motility. It is unlikely that ondansetron
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33 stimulates secretion since data in both animals and man suggest to the contrary that they inhibit
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35 postprandial pancreatic and intestinal secretions (18-23). We feel it is more likely that the
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37 increase in fasting small bowel water content is due to reduced fasting small bowel motility.
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39 Ondansetron is known to inhibit colonic motility (24) and the MMC (25) at least in rodents. That
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41 this effect is mediated via 5-HT₃ receptors in man is also supported by our previous finding that
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43 5-HT₃ agonists appear to have the opposite effect, increasing MMC frequency and accelerating
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45 small bowel transit in the postprandial state (26). We carried out Study 2 to test the hypothesis
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47 that ondansetron would inhibit MMCs in humans.
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51 We found in Study 2 that ondansetron significantly reduced the antroduodenal Motility Index
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53 ($p < 0.04$) and it also reduced the number of peaks per minute, the peak amplitude and the area
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55 under the curve of the fasting MMCs, though these differences did not reach statistical
56
57 significance. This agrees with previous studies in which 5-HT administration increased the
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3 frequency of the MMC (27, 28) whilst an antagonist of 5-HT₃ receptors would be expected to
4
5 reduce MMC frequency (25, 29-32) particularly via action on interneurons of the myenteric
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7 plexus, which are involved in the initiation of MMCs (33).
8
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10 This inhibitory effect is in keeping with the fact that ondansetron was originally
11 developed to inhibit the nausea induced by chemotherapeutic agents, particularly cisplatin
12 which releases large amounts of serotonin from mucosal enterochromaffin cells and induces
13 vomiting by stimulating 5HT₃ receptors on the vagus nerve. This inhibitory effect is also
14 associated with slowing of colonic transit in man (9) which may allow increased time for
15 absorption and contribute to an increase in stool consistency which was a striking benefit in
16 many trials of 5HT₃ receptor antagonists (34). Ondansetron appeared to have a lesser effect
17 postprandially than when fasting. Although SBWC was lower on ondansetron from 180-270
18 minutes postprandially this difference was not significant possibly because while the anti-
19 secretory effects of ondansetron reduce SBWC, its anti-motility effects may produce the
20 opposite effect. Another factor to consider is that both 5HT₃ and 5HT₄ receptors are important in
21 controlling human jejunal secretion and peristalsis (35, 36) thereby reducing the effect of a pure
22 5HT₃ antagonist like ondansetron. The increased fasting small bowel water did not appear to be
23 associated with symptoms like bloating in our volunteers and in one clinical trial ondansetron did
24 not worsen bloating (37).
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42 Our technique only measures volume, although if combined with oro-cecal transit time a
43 flow index can be calculated as we did in a previous study (13) but this measurement was not
44 made in the current study .
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48 We found a striking difference between the fasting small bowel water content of the
49 subjects in the MRI feeding Study 1 and those in the manometry Study 2. The fasting small
50 bowel water was found to be very much lower ($P < 0.0001$) amongst the intubated subjects. We
51 feel the failure to reproduce Study 1's findings of the effect of ondansetron was due to the fact
52 that this effect of intubation was so much stronger than the drug effect.
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Gastrointestinal intubation has been shown previously to affect gastrointestinal function, delaying gastric emptying and accelerating small bowel transit (38). It seems likely that the fall in small bowel water is due to increased propulsive motility induced by mechanoreceptor-mediated stimulation by the catheter. Mechanical stimulation of the mucosa is known to stimulate secretions via serotonin release (18) and to stimulate propulsive motility via 5-HT_{3&4} receptor stimulation (39).

Nausea and stress induced by intubation also have a role in accelerating small bowel transit (40, 41). Stress from the intubation procedures or from disturbed sleep due to the presence of the tube might therefore have had an influence on the amount of small bowel water content. We did not assess formally the level of stress of the subjects in the manometry Study 2, though anecdotally they all found it unpleasant. We did assess Global Well Being and in keeping with our anecdotal impression these subjects did have significantly reduced scores compared to the group of Study 1. It is interesting to note a possible analogy with patients with irritable bowel syndrome with diarrhea (IBS-D), a group in which the role of stress and anxiety is important and who were recently shown to have reduced fasting small bowel water and accelerated small bowel transit which correlates negatively with anxiety (13).

A unique feature of our MRI studies is the ability to describe the physiological changes in an otherwise undisturbed SBWC after feeding. It can also provide information about the overall impact of changes in small bowel motility, absorption and secretion unlike intubated studies which assess motility at only a few discrete points in the bowel and absorption from a limited segment of bowel. The use of magnetic resonance imaging to assess gastric emptying has been documented in the literature (42-45) and this has recently been used in conjunction with validated MRI measurements of small bowel water content (12). This allows an accurate assessment of both gastric volumes and small bowel water content, which yields novel insights in gastrointestinal events, physiology and disease (13). We scanned 3 individuals per session and acquired individual scans at 45 min intervals, a time sufficient to characterize the time

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3 courses while keeping costs reasonable. We performed these initial studies in healthy
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5 volunteers since they are a much more homogenous group than IBS-D patients, hence making
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7 it easier to show a pharmacological effect with small numbers. We are currently in the process
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9 of performing a much larger randomised placebo controlled study (RCT) of ondansetron in IBS-
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11 D to see if these findings can be reproduced in patients. Although this is currently not a licensed
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13 indication we have been using ondansetron for severe IBS-D for some years at doses ranging
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15 from 4mg daily to 8mg t.d.s and find that those who do respond do so in the lower dose range.
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17 Of course definitive recommendations must await the results of our current RCT.
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21 The MRI technique was well tolerated and provided novel insights into the action of this
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23 drug and could be a marker of those who would respond to the drug. It could be applied to
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25 patient studies of other conditions such as celiac disease and lactose intolerance in which
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27 increased small bowel water has been described. It could also be used to study the mode of
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29 action of a range of new drugs designed to alter secretions and motility such as lubiprostone,
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31 linaclotide, 5-HT₃ antagonists, opioids and 5-HT₄ agonists. The time and effort that it takes to
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33 process of the large amount of data collected during serial MRI studies is a significant burden.
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35 However current improvements in semi-automated image processing and an optimized and
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37 reduced scanning schedule will help broaden the use of these functional GI MRI techniques.
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39 Although currently expensive, as experience with the technique grows it is highly likely that
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41 shorter scanning protocols will reduce costs further so that they become comparable in price to
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43 other investigations such as Manometry or endoscopy.
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Table 1. Antroduodenal motility parameters from 2300 hours until tube removal at around 0800 hours the next day while taking either ondansetron or placebo. (mean±SEM reported with *P* values)

		Placebo	Ondansetron	Significance of difference
Phase III MMCs	Duration (seconds)	353±59	331±25	<i>P</i> = <i>n.s.</i> (<0.2)
	Number of peaks	32±5	31±5	<i>P</i> = <i>n.s.</i> (<0.9)
	Mean amplitude (mmHg)	96±15	77±11	<i>P</i> = <i>n.s.</i> (<0.2)
	Frequency (1/min)	5.6±0.6	5.3±0.6	<i>P</i> = <i>n.s.</i> (<0.2)
Overall overnight motility	Peaks/min	0.7±0.1	0.56±0.08	<i>P</i> = <i>n.s.</i> (<0.09)
	Mean amplitude (mmHg)	39±3	35±3	<i>P</i> = <i>n.s.</i> (<0.4)
	AUC (mmHg·s)	6223±484	4692±459	<i>P</i> = <i>n.s.</i> (<0.08)
	Motility Index (mmHg)	9.2±0.4	8.3±0.4	<i>P</i> <0.04

Figure Legends

Figure 1. Schematic diagrams of the study protocols for the magnetic resonance imaging Study 1 (top panel) and the manometry Study 2 (bottom panel).

Figure 2. Examples of the images acquired during the magnetic resonance imaging Study 1. (a) shows an anatomical dual-echo fast field echo coronal slice through the small bowel of a fasted subject. (b) and (c) show the corresponding magnetic resonance cholangiopancreatography coronal slice acquired on the same fasted subject on two different occasions after receiving (b) ondansetron and (c) placebo. This particular sequence yields high intensity (bright) signals from areas with liquid fluid and little (dark) signal from body tissues. Comparing (b) and (c) it can be clearly seen that the amount of fluid in the small bowel of this fasted subject was higher for the ondansetron arm of the study.

Figure 3. The fasting small bowel water content in ml for both the ondansetron and the placebo conditions of the magnetic resonance imaging Study 1. The individual values are connected by the diagonal lines and the median values are indicated by the horizontal lines (* $P < 0.0007$).

Figure 4. The time course of the small bowel water content plotted before and after meal ingestion while taking either ondansetron or placebo. The values are shown here as mean \pm SEM (* $P < 0.0007$).

Figure 5. Comparison between all the fasting small bowel water content values of the subjects in Study 2 immediately after removal of the naso-duodenal tube and of all the subjects of Study

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1, who had not been intubated. The difference between the two groups is highly significant. The median values are indicated by the horizontal lines (* $P < 0.0002$).

For Peer Review

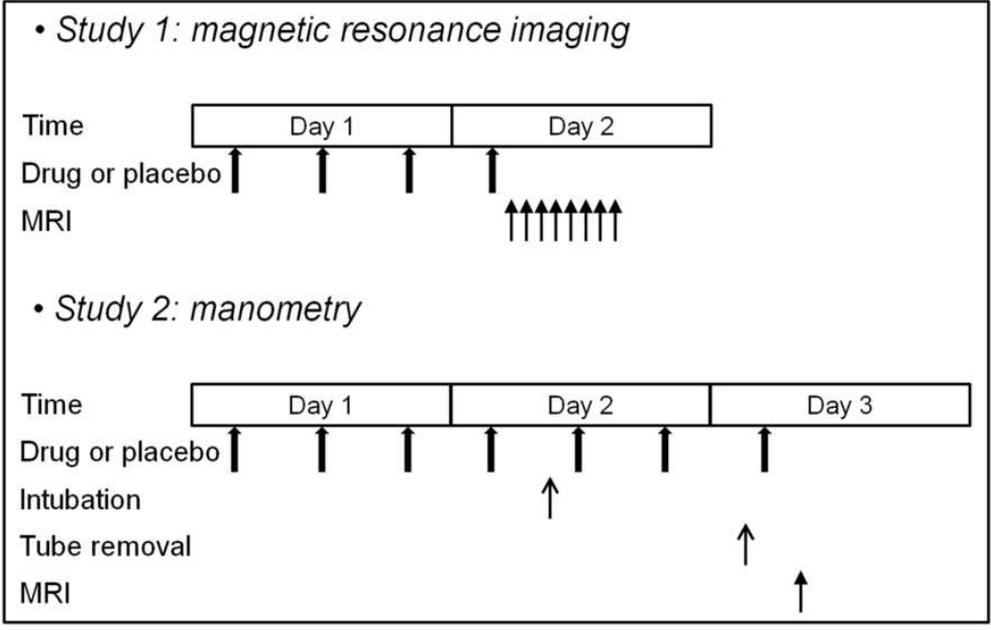
Acknowledgements

Declaration of personal interests: There is no conflict of interest for any of the authors.

Declaration of funding interests: This study was funded in part by the University of Nottingham's Institute of Neuroscience and it was supported by the Nottingham Gastrointestinal and Liver National Institute for Health Research Biomedical Research Unit (NIHR BRU).

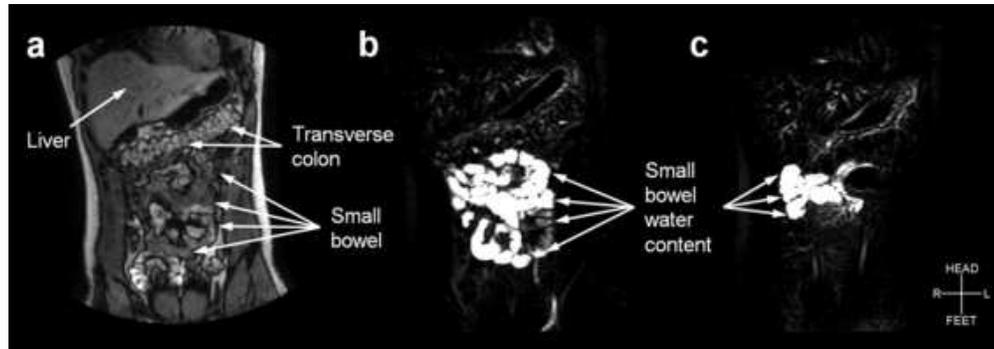
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Review

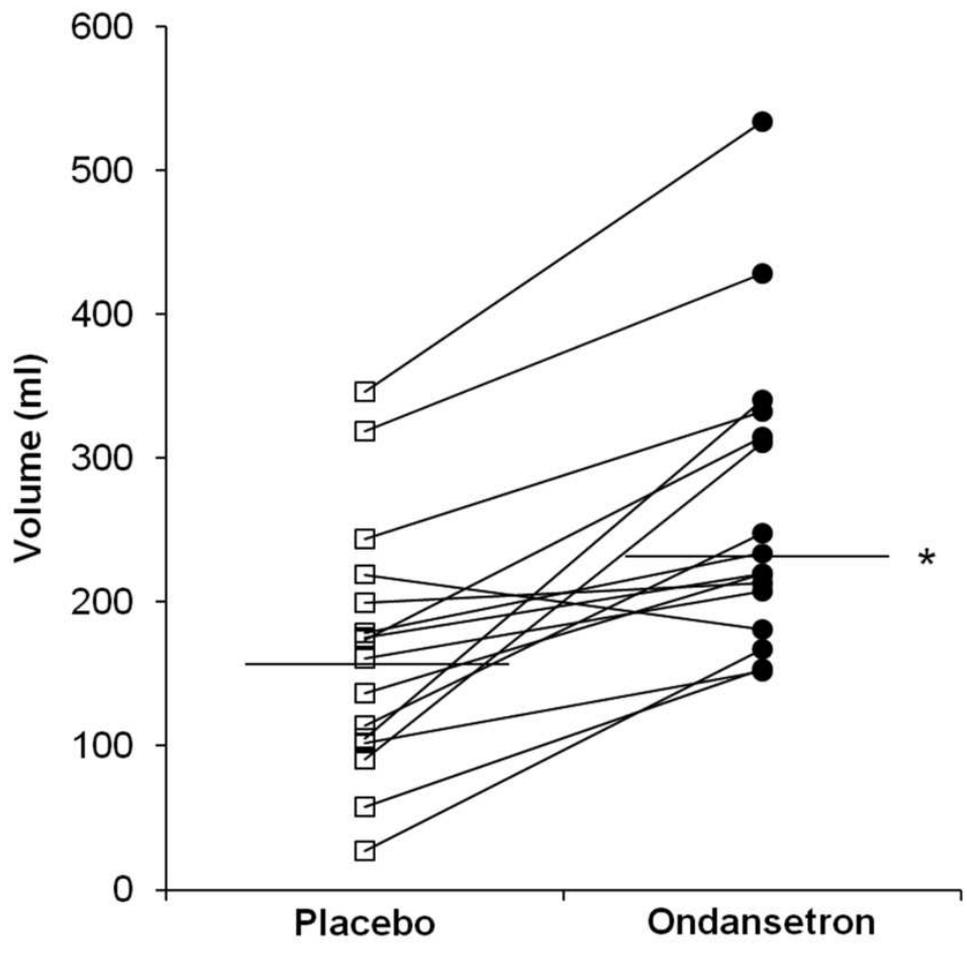


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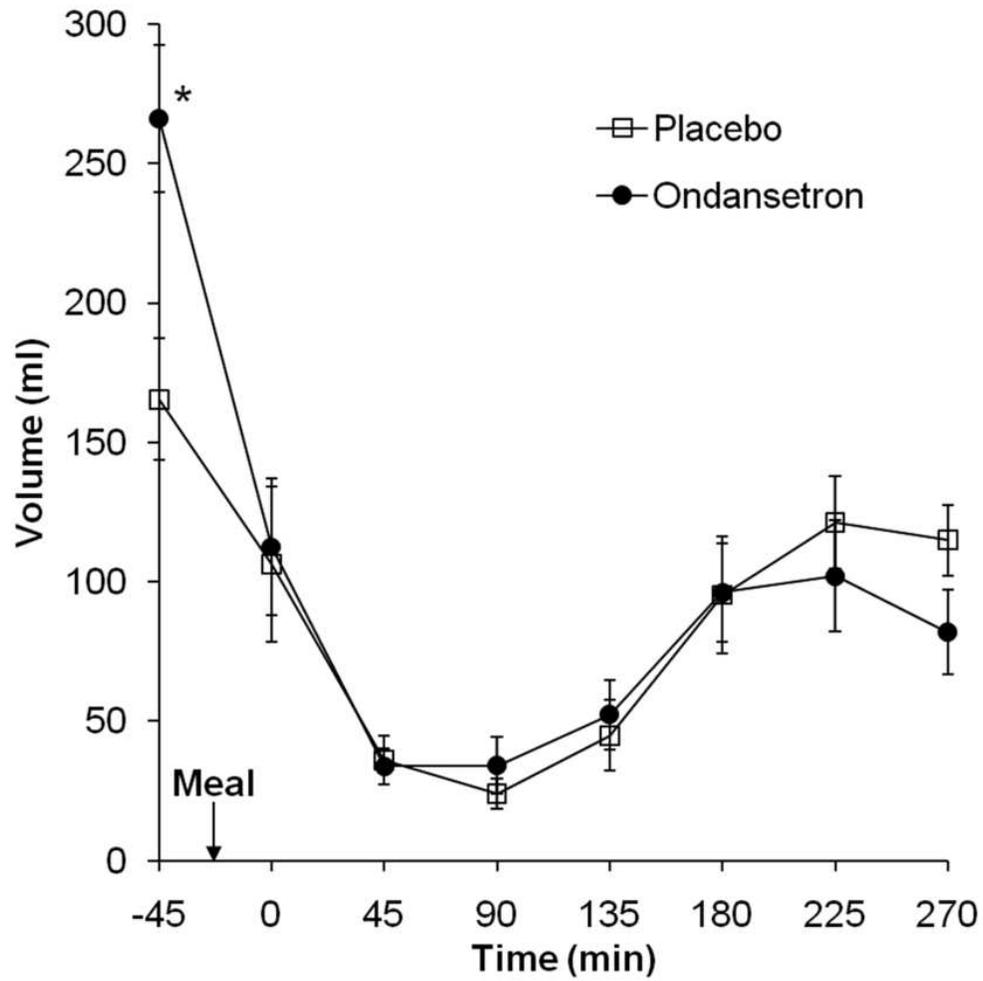
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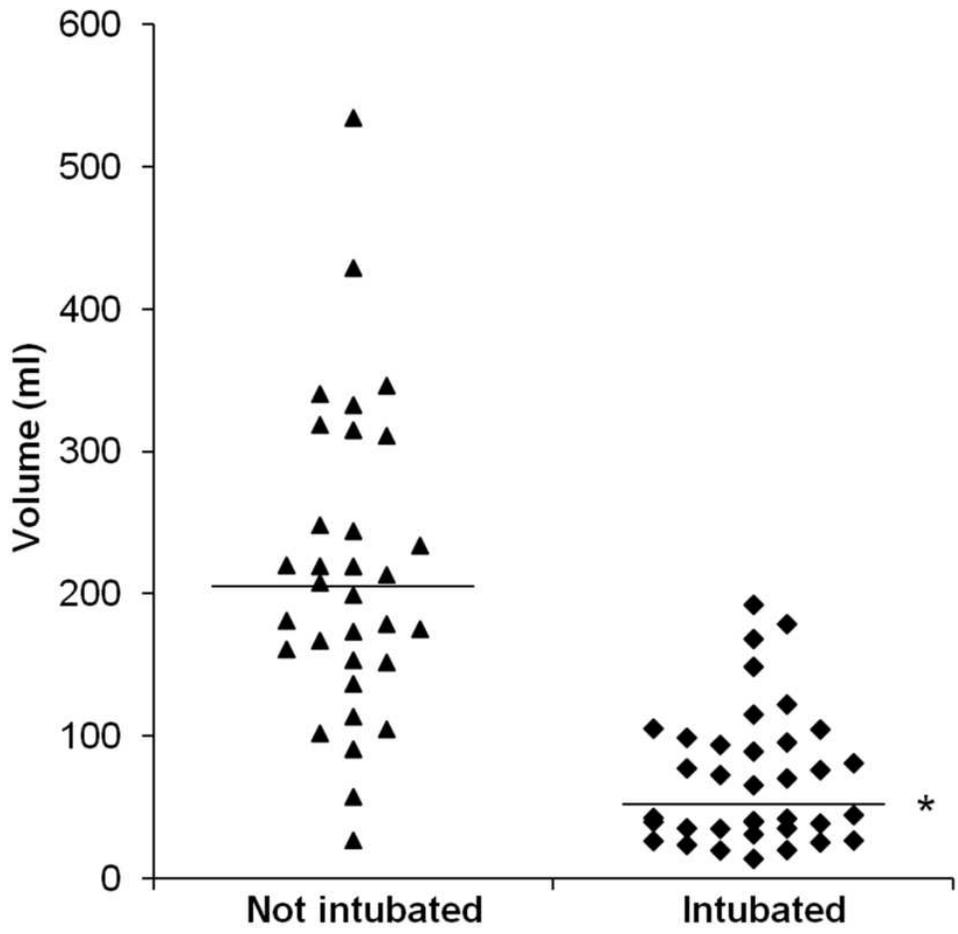


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Professor R E Pounder MD DSc FRCP FRACP FRCPI
Editor
Alimentary Pharmacology & Therapeutics

14th May 2010

Dear Roy

Please find enclosed the revised version of our manuscript entitled "*Effects of a 5-HT₃ antagonist on fasting and postprandial small bowel water content assessed by magnetic resonance imaging*".

We are grateful for the Reviewers' and Editor's comments and suggestions that we have received. We have acted positively upon all of them as specified in the detailed point-by-point answer enclosed. We believe the changes have improved the manuscript. Changes to the enclosed, revised manuscript file are made in red font as requested for ease of reference.

We had already sent by post the original, signed Alimentary Pharmacology & Therapeutics Copyright Transfer Agreement form.

Looking forward to hearing from you

Yours Sincerely

A handwritten signature in black ink that reads "Robin".

R C. Spiller
Professor of Gastroenterology

1 Alimentary Pharmacology & Therapeutics

2 Manuscript APT-0185-2010

3 Title: Effects of a 5-HT₃ antagonist on fasting and postprandial small bowel water content assessed by
4 magnetic resonance imaging
5

6 **ANSWER TO THE REVIEWERS' AND EDITOR'S COMMENTS**
7

8 We are grateful for the Reviewers' and Editor's comments and suggestions that we have received. We
9 have acted positively upon all of them as specified in the detailed point-by-point answer below. We
10 Some of the requests required more explanation but we were also asked to reduce the paper length.
11 It is difficult to satisfy both demands but we have done our best. We believe the changes have
12 improved the manuscript. Changes to the revised manuscript file are made in red font as requested
13 for ease of reference.
14

15 **Reviewer: 1**
16

17 1. A series of big studies - I think it would be worth recording the diameter of the tube used for duodenal intubation.
18

19 We have now indicated in the Methods that the outer diameter of the tube was 3.5 mm
20

21 2. Ondansetron should not have a capital O, unless it's the first word in a sentence.
22

23 Changed accordingly, also in Figures and Table legends
24

25 3. I think the drug should be named in the Title of the article.
26

27 Ondansetron is now named in title
28

29 4. Ondansetron's main clinical use is, as explained in nausea / vomiting associated with chemotherapy; I think this use should be
30 discussed in relation to the results of this study.
31

32 We quite agree. This is mentioned in the introduction and we now add this sentence in the discussion:
33 "This inhibitory effect is in keeping with the fact that ondansetron was originally developed to inhibit
34 the nausea induced by chemotherapeutic agents, particularly cisplatinum which releases large
35 amounts of serotonin from mucosal enterochromaffin cells and induces vomiting by stimulating 5HT₃
36 receptors on the vagus nerve."
37
38

39 **Reviewer: 2**
40

41 This is an interesting and well-designed study that gives lots of original observations that may have significant clinical
42 implications. However, it needs some clarifications/revisions on the following issues mentioned below.
43

44 We thank Reviewer 2 for the very positive comments, we have addressed all the issues raised as
45 outlined below.
46

47 1. Slowing of small bowel transit will also improve absorption as small intestine would get more time for absorption. Authors
48 did not discuss this issue.
49

50 We quite agree. However the only 2 trials of ondansetron which measured this found a slowing of
51 colonic transit not small bowel so we have added the following sentence to the discussion:
52 "This inhibitory effect is also associated with slowing of colonic transit in man (1) which may allow
53 increased time for absorption and contribute to an increase in stool consistency which was a striking
54 benefit in many trials of 5HT₃ receptor antagonists (2)."
55

56 2. Though the authors discussed a lot about IBS-D in the Introduction section, they chose healthy volunteers instead of IBS-D
57 for the study. This should be clarified. Also, discussion on IBS-D can be shortened.
58
59
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1 The real interest in the effects we describe is because of their potential application to IBS patients so
2 we do feel that IBS-D does need to be introduced in the first part of the paper to explain the original
3 rationale for the study. Obviously healthy volunteers are more homogeneous and easier to show a
4 pharmacological effect which is why we started with them. We are in the process of doing a trial in
5 IBS but the results will not be known for many months. We have added this comment to the
6 discussion:

7 "We performed these initial studies in healthy volunteers since they are a much more homogenous
8 group than IBS-D patients, hence making it easier to show a pharmacological effect with small
9 numbers. We are currently in the process of performing a much larger randomised placebo controlled
10 study (RCT) of ondansetron in IBS-D to see if these findings can be reproduced in patients."
11

12
13 3. Fluoroscopy could have helped to know the exact positioning of the catheter.

14
15 We agree that the exact position of the catheter could have been determined using conventional
16 fluoroscopy, however this involves exposing the subjects to ionizing radiation. We have good
17 experience in positioning the catheter with the procedure described in the Methods (3) which allows
18 us to confirm the duodenal positioning by observing from the recordings that the appropriate ports
19 are subject to the relevant contractile activity, gastric or duodenal.

20
21 4. A schematic diagram outlining the study protocol would improve the understanding of it by the
22 readers.

23
24 Agreed. We now provide in the new Figure 1 a schematic diagram as requested.

25
26 5. Table 1. What do the authors mean by p values in this table? P values <0.05 are considered significant. Most of the p values in
27 this table are <0.2, 0.9 and 0.4 etc. What do these values mean? Authors should write p=ns for non-significant p values and
28 present only the significant p values in the table.

29
30 As suggested we have now clearly indicated in Table 1 which outcome measures are significantly
31 different between drug and placebo. We have left indicated aside in brackets the original p values so
32 that the readers can see for themselves which parameters were just marginally outside the usual
33 $p < 0.05$ cut-off.

34
35 6. The paper is quite lengthy. It can be shortened.

36
37 As suggested we have reduced the introduction and discussion by about 300 words and 2 references.
38 Some of the requests of the Reviewers required more explanation and we have done our best to
39 satisfy both demands. We have not taken out any more as we feel it is important to place these
40 findings in their clinical context since it will mainly be clinicians reading it.

41
42 7. "The 5-HT₃ receptor appears to be involved in the control of the migrating motor complex (MMC) (10-12) since 5-HT
43 infusions stimulate migrating motor complexes in man (13) and this effect is inhibited by the 5-HT₃ antagonist Ondansetron" –
44 migrating motor complex has come several times in the same sentence. Also, the full form of it has been used even after
45 indicating an abbreviation of it.

46
47 Thank you for spotting this, now the acronym MMC is used after first definition.

48
49 **Reviewer: 3**

50
51 **General comments**

52
53 Overall, this is a very interesting, important and well-designed study and a very well written manuscript.

54
55 We thank Reviewer 3 for the very positive comments.

56
57 1. 5-HT₃-antagonists are proven to be clinically effective in IBS-D, yet the exact mechanism of action was so far unclear. The
58 results of this study clearly indicate one possible mechanism of action, i.e. the decreased small bowel motility leading to increase
59
60

1 in fasting small bowel water content (SBWC). To my knowledge, this effect of 5-HT₃-antagonists has not been demonstrated in
2 humans before. I am surprised, though, that the drug effects are only seen in the fasting state and not postprandially. This should
3 be discussed.
4

5 We agree it is at first place surprising. We now add this phrase to this effect in the Discussion:
6 "Ondansetron appeared to have a lesser effect postprandially than when fasting. Although SBWC was
7 lower on ondansetron from 180-270 minutes postprandially this difference was not significant possibly
8 because while anti-secretory effects of ondansetron reduce SBWC its anti-motility effects may
9 produce the opposite effect. Another factor to consider is that both 5HT₃ and 5HT₄ receptors are
10 important in controlling human jejunal secretion and peristalsis (4, 5) thereby reducing the effect of
11 a pure 5HT₃ antagonist like ondansetron."
12

13
14 2. This study also underlines that classical manometric motility assessments may be less valuable to assess such drug effects on
15 the gut, because the invasive technique itself has already such strong effects on the motility that additional drug effects cannot be
16 detected. The newly established MRI technique to measure small bowel water content (SBWC), which is used in this study,
17 therefore appears to be superior, yet it should be emphasized that this is a very specialized, expensive and extremely time-
18 consuming technique.
19

20 We quite agree as to the first comment but feel that in the future these techniques may be quite cost
21 effective since an MRI scan at £300 / hour is less than an intubation or indeed an endoscopy. We now
22 add this phrase to this effect at the end of the discussion:
23 "Although currently expensive as experience with the technique grows it is highly likely that shorter
24 scanning protocols will reduce costs further so that they become comparable in price to other
25 investigations such as Manometry and endoscopy."
26

27 3. The MRI is also only able to measure volume as a surrogate marker for motility. Other effects on volume (such as secretion)
28 cannot be differentiated by this technique. This should be discussed regarding the perspective of using this technique in a
29 broader setting.
30

31 We agree. We now add this phrase in the Discussion:
32 "Our technique only measures volume although if combined with orocecal transit time a flow index
33 can be calculated as we did in a previous study (6) but this measurement was not made in the current
34 study."
35
36

37 **Specific comments**

38 **Abstract:**

39
40
41 4. 2nd sentence: Regarding SBWC in intubated vs non-intubated patients, it should be clarified that patients from study 1 are
42 compared with patients in study 2, otherwise it sounds like patients were randomized to intubation versus non-intubation.
43

44 We have now made clearer in the Abstract Aims and in the Abstract Results that these were two
45 studies in two separate groups of subjects
46

47 **Methods:**

48
49 5. Ondansetron is used three times daily for this study. Is this the dosage the authors typically use for IBS-D? Approved dosage
50 for chemotherapy-induced emesis is only twice daily.
51

52 We have been using Ondansetron clinically at doses ranging from 4mg daily to 8 mg t.d.s. Most
53 patients respond to the lower doses. We now add this phrase in the Discussion to clarify this point:
54 "Although this is currently not a licensed indication we have been using ondansetron for severe IBS-D
55 for some years at doses ranging from 4mg daily to 8mg t.d.s and find that those who do respond do
56 so in the lower dose range. Of course definitive recommendations must await the results of our
57 current RCT."
58
59
60

Results/Discussion:

6. While the effect of 5-HT₃-antagonists on fasting SBWC is quite convincing, there seems to be no effect or maybe even a contrary effect on SBWC after a meal, i.e. a marked lower SBWC after 270 min in the ondansetron group compared to the placebo group. Please discuss.

Agreed. The same comment was made by Reviewer 2. We now add this phrase to this effect in the Discussion:

"Ondansetron appeared to have a lesser effect postprandially than when fasting. Although SBWC was lower on ondansetron from 180-270 minutes postprandially this difference was not significant possibly because while anti-secretory effects of ondansetron reduce SBWC its anti-motility effects may produce the opposite effect. Another factor to consider is that both 5HT₃ and 5HT₄ receptors are important in controlling human jejunal secretion and peristalsis (4, 5) thereby reducing the effect of a pure 5HT₃ antagonist like ondansetron."

7. 5-HT₃-antagonists are proven to be effective in IBS-D. This study very nicely indicates a possible mechanism of action, i.e. the decreased small bowel motility. To my knowledge, this has not yet been demonstrated in humans. This effect could very well explain the positive effects on diarrhea. On the other hand, it is very well conceivable that an increased fasting SBWC might also cause other symptoms such as bloating or fullness, such as suggested by the authors in the end of the discussion. These are also typical IBS symptoms. Is there any data from clinical trials that these specific symptoms might actually worsen in IBS-D patients during 5-HT₃-antagonist therapy?

We are currently doing such a trial but it is placebo controlled and we have yet to break the code so we can only refer to one earlier study which reported benefit

We now add to the discussion

"The increased fasting small bowel water did not appear to be associated with symptoms like bloating in our volunteers and in one clinical trial ondansetron did not worsen bloating (7)."

8. I agree with the authors that their MRI technique would be very useful for all kinds of different patients and different research questions. But I do not agree that this could be "easily" done. MRI is an expensive technique and the specific analysis software is probably not even commercially available. The main problem, however, would be the extremely time consuming process of analyzing the data: the authors describe that SB volumes are manually traced on each slice on every single image! This is an enormous workload! (I know from experience). As long as there is no automated way of identifying the volume, this technique has major limitations for a broader use. This should be clarified.

We agree with the Reviewer that the workload of manual segmentation can still be a burden and very time consuming, but in this case the load was much reduced because we had to exclude only regions outside the bowel, so the segmentation was quicker. We have removed the term 'easily' from the concluding remark of the manuscript and added 4 lines at the end of it to this effect:

"The time and effort that it takes to process of the large amount of data collected during serial MRI studies is a significant burden. However current improvements in semi-automated image processing and an optimized and reduced scanning schedule will help broaden the use of these functional GI MRI techniques."

However we are optimistic that things will improve and added:

"Although currently expensive as experience with the technique grows it is highly likely that shorter scanning protocols will reduce costs further so that they become comparable in price to other investigations such as Manometry and endoscopy."

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