Randomised clinical trial: linaclotide vs placebo—a study of bi-directional gut and brain axis

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Summary
Background: Linaclotide, a guanylate cyclase C agonist relieves irritable bowel syndrome with predominant constipation (IBS-C) symptoms, but how it improves pain in humans is unknown.

Aims: To investigate the effects of linaclotide and placebo on the afferent and efferent gut-brain-gut signalling in IBS-C patients, in a randomised clinical trial.

Methods: Patients with IBS-C (Rome III) and rectal hypersensitivity were randomised (2:1) to receive linaclotide (290 µg) or placebo for 10 weeks and undergo bi-directional gut and brain axis assessment using anorectal electrical stimulations and transcranial/transspinal-anorectal magnetic stimulations. Rectal sensations were examined by balloon distention. Assessments included abdominal pain, bowel symptoms and quality of life (QOL) scores. Primary outcomes were latencies of recto-cortical and cortico-rectal evoked potentials.

Results: Thirty-nine patients participated; 26 received linaclotide and 13 received placebo. Rectal cortical evoked potentials latencies (milliseconds) were significantly prolonged with linaclotide compared to baseline (P1: Δ 19 ± 6, P < 0.005; N1: Δ 20 ± 7, P < 0.02) but not with placebo (P1: Δ 3 ± 5; N1: Δ 4.7 ± 5, P = 0.3) or between groups. The efferent cortico-anorectal and spino-anorectal latencies were unchanged. The maximum tolerable rectal volume (cc) increased significantly with linaclotide compared to baseline (P < 0.001) and placebo (Δ 29 ± 10 vs 4 ± 20, (P < 0.03). Abdominal pain decreased (P < 0.001) with linaclotide but not between groups. Complete spontaneous bowel movement frequency increased (P < 0.001), and IBS-QOL scores improved (P = 0.01) with linaclotide compared to baseline and placebo. There was no difference in overall responders between linaclotide and placebo (54% vs 23%, P = 0.13).

Conclusions: Linaclotide prolongs afferent gut-brain signalling from baseline but both afferent and efferent signalling were unaffected compared to placebo. Linaclotide significantly improves rectal hypersensitivity, IBS-C symptoms and QOL compared to placebo. These mechanisms may explain the effects of linaclotide on pain relief in IBS-C patients.

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1 | INTRODUCTION

Irritable bowel syndrome with predominant constipation (IBS-C) affects up to 10% of the US population, predominantly women, and is characterised by abdominal pain with altered bowel habits.1-5 The pathophysiology of IBS has evolved to that of a more complex paradigm that involves altered pain perception (visceral hypersensitivity), dysregulation of brain and gut axis,6-10 and gut microbiome and brain interactions.11

IBS has been linked with significant neuroenteric dysfunction that includes abnormal forebrain activity and interoceptive processing of the somatosensory cortex, insula, rostral anterior cingulate cortex and medial thalamus.6-10 These observations suggest that IBS may be caused by abnormal changes in the gut autonomic nervous system as well as perturbations in the brain and gut neuroenteric axis.11-13

Altered pain perception and visceral hypersensitivity has been widely reported in IBS.10-13 The rectal hypersensitivity in IBS may represent a dysfunction ofafferent gut and brain pathways,7,10,12 hyperexcitability of dorsal neurons, abnormal central processing of afferent information7,10 or abnormal endogenous descending inhibitory pathways12,13 or a combination of these mechanisms.14,15 Two studies of cortical evoked potentials (CEP), either with rectal balloon distension or electrical stimulation in IBS-C and IBS-diarrhoea predominant patients showed shorter latency7,8 indicating accelerated afferent gut and brain signalling. Previously, we showed that IBS subjects exhibit shorter anorectal-brain cortical evoked potential latencies compared to controls, and shorter lumbo-sacral motor evoked potential latencies providing evidence of hyperexcitability and rapid bidirectional neuronal transmission.16 These observations suggest that both the afferent and efferent pathways may be affected in IBS patients.

Linaclotide, a Guanylate Cyclase C (GC-C) agonist, has been shown in large randomised controlled trials to significantly improve abdominal pain, constipation and bloating in IBS-C patients.17-19 In a animal model of chronic visceral hypersensitivity linaclotide has been shown to inhibit colonic nociceptors and reduce distension-induced nociceptive signalling.20,21 These mechanistic studies show that Cyclic Guanosine Monophosphate (cGMP) released in response to linaclotide-induced activation of guanylate cyclase-C, decreases the firing rate of visceral nociceptive fibres, and thereby relieves visceral pain.20-23 However, the mechanism(s) by which linaclotide improves abdominal pain in humans is unknown.

Our hypothesis was that linaclotide improves abdominal pain in patients with IBS-C by decreasing rectal hypersensitivity and altering the bi-directional gut-brain signalling. Our aims were: (a) To conduct a randomised, double blind, placebo-controlled study of linaclotide and placebo in IBS-C patients and to investigate the afferent recto-cortical and ano-cortical axis and rectal sensation, IBS symptoms, and quality of life; (b) To examine the efferent cortico-rectal and cortico-anal axis and the spino-anorectal axis in these patients.

2 | METHODS

Patients with suspected constipation-predominant IBS (IBS-C) assessed at Augusta University Medical Center, Augusta, GA were eligible, if the following criteria were met: (a) During the previous year, all patients reported recurrent abdominal discomfort or pain for at least 3 days per month over previous 3 months associated with two or more of the following (Rome III): (i) improvement with defecation; (ii) onset associated with a change in frequency of stool; and/or (iii) onset associated with a change in form (appearance) of stool; (b) No evidence for structural disease on colonoscopy/barium enema and metabolic problem by laboratory tests; and (c) On a prospective symptom/stool diary patients reported (i) the presence of abdominal pain/discomfort for at least 2 days per week; (ii) hard or lumpy stools >25% and loose or watery stools in <25% of bowel movements; 4) On a rectal balloon distension study, patients had rectal hypersensitivity, defined as two or more thresholds of rectal sensation [first (15-23 cc), desire to defecate (83-123 cc), urgency (150-196 cc) or maximum tolerable volume (205-255 cc)] that were ≤ 2 SD of normal mean values.24 Patients were excluded: (a) if they were taking constipating drugs, (eg opioids), tricyclics (seizure risk), serotonin modulators, antispasmodics, and muscle relaxants, unless discontinued 2 weeks before enrolment; (b) antidepressants (except stable doses of selective serotonin reuptake inhibitors (SSRI); (c) laxative abuse, anorexia nervosa, severe cardiac disease, chronic renal failure; (d) previous gastrointestinal surgery except cholecystectomy and appendectomy; (e) neurologic diseases (eg head injuries, epilepsy, multiple sclerosis, strokes, spinal cord injuries; (f) pregnancy; (g) inflammatory bowel disease; 8) rectal prolapse, anal fissure, anal surgery or inflamed haemorrhoids.

IBS-C patients were randomised in a 2:1 ratio to receive either once-daily linaclotide 290 micrograms or placebo, 30 minutes before breakfast for 10 weeks. The randomisation schedule was generated in advance by the study bio-statistician using permuted blocks. The allocations were placed into sequentially numbered sealed opaque envelopes that were sent to the research pharmacist who dispensed the study medication. The patients and research team were blinded to the allocation. The study drug and placebo were supplied by Ironwood/Forest Laboratories. Patients were asked to record their daily abdominal pain and other symptoms as well as their bowel habits on a prospective bowel diary. Patients were allowed a rescue laxative, bisacodyl 5mg once daily if they had no bowel movement for 3 days, and a maximum of 2 doses per week.

All authors had access to the study data and reviewed and approved the final manuscript.

2.1 | Study protocol

After their screening visit, all subjects were asked to fill out the IBS quality of life (IBS-QOL) score,25 subject global assessment (SGA) of pain on a scale of 0 (no pain) to 10 (severe pain), and maintain a daily stool/pain diary for one more week. A detailed scheme
is shown in Figure S1. Next they underwent rectal sensory testing using standard high resolution anorectal manometry system (Medtronics Ltd, Minneapolis, MN, USA). Sensory thresholds for first sensation, desire and urgency to defecate and maximum tolerable volume were assessed using standard criteria. If eligible, they underwent baseline bi-directional brain gut interaction studies using Cortical Evoked Potential (CEP), Transcranial Magnetic Stimulation (TMS) and Translumbosacral Anorectal Magnetic Stimulation (TAMS) tests.

2.2.2 Measurements and analyses

2.2.2.1 Cortical evoked potential (CEP) measurements

The CEP study was performed by placing a probe with two pairs of bipolar steel ring electrodes, each 2 cm apart (Gaeltec, Gaeltec Devices Ltd. Dunvegan, UK) into the anorectum. The proximal pair was located 10 cm from anus and stimulated the rectal wall and the distal pair at 1 cm from anus. The CEP studies were performed using previously published methodology with the active electrode positioned at 2 cm posterior to the vertex (Cp3). Four runs of 50 stimuli at 0.2 Hz were performed. The order of rectal or anal stimulation was randomised.

The TMS study was performed in a semi-reclined position using magnetic stimulation of the cerebral cortex. A double cone coil (The MAGSTIM Company Limited, Whiteland, Wales, UK) was positioned over the cranium’s vertex and study performed using previously described methodology.

The TAMS study was performed by using a 90-mm circular coil and using previously published methodology (The MAGSTIM Company Limited). For the translumbar study, with the subject in prone position, the coil was discharged on each side, 3-4 cm lateral to the L2/L3 vertebra, and for the transsacral test, 3-4 cm lateral to the S2 and S3 vertebra.

2.2.2.2 Motor evoked potential (MEP) measurements

The cortico-rectal and cortico-anal MEPs, and the bilateral latency for the lumbo-rectal, lumbo-anal, sacro-rectal and sacro-anal MEPs were calculated. The primary outcome measures were the onset latency of anal and rectal MEP response to TMS.

2.2.3 Abdominal and bowel symptom analysis

These were analysed from the daily abdominal pain and stool diaries. We calculated the number of bowel movements (BMIs) per week (stool frequency), the number of complete spontaneous bowel movements (CSBM) per week, the mean stool consistency (Bristol stool scale from 1-7), and the mean straining effort (0-3). We also assessed the mean daily and weekly abdominal pain scores on a Likert-like scale (0 = none, 4 = very severe) and the overall subject global assessment (SGA) of pain at baseline and end of study. A responder was defined as an individual who showed ≥30% improvement in abdominal pain and an increase of ≥1 CSBM/week during the last week of study when compared to baseline stool and pain diary.

2.2.4 IBS-quality of life analysis

The quality of life (QOL) was assessed using the eight domains of IBS-QOL by averaging and grouping the various questions under the specific domains as well as the overall IBS-QOL score.

2.3 Statistical analyses

Sample Size analysis: For a proposed sample size of 40 (2:1) subjects, and assuming a SD of 15 and 29 milliseconds respectively for the anal and rectal N1 latency (CEP), and a correlation of r = 0.5 between the baseline and post-treatment measures of latency from the same subject, the statistical test will be able to detect a mean change in N1 latency of at least 6 milliseconds for the anal and 16 milliseconds for the rectal latency at the 0.05 significance level, with 0.80 power. In a previous study, a mean change of 32 milliseconds for the anal and 19 milliseconds for the rectal N1 latency was observed following a therapeutic intervention.

Descriptive statistics were calculated for all patients and for each treatment arm (linaclootide vs placebo). To examine whether the changes from baseline within arms for the MEP latencies, SGA, and stool diary data (CSBM, consistency and strain) were statistically significant, a paired Wilcoxon rank-sum test was used. A two-sample Wilcoxon rank-sum test was performed to determine if the differences between arms were significant. Because the CEP data were not normally distributed, the Wilcoxon signed-rank test was used to assess the changes from baseline and comparisons between arms. For responder analysis a test for binomial proportion was used. For IBS-QOL, each of the 34 question scores was transformed from a Likert scale of 0-4 to 1-5 in keeping with the scoring guidelines. Sum scores were calculated for the eight subscales, and a change from baseline (∆ Score) was calculated. To accommodate the multiple
comparisons within each hypothesis, a multiple testing correction using the Benjamini-Hochberg false discovery rate method was implemented. SAS 9.4 was used for all statistical analyses. An alpha level of 0.05 was used to assess significance. An intention-to-treat analysis was performed on all subjects who were enrolled and received at least 1 day of study medication. For those subjects with missing data, the last observation was carried forward. For correlation analysis, Pearson’s correlation coefficient was used to measure the level and test the significance of association between two continuous measures. For evaluating the association between a continuous measure and a dichotomous variable, the adjusted $R^2$ from the logistic regression model was used, and its significance assessed the degree of correlation.

3 | RESULTS

3.1 | Demographics

Thirty-nine patients (38F) participated, of whom 26 received linaclotide and 13 received placebo (Figure 1). The baseline demographic features were comparable between the two groups (Table 1).

3.2 | Effects of linaclotide on recto-cortical and ano-cortical CEPs (afferent gut-brain)

A typical CEP recto-cortical response before and after linaclotide and placebo is shown in Figure 2A. The mean recto-cortical latencies for $P_1(\Delta 19 \pm 6, P < 0.005)$, and $N_1(\Delta 20 \pm 7, P < 0.02)$ waveform responses, and likewise for $P_2 (P = 0.001)$ and $N_2 (P = 0.0001)$ responses were all significantly prolonged in the linaclotide group when compared to baseline but not in placebo group ($P_1: \Delta 3 \pm 5$; $N_1: \Delta 4.7 \pm 5, P = 0.3$) (Figure 2B and Table 2).

The mean ano-cortical latencies for the $P_1 (P = 0.003)$, $N_1 (P = 0.0001)$, $P_2 (P = 0.009)$ and $N_2 (P = 0.022)$ waveform responses of the CEP were all significantly prolonged when compared to baseline in the linaclotide group (Table 2). The $N_1$ latency was prolonged ($P = 0.037$) in the placebo group but not the $P_1$, $P_2$ and $N_2$ responses (Table 2). Although there were no statistical differences between the linaclotide and placebo groups, there was at least twofold greater prolongation of the recto-cortical and ano-cortical latencies in the linaclotide group compared to placebo (Table 2).

### TABLE 1 Baseline characteristics and demographic data (Mean ± SEM)

|                     | Linaclotide  | Placebo    |
|---------------------|--------------|------------|
|                     | (N = 26)     | (n = 13)   |
| Age (y)             | 40.1 ± 2.6   | 46.4 ± 2.1 |
| Female/male         | 25/1         | 13/0       |
| Duration of IBS symptoms (y) median (range) | 3(0.8-20) | 3.6(1-18) |
| Abdominal pain score | 1.9 ± 1.0 | 2.1 ± 0.7 |
| Stool consistency (BSFS) | 1.8 ± 0.2 | 1.4 ± 0.3 |
| No. of bowel movements/week | 4.2 ± 0.5 | 4.4 ± 0.6 |
| No. of CSBMs/Week   | 0.7 ± 0.2    | 1.0 ± 0.4  |

FIGURE 1 Consort flow diagram for the study
3.3 | Effects of linaclotide on transcranial-anorectal MEPs (efferent brain-gut)

The cortico-rectal and cortico-anal MEPs as well as the spino-rectal and spino-anal MEPs were largely unchanged with either linaclotide or placebo, except the right cortico-anal and the right sacro-anal responses that were significantly prolonged (P < 0.05) with linaclotide (Table S1).

3.4 | Rectal sensory thresholds and compliance

The maximum tolerable rectal volume (MTV) increased significantly in the linaclotide group compared to baseline (143.5 ± 8.0 cc vs 172.7 ± 10.5 cc, P = 0.001), and when compared to placebo (Δ 29 ± 10 vs 4 ± 20 cc, P < 0.03), but not in placebo group (P = 0.985), (Table 2, Figure S2). The thresholds for first sensation and desire to defecate and those between groups were not significantly different (Table 2). The rectal compliance significantly increased (P < 0.01) in the linaclotide group, but not in the placebo group (P > 0.1), and there were no differences between the two groups (Table 2). There was no significant correlation between MTV and either abdominal pain (r = 0.3) or CEP data (r = 0.3) in the linaclotide group as well as placebo group, (P = 0.4).

3.5 | Abdominal pain

Abdominal pain was assessed using a daily abdominal pain score as well as overall subject global assessment scores (SGA). Mean daily abdominal pain score decreased significantly with linaclotide when compared to baseline (P = 0.0003), but not after placebo (P = 0.12), but there was no difference between the two groups (P = 0.4) (Figure 3A,B). Mean SGA score also decreased with linaclotide when compared to baseline (P = 0.0002) but not with placebo (P = 0.9), and there was no difference between the two groups (Table 3).

3.6 | Bowel symptoms

The mean number of CSBMs significantly increased in the linaclotide group when compared to baseline (P < 0.0001) and when compared to placebo (P < 0.003) but not in the placebo group (P = 0.5, Figure 3C,D). The mean stool frequency was also significantly higher after linaclotide (P < 0.0001), but not after placebo (P = 0.1), but there was no difference between the two arms (Table 3). The mean stool consistency also improved significantly with linaclotide (P < 0.0001) but not with placebo (P = 0.06), but there was no difference between groups (P = 0.28) (Table 3). The mean straining effort did not change with either linaclotide or placebo (Table 3).
TABLE 2  Effects of Linaclotide and placebo on the Rectal CEP and Anal CEP responses and rectal sensory thresholds and rectal compliance. (Mean ± SEM)

|                      | Linaclotide (n = 26) | Placebo (n = 13) |
|----------------------|-----------------------|------------------|
|                      | Before                | After            | P       | Before                | After            | P       |
| Rectal P1 latency (ms)| 73.1 ± 5.1            | 91.6 ± 6.2       | 0.005   | 64.8 ± 5.5            | 67.9 ± 4.0       | 0.577   |
| Rectal N1 latency (ms)| 110.3 ± 6.8           | 130 ± 7.6        | 0.020   | 96.2 ± 6.2            | 100.9 ± 6.8      | 0.320   |
| Rectal P2 latency (ms)| 187.8 ± 9.5           | 223.7 ± 9.1      | 0.001   | 175 ± 16.2            | 177.7 ± 13.2     | 0.789   |
| Rectal N2 latency (ms)| 221.3 ± 11.4          | 278.1 ± 12.0     | 0.0001  | 209.8 ± 20.4          | 233.9 ± 19.9     | 0.594   |
| Anal P1 latency (ms)  | 77.18 ± 20.4          | 107.2 ± 36.5     | 0.003   | 72.9 ± 21.2           | 87.3 ± 37.0      | 0.1973  |
| Anal N1 latency (ms)  | 106.8 ± 34.4          | 147.8 ± 49.9     | 0.0001  | 99.8 ± 26.6           | 125.4 ± 51.6     | 0.0371  |
| Anal P2 latency (ms)  | 175.4 ± 12.1          | 209.0 ± 13.2     | 0.009   | 182.7 ± 18.8          | 204.2 ± 20.0     | 0.424   |
| Anal N2 latency (ms)  | 207.4 ± 13.9          | 243.4 ± 14.7     | 0.022   | 225.7 ± 22.5          | 239.2 ± 23.4     | 0.722   |
| Rectal sensory thresholds | First sensation (mL) | 15.4 ± 1.3       | 18.5 ± 1.9 | 0.073   | 15.4 ± 1.4          | 20.0 ± 2.8       | 0.156   |
| Rectal sensory thresholds | Desire to defecate (mL) | 66.9 ± 6.4      | 72.3 ± 6.2      | 0.452   | 100.8 ± 16.1      | 92.3 ± 11.5     | 0.879   |
| Rectal sensory thresholds | MTV (mL)              | 143.5 ± 8.0      | 172.7 ± 10.5  | 0.001   | 194.6 ± 22.5      | 190.8 ± 20.1    | 0.985   |

Rectal volume rectal pressure (mm Hg)

| Rectal compliance | Before | After | P       | Before | After | P       |
|-------------------|--------|-------|---------|--------|-------|---------|
| 20 mL             | 19.3 ± 2.2 | 14.2 ± 2.8 | 0.015   | 20.2 ± 3.1 | 23.9 ± 6.7 | 0.095   |
| 40 mL             | 31.7 ± 2.2 | 26.0 ± 2.4 | 0.018   | 30.9 ± 2.3 | 32.0 ± 5.9 | 0.534   |
| 70 mL             | 32.6 ± 1.9 | 25.8 ± 1.8 | 0.004   | 33.3 ± 2.6 | 32.0 ± 6.1 | 0.909   |
| 100 mL            | 37.9 ± 2.0 | 30.9 ± 2.1 | 0.011   | 33.4 ± 2.0 | 34.9 ± 5.6 | 0.421   |

Abbreviations: MTV, maximum tolerable volume.

FIGURE 3  Effects of linaclotide and placebo on A, abdominal pain scores; B, % abdominal pain responders; C, number of complete spontaneous bowel movements (CSBMs)/week; D, % complete spontaneous bowel movement (CSBM) responder; E, % of Composite responders
3.7 | Responder analysis

Patients receiving linaclotide were more likely to be responders (composite endpoint) than placebo (54% vs 23%), but the differences between the two patient groups were not significant ($P = 0.13$, Figure 3 E). There was a significant correlation between CEP data (N1 latency) and abdominal pain responders ($r = 0.42$, $P < 0.03$), and the composite responders ($r = 0.40$, $P < 0.04$) in the linaclotide group, but no correlations were seen in the placebo group ($r = 0.2$, $P = 0.4$ and $r = 0.04$, $P = 0.8$).

3.8 | Quality of life

There were significant ($P < 0.026$) improvements in seven of eight domains of the IBS-QOL survey in patients who received linaclotide when compared to baseline, but no changes in any of domains in patients who received placebo (Table 3). Also, four domains notably, dysphoria, health worry, food avoidance and sexual relationships improved significantly in the linaclotide group when compared to placebo group. Furthermore, the change in total IBS-QOL score significantly improved in the linaclotide group when compared to the baseline score ($P = 0.0006$, Table 3) as well as when compared to the placebo group ($P = 0.0166$), but not in the placebo group when compared to its baseline ($P = 0.9186$) (Figure S3).

3.9 | Adverse events

Seven patients had adverse events. Two patients had AEs before randomisation; one had vaginal bleeding and another deep venous thrombosis and were withdrawn. Three patients on linaclotide had severe diarrhoea and withdrew, and one of these also experienced transient headaches and myalgia. One patient reported nausea and another developed streptococcal throat infection on day 49 and received antibiotics but both patients in the linaclotide group completed the study.

4 | DISCUSSION

We found that linaclotide significantly prolonged the latencies of the afferent signals between the gut and the brain as measured by both the recto-cortical and ano-cortical evoked potentials in IBS-C patients when compared to baseline, but these changes were not significant when compared to placebo. In comparison, placebo had little to no effect on these latencies. The CEP measures the electrical potentials generated within the cortical neurons in response to targeted sensory stimulation and are recorded using scalp surface electrodes. $^7,8,24,28,29,32$ Previously, we have shown that CEP is reproducible and provides reliable data in healthy subjects and in patients with dyssynergic defecation. $^{26,27}$ Also, previous studies have shown that the gut and brain axis is aberrant in IBS patients when compared to healthy controls. $^{16}$ We found that although the CEP response in patients who received linaclotide and placebo were comparable at baseline, there was a significant prolongation of the rectal and anal CEP responses in patients who received linaclotide. This suggests that linaclotide may improve nociceptive signalling between the gut and brain.

It is possible that the CEP assessments may be influenced by cognitive/psychological function and habituation, but these effects should be similar for IBS patients participating in the placebo and
linaclootide arms. We found that baseline psychological features that were assessed were similar between the two groups. Consequently, the changes in CEP after taking linaclootide were most likely due to a pharmacophysical effect of the drug.

All patients who were selected for this study had IBS with rectal hypersensitivity. In this group, linaclootide significantly increased the rectal sensory thresholds for the maximum tolerable volume when compared to baseline and placebo providing corroborative evidence for an improvement in the rectal visceral hypersensitivity and sensitivity, whereas placebo had no effect. The increased thresholds for rectal sensation and improvement in rectal capacity provide evidence for an improvement in rectal hypersensitivity and that linaclootide could improve visceral hypersensitivity in the gut.

These findings in humans are also consistent with the observations in animal models where acute or chronic linaclootide use significantly reduced the firing of sensitised visceral nociceptive fibres, and also relieved colorectal distension-evoked visceral pain and reduced nociceptive signalling within the spinal cord. Furthermore, linaclootide was effective in both stress-induced, inflammatory and chronic visceral hypersensitivity models. In contrast, linaclootide had no effect on guanylate cyclase-c knockout mice suggesting that these effects on reducing visceral hypersensitivity were mediated by the release of cyclic guanosine monophosphate (cGMP). Studies in rodent models also showed an extracellular mechanism of anti-nociception that was linked with cGMP. Studies have also demonstrated that exogenous cGMP can inhibit action potential firing of human dorsal root ganglion sensory neurons. Overall, these studies suggest that linaclootide may exert its pain-relieving effect, and nociceptive signalling effect through the release of cGMP.

Because efferent descending inhibitory signals have been shown to be altered in IBS, we used transcranial magnetic stimulation that relies on Faraday's principle to assess efferent signalling between the cortex and anorectum. When a brief surge of current is passed through a magnetic coil, it induces a rapidly changing magnetic field that passes unimpeded through the skin and bones and generates an electric current that can be focused to a small area. The magnetic field upon contact with nerves induces excitatory post-synaptic potentials that activates peripheral nerve axons which in turn activates muscles. In patients with IBS and interstitial cystitis, the efferent (brain-spino-fugal) pathway has been shown to be abnormal. However, here we found that neither the efferent signalling between the brain and the rectum or anus, nor between the spinal cord and anorectum were altered by linaclootide. This new finding suggests that linaclootide has no significant effects on the efferent cortico-anorectal or peripheral spino-anorectal signalling.

In addition to the mechanistic improvements in the gut and brain interactions and rectal sensory function with linaclootide, we also observed significant improvements in daily abdominal pain scores as well as overall pain score when compared to baseline but not when compared to placebo. Also, linaclootide significantly increased the number of CSBMs/week when compared to baseline and placebo. Together these findings extend previous observations from large RCTs that linaclootide improves both pain and bowel symptoms in IBS-C. The overall percentage of treatment responders was more than twofold higher with linaclootide when compared to placebo (54% vs 23%). This composite responder rate was higher compared to the published randomised controlled trials of linaclootide, possibly because of patient selection, as we included a group of patients with demonstrable rectal hypersensitivity and possibly due to symptom(s) fluctuation that is well known in this population. However, this difference did not reach statistical significance, possibly due to a type 2 error. Also, there was a significant correlation between the abdominal pain responder and CEP latency but not between pain and maximum tolerable volume. There were also improvements in stool frequency and stool consistency with linaclootide, but not with placebo or between the two groups.

Importantly, we observed a significant improvement in seven of the eight IBS specific QOL domains among patients who received linaclootide when compared to their baseline scores, whereas there were no changes in patients who received placebo. Also, the QOL scores were significantly better with linaclootide when compared to placebo, indicating that in addition to improvements in pain and bowel symptoms, linaclootide significantly improved QOL in patients with IBS-C, and in part this may have also contributed to the improvement in visceral perception and well-being.

Our study limitations include a smaller sample size, and this was in part due to strict inclusion criteria, although we screened a large population of IBS patients. We only included patients with rectal hypersensitivity, because previous rodent studies showed that linaclootide reduced colorectal sensitivity only in hypersensitive but not in naive rats. Also, by evaluating the hypersensitive group, a population of IBS that has not been examined previously in this manner, we felt that we could more optimally and objectively assess whether linaclootide induces mechanistic changes similar to those observed in animal models of hypersensitivity. Thus, our findings may not be applicable to all IBS patients. The CEP study measures changes in the anal and rectal sensory cortex, but the precise brain regions involved in the linaclootide-induced sensory modulation could not be defined, unlike previous positron emission tomography or functional magnetic resonance imaging studies with other agents. We have shown that both CEP and TMS data have excellent inter-observer agreements. We chose this approach, as our objectives were to examine both the afferent and efferent gut-brain function and peripheral spino-anorectal pathways, and at present such a comprehensive assessment in humans can only be performed using this methodology.

Finally, in this mechanistic study of bi-directional gut and brain axis in IBS patients, using a novel methodology and an assessment of rectal hypersensitivity, we showed that linaclootide alters the rapid afferent conduction of signalling from the gut when compared to baseline, and thereby may diminish the magnitude of perception in the sensory cortex. However, some of the effects observed with linaclootide did not differ from placebo. It is likely that the effects of linaclootide are mediated by the release of cyclic GMP as shown in animal models previously. Our findings provide a mechanism for...
how a GC-C agonist, linaclotide, may improve visceral hypersensitivity and thereby improve pain in IBS-C patients, but these findings merit further confirmation in larger studies. Also this model of testing bi-directional gut and brain interactions may be useful for mechanistic studies of novel therapeutic agents in IBS and other motility disorders.

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AUTHORSHIP

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Author contributions: Satish Rao, project director and principal investigator, was involved in study concept and design, grant support, data analysis and interpretation, manuscript preparation, overall supervision of brain and gut axis studies, and anorectal function and critical revision. Xuelian Xiang was involved in performing neurophysiology tests, conducting anorectal physiology test, data analysis, tables and figures, and manuscript preparation. Kulthep Ratanakovit and Tanisa Patcharatrakul were involved in performing neurophysiology tests and anorectal physiology test, and study recruitment. Yun Yan was involved in conducting neurophysiology tests, data analysis, tables and figures, and manuscript preparation. Rachael Parr, study coordinator, was involved in data collection. Deepak Ayyala was involved in statistical design, statistical methods and data analysis. Amol Sharma, study co-investigator, was involved in recruitment, manuscript preparation and critical revisions.

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REFERENCES

1. Chang L, Heitkemper M, Wiley J, Camilleri M. 2015 James W. Preston single topic conference: a renaissance in the understanding and management of irritable bowel syndrome. *Cellular and Molecular Gastroenterology and Hepatology*. 2016. https://doi.org/10.1016/j.ccmgh
2. Gralnek IM, Hays RD, Kilbourne A, et al. The impact of irritable bowel syndrome on health-related quality of life. *Gastroenterology*. 2000;119:654-660.
3. Camilleri M, Boeckxstaens G. Dietary and pharmacological treatment of abdominal pain in IBS. *Gut*. 2017;66:966-974.
4. Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders. *Gastroenterology*. 2006;130:1480-1491.
5. Drossman DA, Camilleri M, Mayer EA, et al. AGA technical review on irritable bowel syndrome. *Gastroenterology*. 2002;123:2108-2131.
6. Pezzone MA, Liang R, Fraser MO. A model of neural cross-talk and irritation in the pelvis: implications for the overlap of chronic pelvic pain disorders. *Gastroenterology*. 2005;128:1953-1964.
7. Chan YK, Herkes GK, Badcock C, et al. Alterations in cerebral potentials evoked by rectal distension in irritable bowel syndrome. *Am J Gastroenterol*. 2001;96:2413-2417.
8. Sinhamahapatra P, Saha SP, Chowdhury A, et al. Visceral afferent hypersensitivity in irritable bowel syndrome—evaluation by cerebral evoked potential after rectal stimulation. *Am J Gastroenterol*. 2001;96:2150-2157.
9. Naliboff BD, Derbyshire SWG, Munakata J, et al. Cerebral activation in patients with irritable bowel syndrome and control subjects during rectosigmoid stimulation. *Psychosom Med*. 2001;63:365-375.
10. Kwan CL, Diamant NE, Mikula K, et al. Characteristics of rectal perception are altered in irritable bowel syndrome. *Pain*. 2005;113:160-171.
11. Mayer EA, Tillisch K, Gupta A. Gut/brain axis and the microbiota. *J Clin Invest*. 2015;125:926-938.
12. Coss-Adame E, Rao SS. Brain and gut interactions in irritable bowel syndrome: new paradigms and new understandings. *Curr Gastroenterol Rep*. 2014;16:379.
13. Wilder-Smith CH, Schindler D, Lovblad K, et al. Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. *Gut*. 2004;53:1595-1601.
14. Brierley SM, Linden DR. Neuroplasticity and dysfunction after gastrointestinal inflammation. *Nat Rev Gastroenterol Hepatol*. 2014;11:611-627.
15. Hobson AR, Aziz Q. Brain imaging and functional gastrointestinal disorders: has it helped our understanding? *Gut*. 2004;53:1198-1206.
16. Coss-Adame E, Valestin J, Bradley C, et al. Investigation of the effenter spinofugal axis by translumbar and trans-sacral magnetic stimulation in irritable bowel syndrome (IBS) and interstitial cystitis (IC). *Neurogastroenterol Motil*. 2012;24:175.
17. Rao S, Lembo AJ, Shiff SJ, et al. A 12-week, randomized, controlled trial with a 4-week randomized withdrawal period to evaluate the efficacy and safety of linaclotide in irritable bowel syndrome with constipation. *Am J Gastroenterol*. 2012;107:1714-1724.
18. Chey WD, Lembo AJ, Lavins BJ, et al. Linaclotide for irritable bowel syndrome with constipation: a 26-week, randomized, double-blind, placebo-controlled trial to evaluate efficacy and safety. *Am J Gastroenterol*. 2012;107:1702-1712.
19. Rao SSC, Quigley EMM, Shiff SJ, et al. Effect of linaclotide on severe abdominal symptoms in patients with irritable bowel syndrome with constipation. *Clin Gastroenterol Hepatol*. 2014;12:616-623.
20. Castro J, Harrington AM, Hughes PA, et al. Linaclotide inhibits colonic nociceptors and relieves abdominal pain via guanylate cyclase-C and extracellular cyclic guanosine 3’,5’-monophosphate. *Gastroenterology*. 2013;145(6):1334-1346. e1–11.
21. Grundy L, Harrington AM, Castro J, et al. Chronic linaclotide treatment reduces colitis-induced neuroplasticity and reverses persistent bladder dysfunction. *JCI Insight*. 2018;3(19). PMID 30282832.
22. Mohammad E, Ligon CO, Silos-Santiago A, et al. Linaclotide attenuates visceral organ crosstalk: role of guanylate cyclase-C activation in reversing bladder-colon cross-sensitization. *J Pharmacol Exp Ther*. 2018;366:274-281.
23. Castro J, Martin C, Hughes PA, et al. A novel role of cyclic GMP in colonic sensory neurotransmission in healthy and TNBS-treated mice. *Gastroenterology*. 2011;140:S-538.
24. Rao SSC, Hatfield R, Soffer E, et al. Manometric tests of anorectal function in healthy adults. Am J Gastroenterol. 1999;94:773-783.
25. Patrick DL, Drossman DA, Frederick IO, et al. Quality of life in persons with irritable bowel syndrome: development and validation of a new measure. Dig Dis Sci. 1998;43:400-411.
26. Remes-Troche JM, Tantiphlachiva K, Attaluri A, et al. Bi-directional assessment of the human brain-anorectal axis. Neurogastroenterol Motil. 2011;23:240-248, e117–e118.
27. Remes-Troche JM, Yamada T, Hamdy S, Rao SSC. Anorectal-cortical function is impaired in patients with dyssynergic defecation. Gastroenterology. 2007;132:A20.
28. Coss-adame E, Remes-Troche JM, Attaluri A, et al. Does biofeedback therapy improve brain-gut axis in dyssynergic defecation? Neurogastroenterol Motil. 2012;24:S-367.
29. Xiang X, Patcharatrakul T, Sharma A, et al. Cortico-anorectal, Spino-anorectal, and Cortico-spinal Nerve Conduction and Locus of Neuronal Injury in Patients With Fecal Incontinence. Clin Gastroenterol Hepatol. 2019;17(1130–1137):e2.
30. Tantiphlachiva K, Attaluri A, Valestin J, et al. Translumbar and transsacral motor-evoked potentials: a novel test for spino-anorectal neuropathy in spinal cord injury. Am J Gastroenterol. 2011;106:907-914.
31. Rao SS, Tantiphlachiva K, Remes-Troche J, et al. Does biofeedback therapy modulate Anorectal (gut)-brain axis in patients with dyssynergic defecation? Gastroenterology. 2011;140:S-367.
32. Loening-Baucke V, Yamada T. Is the afferent pathway from the rectum impaired in children with chronic constipation and encopresis? Gastroenterology. 1995;109:397-403.
33. Ligon C, Mohammadi E, Ge P, et al. Linaclotide inhibits colonic and urinary bladder hypersensitivity in adult female rats following unpredictable neonatal stress. Neurogastroenterol Motil. 2018;30:e13375.
34. Hannig G, Tchernychev B, Kurtz CB, et al. Guanylate cyclase-C/cGMP: an emerging pathway in the regulation of visceral pain. Front Mol Neurosci. 2014;7:31.
35. Wilder-Smith CH. The balancing act: endogenous modulation of pain in functional gastrointestinal disorders. Gut. 2011;60:1589-1599.
36. Faraday M. Experimental research in electricity. Quaritch. 1839:1-15.
37. Barker AT, Jalinos R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. Lancet. 1985;1:1106-1107.
38. Turnbull GK, Hamdy S, Aziz Q, et al. The cortical topography of human anorectal musculature. Gastroenterology. 1999;117:32-39.
39. Bharucha AE, Linden DR. Linaclotide - a secretagogue and antihyperalgesic agent - what next? Neurogastroenterol Motil. 2010;22:227-231.
40. Eutamene H, Bradesi S, Larauche M, et al. Guanylate cyclase C-mediated antinociceptive effects of linaclotide in rodent models of visceral pain. Neurogastroenterol Motil. 2010;22:s12:e84.
41. Mayer EA, Berman S, Derbyshire SWG, et al. The effect of the 5-HT3 receptor antagonist, alosetron, on brain responses to visceral stimulation in irritable bowel syndrome patients. Aliment Pharmacol Ther. 2002;16:1357-1366.
42. Silos-Santiago I, Hannig G, Eutamene H, et al. Gastrointestinal pain: unraveling a novel endogenous pathway through uroguanylin/guanylate cyclase-C/cGMP activation. Pain. 2013;154:1820-30.

SUPPORTING INFORMATION
Additional supporting information will be found online in the Supporting Information section.

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