Received: 27 December 2019 | First decision: 13 January 2020 | Accepted: 15 April 2020

DOI: 10.1111/apt.15772

AP&T Alimentary Pharmacology & Therapeutics WILEY

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

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Funding information
This study was an investigator-initiated study that was awarded an unrestricted research grant support from Forest Research Institute.

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. ClinicalTrials.Gov: Registered at Clinical trials.gov no NCT02078323.

The Handling Editor for this article was Professor Jonathan Rhodes, and it was accepted for publication after full peer-review.

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 of afferent gut and brain pathways,[7-10,12] hyperexcitability of dorsal neurons, abnormal central processing of afferent information [7-10] or abnormal endogenous descending inhibitory pathways [12,13] 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 latency [7,8] 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)[4]: (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.[24] 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.[26-30]

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 (C p3).[26-29] Four runs of 50 stimuli at 0.2 H_z 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 methoodology.[16,26-29]

The TAMS study was performed by using a 90-mm circular coil and using previously published methodology[26,29] (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 S2 and S3 vertebra.

2.2 | Measurements and analyses

2.2.1 | Cortical evoked potential (CEP) measurements

The four runs of CEPs, following anal and rectal stimulation from each subject were averaged, and the data compared between the groups. The investigator performing data analysis was blinded to the patient's randomisation status. The latency was defined as the time interval (milliseconds) from triggering the stimulus to the onset of each CEP component.[16,26,28,29] Positive CEP peaks were labelled P1 and P2, and negative peaks were labelled N1 and N2. Latency of the rectal and anal CEPs from each subject were calculated separately and group means were calculated. The primary outcome measures were the latency of the P1 and N1 recto-cortical responses.

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.[16,26,27,29,30]

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 (BMs) per week (stool frequency), the number of complete spontaneous bowel movements (CSBMs) 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.[25]

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.[31]

Descriptive statistics were calculated for all patients and for each treatment arm (linaclotide 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 (Δ 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).

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

	Linaclotide (N = 26)	Placebo (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

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 P1(Δ 19 ± 6, $P < 0.005$), and N1 (Δ 20 ± 7, $P < 0.02$) waveform responses, and likewise for P2 ($P = 0.001$) and N2 ($P = 0.0001$) responses were all significantly prolonged in the linaclotide group when compared to baseline but not in placebo group (P1:Δ 3 ± 5; N1:Δ 4.7 ± 5,$P = 0.3$) (Figure 2B and Table 2).

The mean ano-cortical latencies for the P1 ($P = 0.003$), N1 ($P = 0.0001$), P2 ($P = 0.009$) and N2 ($P = 0.022$) waveform responses of the CEP were all significantly prolonged when compared to baseline in the linaclotide group (Table 2). The N1 latency was prolonged ($P = 0.037$) in the placebo group but not the P1, P2 and N2 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).

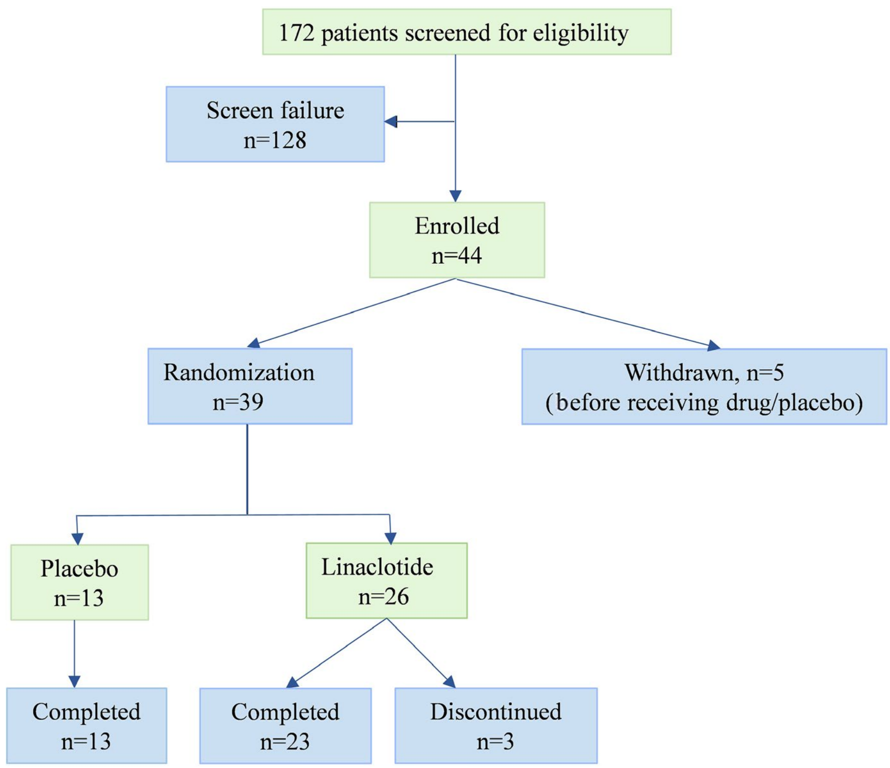

FIGURE 1 Consort flow diagram for the study

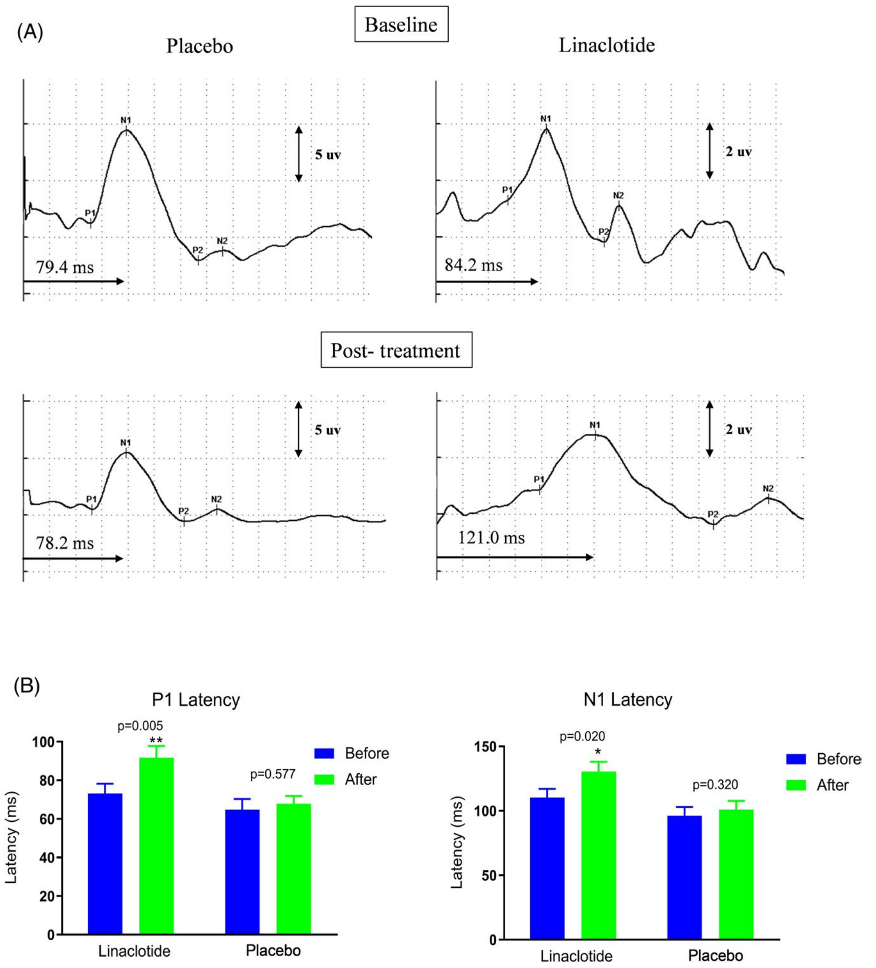

FIGURE 2 A, Typical recto-cortical evoked potential (CEP) responses in IBS-C patients, before and after treatment with linaclotide and placebo. The latency (onset time) of the P1 and N1 waveforms is significantly prolonged after linaclotide but not after placebo. B, Mean (± SEM) latency time for the onset of P1 and N1 waveforms of the rectal CEP response

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
	N1 latency (ms)	110.3 ± 6.8	130 ± 7.6	0.020	96.2 ± 6.2	100.9 ± 6.8	0.320
	P2 latency (ms)	187.8 ± 9.5	223.7 ± 9.1	0.001	175 ± 16.2	177.7 ± 13.2	0.789
	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
	N1 latency (ms)	106.8 ± 34.4	147.8 ± 49.9	0.0001	99.8 ± 26.6	125.4 ± 51.6	0.0371
	P2 latency (ms)	175.4 ± 12.1	209.0 ± 13.2	0.009	182.7 ± 18.8	204.2 ± 20.0	0.424
	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
	Desire to defecate (mL)	66.9 ± 6.4	72.3 ± 6.2	0.452	100.8 ± 16.1	92.3 ± 11.5	0.879
	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	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.

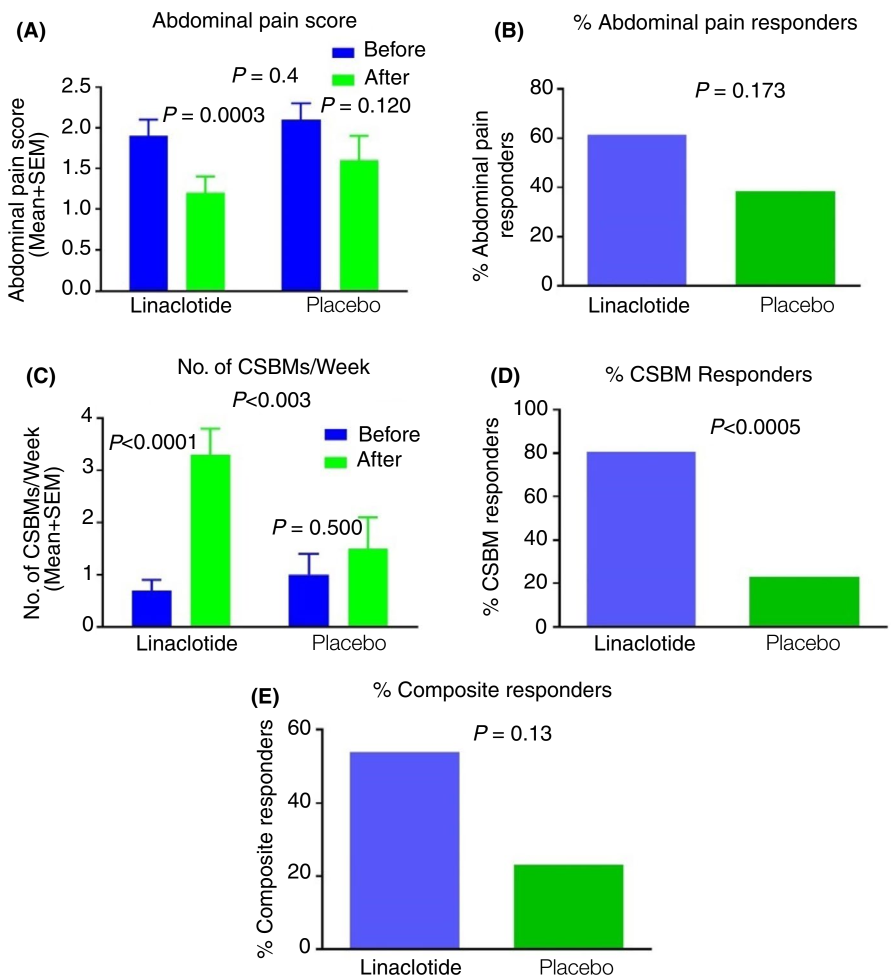

FIGURE 3 Effects of linaclotide and placebo on A, abdominal pain scores; B, % abdominal pain responders; C, number of complete spontaneous bowel movements (CSBMs)/week; D, % complete spontaneous bowel movement (CSBM) responder; E, % of Composite responders

TABLE 3 Effects of linaclotide and placebo on the subject global assessment of pain (SGA), bowel symptoms, and IBS-QOL score (shown as change from baseline) (Mean ± SEM)

	Linaclotide			Placebo		
	Before	After	*P*	Before	After	*P*
SGA	6.14 ± 0.41	3.89 ± 0.50	0.0002	6.70 ± 0.39	4.99 ± 0.84	0.9
Stool frequency	4.16 ± 0.49	6.4 ± 0.55	0.0001	4.38 ± 0.56	5.15 ± 0.52	0.1
Stool consistency (1-7)	1.82 ± 0.23	3.56 ± 0.32	<0.0001	1.35 ± 0.28	2.44 ± 0.28	0.06
Straining (0-3)	1.29 ± 0.15	1.25 ± 0.12	0.7279	1.16 ± 0.17	1.15 ± 0.16	0.82
	Δ from baseline	*P*		Δ from baseline	*P*	
Dysphoria	−6.52 ± 1.55	0.0004		0.92 ± 1.70	0.7051	
Interference with activity	−4.26 ± 1.62	0.0154		−0.42 ± 0.98	0.7635	
Body image	−2.52 ± 0.80	0.0045		3.95 ± 0.82	1.000	
Health worry	−3.65 ± 0.67	<0.0001		−0.58 ± 0.21	0.6033	
Food avoidance	−2.30 ± 0.76	0.0063		0.83 ± 0.79	0.1567	
Social Reaction	−2.17 ± 0.91	0.0256		0.29 ± 0.62	0.7404	
Sexuality	−0.78 ± 0.40	0.0647		0.83 ± 0.34	0.1065	
Relationships	−1.70 ± 0.66	0.0181		−0.42 ± 0.60	0.6255	
Total score	−27.57 ± 6.86	0.0006		0.88 ± 6.04	0.9186	

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.[7,8,26,28,29,32] 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 linaclotide arms. We found that baseline psychological features that were assessed were similar between the two groups. Consequently, the changes in CEP after taking linaclotide were most likely due to a pharmacophysiological effect of the drug.

All patients who were selected for this study had IBS with rectal hypersensitivity. In this group, linaclotide 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 distensibility, 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 linaclotide could improve visceral hypersensitivity in the gut.

These findings in humans are also consistent with the observations in animal models where acute or chronic linaclotide 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.[20,21,23] Furthermore, linaclotide was effective in both stress-induced, inflammatory and chronic visceral hypersensitivity models.[21,22,33] In contrast, linaclotide 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 (GMP).[20-23,34] Studies in rodent models also showed an extracellular mechanism of anti-nociception that was linked with cGMP.[20,21] Studies have also demonstrated that exogenous cGMP can inhibit action potential firing of human dorsal root ganglion sensory neurons.[20] Overall, these studies suggest that linaclotide 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,[13,35] we used transcranial magnetic stimulation that relies on Faraday's [36] 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.[29,37,38] 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-spinofugal) pathway has been shown to be abnormal.[16] 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 linaclotide. This new finding suggests that linaclotide 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 linaclotide, 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, linaclotide significantly increased the number of CSBMs/week when compared to baseline and placebo. Together these findings extend previous observations from large RCTs[17-19] that linaclotide improves both pain and bowel symptoms in IBS-C. The overall percentage of treatment responders was more than twofold higher with linaclotide when compared to placebo (54% vs 23%). This composite responder rate was higher compared to the published randomised controlled trials of linaclotide,[17,18] 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 linaclotide, 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 linaclotide when compared to their baseline scores, whereas there were no changes in patients who received placebo. Also, the QOL scores were significantly better with linaclotide when compared to placebo, indicating that in addition to improvements in pain and bowel symptoms, linaclotide 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 linaclotide reduced colorectal sensitivity only in hypersensitive but not in naïve rats.[39,40] 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 linaclotide 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 linaclotide-induced sensory modulation could not be defined, unlike previous positron emission topography or functional magnetic resonance imaging studies with other agents.[41] Also, we have shown that both CEP and TMS data have excellent inter-observer agreements.[26] We chose this approach, as our objectives were to examine both the afferent and efferent gut-brain-gut 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 linaclotide 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 linaclotide did not differ from placebo. It is likely that the effects of linaclotide are mediated by the release of cyclic GMP as shown in animal models previously.[21,42] 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.

ACKNOWLEDGEMENT

We sincerely appreciate the unrestricted grant support from Forest Research Institute for this study. We sincerely acknowledge the research assistance of Mrs Amanda Schmeltz, Mrs Meagan Gibbs O'Banion, Mrs Ijeoma Azih, and the statistical analysis and support of Mrs Patricia Hall. Importantly, we thank Mrs Helen Smith for superb secretarial assistance.

Declaration of personal interests: Dr S. Rao has served on the advisory board of Forest Labs and Ironwood Pharmaceuticals. Dr A. Sharma has served on advisory board for Ironwood Pharmaceuticals. All other authors declare no conflict of interests with this study.

AUTHORSHIP

Guarantor of the article: Satish SC Rao, MD, PhD, FRCP.

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 Rattanakovit 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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SUPPORTING INFORMATION

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

How to cite this article: Rao SSC, Xiang X, Yan Y, et al. Randomised clinical trial: linaclotide vs placebo—a study of bi-directional gut and brain axis. *Aliment Pharmacol Ther*. 2020;51:1332–1341. https://doi.org/10.1111/apt.15772