Drugs of the Future 2008, 33(7): 570-576

CCC: 0377-8282/2008
DOI: 10.1358/dof.2008.033.07.1214164

Linaclotide Acetate

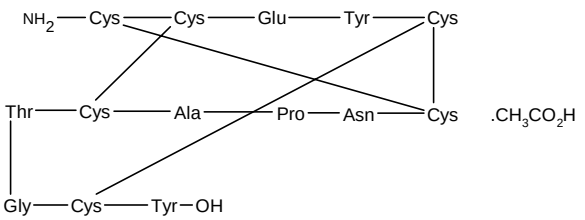
Prop INNM; USAN

Guanylate Cyclase C Receptor Agonist
Treatment of Irritable Bowel Syndrome
Treatment of Constipation

MD-1100
MM-416775

L-Cysteinyl-L-cysteinyl-L-glutamyl-L-tyrosyl-L-cysteinyl-L-cysteinyl-L-asparaginyl-L-prolyl-L-alanyl-L-cysteinyl-L-threonyl-glycyl-L-cysteinyl-L-tyrosine cyclic(*S*-3.1-*S*-3.6:*S*-3.2-*S*-3.10:*S*-3.5-*S*-3.13)-tris(disulfide) acetate

[9-L-Tyrosine]heat-stable enterotoxin (*Escherichia coli*)-(6-19)-peptide



$C_{61}H_{83}N_{15}O_{23}S_6$
Mol wt: 1586.795

CAS: 851199-60-5

CAS: 742095-77-8 (reduced)

CAS: 851199-59-2 (free base)

EN: 379154

Abstract

Linaclotide acetate (MD-1100) is a novel, orally administered agent currently in development for the treatment of gastrointestinal disorders, including irritable bowel syndrome with predominant constipation (IBS-C) and chronic constipation. This 14-amino-acid peptide is a first-in-class compound that acts as an agonist of human guanylate cyclase C (GC-C), a transmembrane protein located in intestinal epithelial cells. Activation of intestinal GC-C induces secretion of fluid, sodium and bicarbonate in the intestinal lumen. In animal studies, linaclotide accelerated gastrointestinal transit, decreased stool consistency and decreased visceral pain measured by surrogate markers. In clinical studies in healthy volunteers and patients with chronic constipation or IBS-C, linaclotide had a significant effect on stool consistency, ease of passage of stools and increase in stool frequency, as well as improving bowel function and abdominal discomfort. In all animal and human studies, linaclotide appeared to be safe and well tolerated, with minimal bioavailability. Further randomized, controlled trials of clinical efficacy and safety in larger patient populations are warranted.

Synthesis

The title compound can be chemically synthesized by solid-phase technology using conventional Fmoc procedures. Amino acids are coupled using DCC/HOBT and Fmoc-protecting groups are removed by means of piperidine. Cysteine thiol groups are protected with trityl and cleavage of the peptide from the resin is done by means of TFA (1, 2).

Background

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders, with a prevalence estimated at 3-15% of the general population in Western countries (3, 4). IBS is characterized by abdominal pain and discomfort in association with altered bowel habits; symptoms can not be explained by any structural abnormalities using current standard diagnostic tests. In addition to abdominal pain, diarrhea or constipation, typical symptoms include bloating, flatulence, stool urgency or straining and the feeling of incomplete evacuation (5). Characteristic symptom patterns, as in the IBS consensus “Rome III” criteria and the absence of alarm features or structural gut disease, allow a positive diagnosis of IBS (6). Patients may be classified into symptom subgroups as diarrhea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C), IBS with mixed bowel movements (IBS-M) or IBS with alternating bowel movements (IBS-A).

The pathophysiology of IBS is still not well understood, but is most likely multifactorial. Several factors such as motor and sensory dysfunction, disturbed brain–

Viola Andresen[1,2], Michael Camilleri[1]*. [1]Clinical Enteric Neuroscience Translational and Epidemiological Research (C.E.N.T.E.R.) Group, Mayo Clinic College of Medicine, Rochester, MN, USA; [2]Israelitic Hospital, University of Hamburg, Germany. *Correspondence: Mayo Clinic, Charlton 8-110, 200 First St. S.W., Rochester, MN 55905, USA.

gut interaction, neuroimmune mechanisms and changes in the intraluminal milieu appear to play a role (5, 7). In a recent study in 120 patients with IBS and 40 controls, it was demonstrated that the most prevalent pathophysiology in IBS was impaired colonic transit: 32% had abnormal colonic transit, 21% had heightened visceral sensitivity and 16% had hyposensitivity (8).

Chronic constipation without abdominal pain is another very common clinical problem. Clinical practice suggests that many patients transition between chronic constipation and IBS-C, and it is also evident that the symptoms of these disorders overlap those of evacuation disorders such as pelvic floor dyssynergia. In some patients, constipation is caused by underlying medical conditions (*e.g.*, hypothyroidism) or medical therapies (*e.g.*, opioids).

The cause of abnormal colonic transit in functional constipation and IBS-C is still incompletely understood. There is evidence that an imbalance of excitatory and inhibitory nerves in the myenteric plexus and deficiencies of the interstitial cells of Cajal (ICCs) may play a role in chronic idiopathic, severe constipation that is treated by surgical resection of the colon. It is unclear whether such enteric degeneration occurs in less severe forms of colonic hypomotility; however, treatments directed at stimulating colonic motility or transit result in improved symptoms.

While patients with mild to moderate slow-transit constipation may benefit from fiber or laxatives, the efficacy of these treatments in IBS-C or severe constipation is rather limited. Efficacious prokinetic drugs such as the 5-HT_4 agonists cisapride and tegaserod are no longer available because they have been associated with rare but serious side effects. Tegaserod, however, can be provided in emergency situations and requests need to be directed to the FDA. Other 5-HT_4 agonists are in development, such as prucalopride, renzapride and ATI-7505, which accelerate colonic transit in health or patients with chronic constipation (9-14) and also improve patients' symptoms (15-17). However, 5-HT_4 receptor agonists may interact with other receptors, such as 5-HT_3, 5-HT_{1B} and hERG channels, at concentration ranges relevant to their action on 5-HT_4 receptors (18). The effects on the hERG channel or 5-HT_{1B} receptor may lead to an unfavorable cardiovascular profile. Recently, it was announced that renzapride proved ineffective in phase III clinical trials and is no longer in development for IBS-C. Therefore, there is still a need to develop novel approaches to treat conditions associated with reduced colonic motility or transit, such as through stimulation of different mechanisms, including enterocyte secretion, as with the chloride channel activator lubiprostone (19-22).

An agent that is in a new class for development in the treatment of chronic constipation and IBS-C is linaclotide acetate (MD-1100). This compound has a truly novel mechanism of action, targeting the intestinal guanylate cyclase C (GC-C) receptor. Current knowledge regarding the mechanism of action of linaclotide and results from animal studies and early human phase I and II studies will be presented in this review.

Preclinical Pharmacology

Linaclotide is a first-in-class, 14-amino-acid peptide (Fig. 1) that acts in the intestine via binding and activation of the receptor guanylate cyclase C (GC-C), located on the luminal membrane of the enterocyte (Fig. 2). The physiological agonists of the intestinal GC-C receptor are the endogenous hormones guanylin (15 amino acids) (23) and uroguanylin (16 amino acids) (24, 25), which are suggested to be involved in sodium homeostasis of the organism. In response to intestinal sodium load, the natural peptide hormones are secreted into the intestinal lumen, where they bind to the transmembrane GC-C receptor. GC-C is located predominantly on the luminal surface of epithelial cells throughout the small intestine and colon. Like the natural peptides guanylin and uroguanylin, linaclotide binds to the extracellular domain of GC-C and activates the protein, which leads to cyclic guanosine monophosphate (cGMP)-dependent inhibition of Na^+/H^+ exchange and activation of the cystic fibrosis transmembrane conductance regulator (CFTR). This activation results in water, chloride and bicarbonate secretion in the intestinal lumen (26) (Fig. 2). In the jejunum and colon, GC-C activation inhibits Na^+ and fluid absorption (27, 28). Other natural agonists of the intestinal GC-C receptor are enteric bacterial peptides of the heat-stable enterotoxin family (ST peptides; 19 amino acids) (29), which are known to cause secretory diarrhea.

Studies in intestinal epithelial cells from wild-type mice and GC-C knockout mice confirmed the mechanism of action of linaclotide, showing high affinity for the intestinal GC-C receptor, higher potency than the natural hormones, as measured by the intracellular cGMP response, and the induction of fluid secretion into the intestinal lumen (30).

In vitro and *in vivo* studies have shown that carboxypeptidase A converts linaclotide by cleavage of the *C*-terminal tyrosine to the active metabolite MM-419447 (Fig. 1) (31, 32). This metabolite has GC-C receptor-agonist activity equivalent to that of linaclotide. Both linaclotide and its active metabolite MM-419447 have very low bioavailability in rats (0.07% and 0.08%, respectively) (32).

A study in mice compared the effects of linaclotide to those of control vehicle and the 5-HT_4 agonist tegaserod on intestinal secretion and gastrointestinal transit time; other studies compared the effects of linaclotide on visceral pain to those of indomethacin (33). In the former study, oral linaclotide increased transit time by 27.3% (± 12%) over control, and the effects were comparable to those of tegaserod. On the other hand, intestinal secretion was stimulated 45% on average by linaclotide compared to tegaserod and vehicle control. In the animal model of visceral pain induced by i.p. injection of phenylbenzoquinone, both linaclotide and indomethacin reduced writhing (a biomarker of pain) by 30% and 51%, respectively.

Oral linaclotide also dose-dependently accelerated intestinal transit and secretion in rats, with minimal absorption of the drug and no adverse effects noted in the

Fig. 1. Structure of linaclotide and its active metabolite MM-419447.

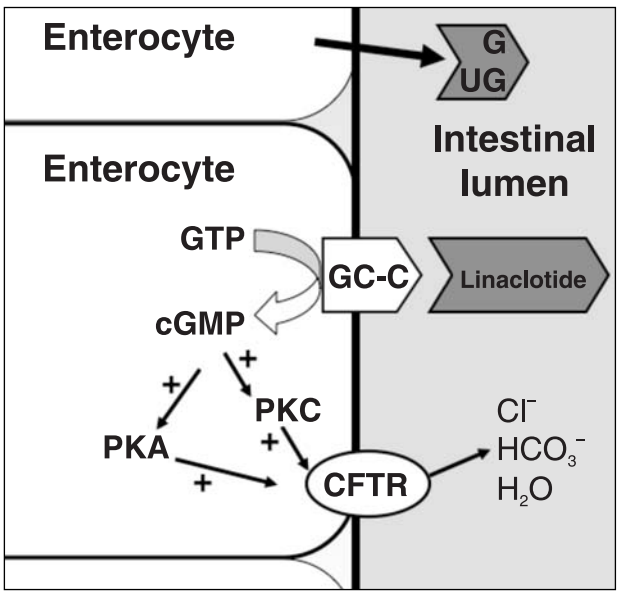


Fig. 2. Proposed mechanism of action of linaclotide and the closely related natural peptide hormones guanylin (G) and uroguanylin (UG). Like the natural peptides, linaclotide binds to the extracellular domain of guanylate cyclase C (GC-C) and activates the protein, which leads to intracellular cyclic guanosine monophosphate (cGMP) formation and activation of the cystic fibrosis transmembrane conductance regulator (CFTR) via protein kinases A and C (PKA and PKC). CFTR activation results in water, chloride and bicarbonate secretion in the intestinal lumen.

therapeutic dose range (29). Moreover, linaclotide (0.3 μg/kg) significantly reduced the abdominal contractile response to colorectal distension at a distending pressure of 15 mmHg following trinitrobenzene sulfonic acid-induced colonic inflammation and following stress (34). The latter findings suggest that linaclotide may also influence visceral hypersensitivity. The mechanism by which linaclotide may affect visceral sensation is currently unknown. It is conceivable that the second messenger cGMP, which is induced by GC-C activation, may stimulate additional effector systems potentially involved in visceral sensation.

The effects of linaclotide were investigated in two rat models of reduced intestinal motor function: postoperative ileus and opioid-induced constipation. In both models, linaclotide accelerated gastrointestinal transit compared to animals that received vehicle (35).

In summary, the results of animal studies demonstrated the efficacy of linaclotide in increasing intestinal secretion and accelerating gastrointestinal transit, as well as reducing visceral pain, thereby supporting the potential utility of linaclotide for the treatment of disorders associated with constipation and visceral pain, such as chronic constipation, IBS-C and opioid-induced constipation.

Clinical Studies

The safety and efficacy of linaclotide at single doses ranging from 30 to 3000 μg or multiple doses (7-day treatment) of 30-1000 μg were evaluated in phase I studies in healthy volunteers. Linaclotide was safe and well tolerated in these studies and there was no evidence of systemic exposure to linaclotide or its active 13-amino-acid metabolite MM-419447 after oral administration. These data are consistent with the limited bioavailability of linaclotide. During these safety studies, there was a significant effect of linaclotide on stool consistency and ease of passage of stool, and evidence for an increase in stool frequency (36, 37).

The effects of linaclotide in patients with chronic constipation have been evaluated in a 14-day phase IIA study (38). In this placebo-controlled, double-blind, randomized study of multiple oral ascending doses in 42 patients with chronic constipation, linaclotide was well tolerated across a daily dose range of 100-1000 μg. Linaclotide increased baseline stool frequency by 4.0-6.8 spontaneous bowel movements (SBM)/week compared to 1.8 SBM/week for placebo, baseline stool consistency measured by the Bristol Stool Form Scale (BSFS) by 1.5-2.7 compared to 0.5 for placebo, and baseline ease of passage by 0.7-2.1 compared to 0.2 for placebo. Abdominal discomfort decreased in severity in linaclotide-treated patients by approximately 26% compared to 8% for placebo. In addition, a responder analysis (responder = ≥ 3 SBM/week + increase of ≥ 1 SBM/week) demonstrated a 71-89% response rate in the linaclotide groups compared to 50% in the placebo group. There were no serious adverse events (SAEs) and only 7 patients experienced treatment-related AEs. The majority of AEs were mild, with diarrhea being the most common.

A single-site, double-blind, placebo-controlled, randomized study evaluated the pharmacodynamic effects of oral linaclotide 100 and 1000 μg and placebo in 36 women with IBS-C (39). Participants underwent 5-day baseline and 5-day treatment periods, during which gastrointestinal transit (by validated scintigraphy [40]) and bowel function (by daily diaries, including the Bristol Stool Form Scale [41]) were assessed. The primary endpoint was the effect

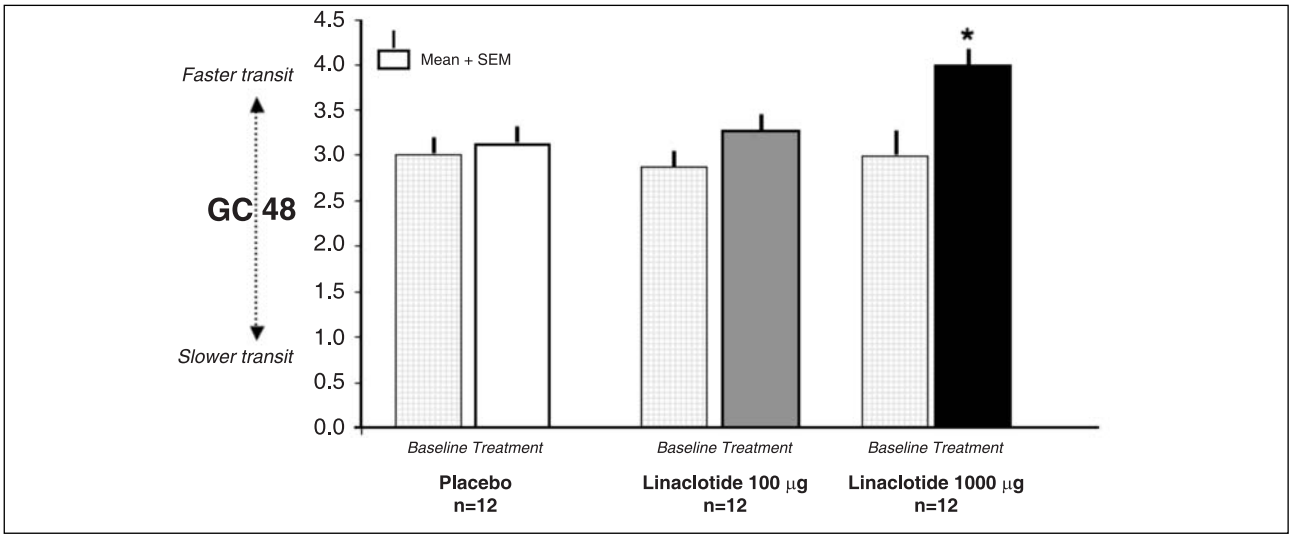


Fig. 3. Effect of linaclotide on overall colonic transit at 48 h in patients with IBS-C. Overall $p = 0.02$; *pairwise comparison $p = 0.01$ *versus* placebo. Reprinted from Andresen, V., Camilleri, M., Busciglio, I. et al. *Effects of linaclotide, a novel guanylate cyclase-C agonist, on gastrointestinal transit and bowel function in patients with constipation-predominant irritable bowel syndrome*. Gastroenterology 2007, 133(3): 761-8, Copyright 2007, with permission from Elsevier.

of linaclotide on gastrointestinal transit. Secondary endpoints were the effects on time to first bowel movement after first drug intake, and on stool frequency, stool consistency, ease of passage and sensation of complete evacuation during the treatment period relative to a pre-drug baseline period. Effects of linaclotide on gastric emptying and orocecal transit times were not detected, but there was a significant ($p = 0.015$) overall treatment effect on ascending colon half-emptying time ($t_{1/2}$), with a significant acceleration by linaclotide 1000 μg ($p = 0.004$). Moreover, treatment effects on overall colonic transit were also observed ($p = 0.020$ for the geometric center [GC] at 48 h), with a significant acceleration by linaclotide 1000 μg once daily *vs.* placebo ($p = 0.010$) (Fig. 3). Finally, there were significant overall treatment effects on stool frequency, stool consistency, ease of passage and time to first bowel movement, with a strong dose–response for stool consistency (overall $p < 0.001$) (Fig. 4). Given the known mechanisms whereby intestinal GC-C activation activates secretion or reduces absorption in the intestine, the effects of linaclotide on colonic transit and the improved bowel function are currently considered to most likely reflect increased luminal water content. This results in acceleration of transit, especially through the ascending colon. Accelerating transit in the proximal colon reduces the ability of the colon to reabsorb water and electrolytes, thereby resulting in looser stool consistency. There were no SAEs, and no patient had to stop treatment due to an AE. Recorded AEs occurring in more than 1 patient are shown in Table I. There were no differences detected among the treatment groups in the proportions of subjects with any AEs ($p = 0.683$) or overall gastrointestinal AEs ($p = 0.108$), which predominantly reflected the pharmacological effects of linaclotide, such as diarrhea/altered bowel movements or borborygmi. There was no increase in AEs with increasing doses of linaclotide.

In early March 2008, a public communication (42) summarized the results of two randomized, double-blind, placebo-controlled phase IIB studies assessing the safety, therapeutic effect and dose–response of four different once-daily doses of linaclotide (75, 150, 300 and 600 μg) in patients with chronic constipation or IBS-C. In patients with chronic constipation treated for 4 weeks, linaclotide was associated with an increase in the weekly SBM frequency rate, which was significant at all doses above 75 μg. Linaclotide also improved the complete spontaneous bowel movement (CSBM) frequency, stool consistency, straining, abdominal pain, bloating and abdominal discomfort. Patients with IBS-C who received once-daily treatment with linaclotide experienced a significant increase in the weekly CSBM frequency rate —the primary endpoint chosen for the study— at all doses except for 150 μg. Linaclotide-treated patients also experienced improvements in SBM frequency, stool consistency, abdominal pain, bloating, abdominal discomfort, adequate relief and IBS-C symptom severity. In both studies, linaclotide was well tolerated at all doses, with no SAEs in any patient attributed to the treatment. The most common AE in both studies was diarrhea. These promising results suggest that the medication will likely move to a phase III program, and its safety will need to be further evaluated in long-term trials. However, it is unclear from the public communication whether the endpoints used in the phase IIB trial in IBS-C are validated and consistent with the PRO guidance from the regulatory agencies (43).

Summary

Linaclotide is a novel, first-in-class agonist of intestinal epithelial GC-C. Activation of intestinal GC-C induces intestinal fluid secretion and inhibits colonic fluid absorption. In animal studies, linaclotide accelerated gastroin-

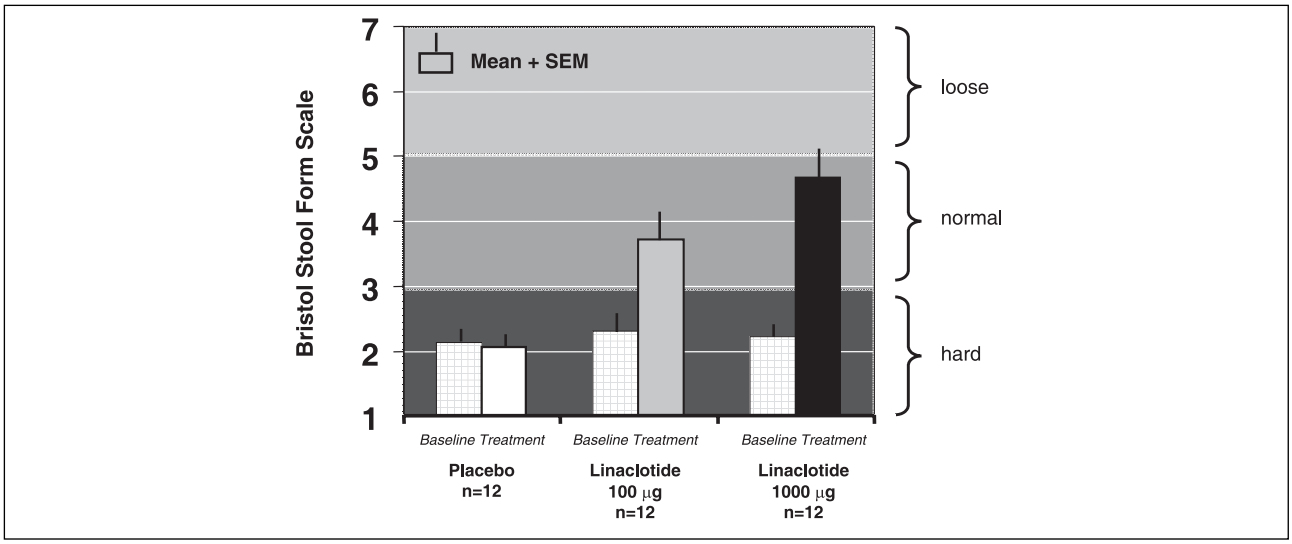


Fig. 4. Effect of linaclotide on stool consistency measured by the Bristol Stool Form Scale in patients with IBS-C. Reprinted from Andresen, V., Camilleri, M., Busciglio, I. et al. *Effects of linaclotide, a novel guanylate cyclase-C agonist, on gastrointestinal transit and bowel function in patients with constipation-predominant irritable bowel syndrome*. Gastroenterology 2007, 133(3): 761-8, Copyright 2007, with permission from Elsevier.

Table I: Adverse effects recorded in the entire study population of 36 women with IBS-C.*

		Placebo (n=12)	Linaclotide 100 μg (n=12)	Linaclotide 1000 μg (n=12)	Total (n=36)
Non-GI-related	Headache	2	5	3	10
	Drowsiness	0	1	1	2
GI-related	Bloating	1	4	2	7
	Abdominal pain	3	2	1	6
	Borborygmi	0	5	1	6
	Loose stools	0	2	3	5
	Urgency	1	2	0	3
	Flatulence	0	3	0	3
	Nausea	1	1	0	2

*Patients may have experienced more than one adverse effect and therefore the total number of adverse events may exceed the number of patients per group. GI, gastrointestinal. Reprinted from Andresen, V., Camilleri, M., Busciglio, I. et al. *Effects of linaclotide, a novel guanylate cyclase-C agonist, on gastrointestinal transit and bowel function in patients with constipation-predominant irritable bowel syndrome*. Gastroenterology 2007, 133(3): 761-8, Copyright 2007, with permission from Elsevier.

testinal transit and decreased surrogates of visceral pain. In phase I studies in healthy volunteers, linaclotide was safe and well tolerated, decreased stool consistency, increased stool frequency and increased ease of passage of stools. A phase II trial in patients with chronic constipation showed improvement in bowel habits and abdominal discomfort compared to placebo across all linaclotide treatment groups. In a phase II pharmacodynamic study in women with IBS-C, linaclotide significantly accelerated colonic transit and improved stool consistency, frequency, ease of passage and time to first bowel movement. These results were predictive of the positive outcomes in phase IIB trials. The actions of linaclotide in the intestine ultimately appear to provide a desirable alteration in stool consistency and ease of passage in patients with IBS-C or chronic constipation. In all animal and human studies, linaclotide appeared to be safe and well tolerated, with minimal bioavailability. This minimal systemic absorption with no detectable plasma levels in humans is important, because it may avoid systemic effects that have resulted in cessation of drug development programs for other drugs in development for the treatment of bowel disorders.

Overall, linaclotide may be a promising new agent for the treatment of conditions such as IBS-C and chronic constipation. Further randomized, controlled trials of clinical efficacy and safety in larger patient populations and with longer treatment durations are warranted.

Acknowledgements

The excellent secretarial support of Mrs. Cindy Stanislav is gratefully acknowledged.

Disclosure

Drs. Camilleri and Andresen received a research grant for a single-center pharmacodynamic study of linaclotide.

Sources

Ironwood Pharmaceuticals, Inc. (formerly Microbia) (US); codeveloped with Forest Laboratories, Inc. (US).

References

1. Currie, M.G., Mahajan-Miklos, S., Fretzen, A., Sun, L.J. (Microbia, Inc.). *Methods and compositions for the treatment of gastrointestinal disorders*. EP 1730172, JP 2008501310, US 2006258593, US 2006281682, WO 2005087797.

2. Currie, M.G., Mahajan-Miklos, S., Fretzen, A. et al. (Microbia, Inc.). *Methods and compositions for the treatment of gastrointestinal disorders*. WO 2007022531.

3. Drossman, D.A., Li, Z., Andruzzi, E. et al. *U.S. householder survey of functional gastrointestinal disorders. Prevalence, sociodemography, and health impact*. Digest Dis Sci 1993, 38(9): 1569-80.

4. Cremonini, F., Talley, N.J. *Irritable bowel syndrome: Epidemiology, natural history, health care seeking and emerging risk factors*. Gastroenterol Clin North Am 2005, 34(2): 189-204.

5. Drossman, D.A., Camilleri, M., Mayer, E.A., Whitehead, W.E. *AGA technical review on irritable bowel syndrome*. Gastroenterology 2002, 123(6): 2108-31.

6. Longstreth, G.F., Thompson, W.G., Chey, W.D., Houghton, L.A., Mearin, F., Spiller, R.C. *Functional bowel disorders*. Gastroenterology 2006, 130(5): 1480-91.

7. Camilleri, M. *Mechanisms in IBS: Something old, something new, something borrowed*. Neurogastroenterol Motil 2005, 17(3): 311-6.

8. Camilleri, M., McKinzie, S., Busciglio, I. et al. *Prospective study of motor, sensory, psychologic and autonomic functions in patients with irritable bowel syndrome*. Clin Gastroenterol Hepatol 2008, 6(7): 772-81.

9. Bouras, E.P., Camilleri, M., Burton, D.D., Thomforde, G., McKinzie, S., Zinsmeister, A.R. *Prucalopride accelerates gastrointestinal and colonic transit in patients with constipation without a rectal evacuation disorder*. Gastroenterology 2001, 120(2): 354-60.

10. Bouras, E.P., Camilleri, M., Burton, D.D., McKinzie, S. *Selective stimulation of colonic transit by the benzofuran 5HT4 agonist, prucalopride, in healthy humans*. Gut 1999, 44(5): 682-6.

11. Emmanuel, A.V., Roy, A.J., Nicholls, T.J., Kamm, M.A. *Prucalopride, a systemic enterokinetic, for the treatment of constipation*. Aliment Pharmacol Ther 2002, 16(7): 1347-56.

12. Sloots, C.E., Poen, A.C., Kerstens, R. et al. *Effects of prucalopride on colonic transit, anorectal function and bowel habits in patients with chronic constipation*. Aliment Pharmacol Ther 2002, 16(4): 759-67.

13. Camilleri, M., McKinzie, S., Fox, J. et al. *Effect of renzapride on transit in constipation-predominant irritable bowel syndrome*. Clin Gastroenterol Hepatol 2004, 2(10): 895-904.

14. Camilleri, M., Vazquez-Roque, M.I., Burton, D., Ford, T., McKinzie, S., Zinsmeister, A.R., Druzgala, P. *Pharmacodynamic effects of a novel prokinetic 5-HT receptor agonist, ATI-7505, in humans*. Neurogastroenterol Motil 2007, 19(1): 30-8.

15. Coremans, G., Kerstens, R., De Pauw, M., Stevens, M. *Prucalopride is effective in patients with severe chronic constipation in whom laxatives fail to provide adequate relief. Results of a double-blind, placebo-controlled clinical trial*. Digestion 2003, 67(1-2): 82-9.

16. Tack, J., Middleton, S.J., Horne, M.C., Piessevaux, H., Bloor, J.S., Meyers, N.L., Palmer, R.M. *Pilot study of the efficacy of renzapride on gastrointestinal motility and symptoms in patients with constipation-predominant irritable bowel syndrome*. Aliment Pharmacol Ther 2006, 23(11): 1655-65.

17. George, A.M., Meyers, N.L., Hickling, R.I. *Clinical trial: Renzapride therapy for constipation-predominant irritable bowel syndrome - Multicentre, randomized, placebo-controlled, double-blind study in the primary healthcare setting*. Aliment Pharmacol Ther 2008, 27(9): 830-7.

18. De Maeyer, J.H., Lefebvre, R.A., Schuurkes, J.A. *5-HT4 receptor agonists: Similar but not the same*. Neurogastroenterol Motil 2008, 20(2): 99-112.

19. Camilleri, M., Bharucha, A.E., Ueno, R. et al. *Effect of a selective chloride channel activator, lubiprostone, on gastrointestinal transit, gastric sensory, and motor functions in healthy volunteers*. Am J Physiol Gastrointest Liver Physiol 2006, 290(5): G942-7.

20. Johanson, J.F., Drossman, D.A., Panas, R., Wahle, A., Ueno, R. *Clinical trial: Phase 2 trial of lubiprostone for irritable bowel syndrome with constipation*. Aliment Pharmacol Ther 2008, 27(8): 685-96.

21. Johanson, J.F., Morton, D., Geenen, J., Ueno, R. *Multicenter, 4-week, double-blind, randomized, placebo-controlled trial of lubiprostone, a locally-acting type-2 chloride channel activator, in patients with chronic constipation*. Am J Gastroenterol 2008, 103(1): 170-7.

22. Johanson, J.F., Ueno, R. *Lubiprostone, a locally acting chloride channel activator, in adult patients with chronic constipation: A double-blind, placebo-controlled, dose-ranging study to evaluate efficacy and safety*. Aliment Pharmacol Ther 2007, 25(11): 1351-61.

23. Currie, M.G., Fok, K.F., Kato, J., Moore, R.J., Hamra, F.K., Duffin, K.L., Smith, C.E. *Guanylin: An endogenous activator of intestinal guanylate cyclase*. Proc Natl Acad Sci USA 1992, 89(3): 947-51.

24. Kita, T., Smith, C.E., Fok, K.F. et al. *Characterization of human uroguanylin: A member of the guanylin peptide family*. Am J Physiol 1994, 266(2, Pt. 2): F342-8.

25. Hamra, F.K., Forte, L.R., Eber, S.L. et al. *Uroguanylin: Structure and activity of a second endogenous peptide that stimulates intestinal guanylate cyclase*. Proc Natl Acad Sci USA 1993, 90(22): 10464-8.

26. Forte, L.R. *Guanylin regulatory peptides: Structures, biological activities mediated by cyclic GMP and pathobiology*. Regul Pept 1999, 81(1-3): 25-39.

27. Ieda, H., Naruse, S., Kitagawa, M., Ishiguro, H., Hayakawa, T. *Effects of guanylin and uroguanylin on rat jejunal fluid and electrolyte transport: Comparison with heat-stable enterotoxin*. Regul Pept 1999, 79(2-3): 165-71.

28. Nobles, M., Diener, M., Rummel, W. *Segment-specific effects of the heat-stable enterotoxin of E. coli on electrolyte transport in the rat colon*. Eur J Pharmacol 1991, 202(2): 201-11.

29. Giannella, R.A *Escherichia coli heat-stable enterotoxins, guanylins, and their receptors: What are they and what do they do?* J Lab Clin Med 1995, 125(2): 173-81.

30. Busby, R., Bryant, A., Cordero, E. et al. *The molecular target of MD-1100 is guanylate cyclase C (GC-C), an apical receptor on intestinal epithelial cells*. Dig Dis Week (May 14-19, Chicago) 2005, Abst T1136.

31. Bryant, A., Busby, R., Cordero, E. et al. *MD-1100, a therapeutic agent in development for the treatment of IBS-C, enhances intestinal secretion and transit, decreases visceral pain and is minimally absorbed in rats*. Dig Dis Week (May 14-19, Chicago) 2005, Abst T1135.

32. Busby, R., Bryant, A., Bartolini, W. et al. *MM-419447 is an active in vivo metabolite of linaclotide, a therapeutic agent in development for the treatment of IBS-C and chronic constipation*. Drug Metab Rev 2006, 38(Suppl. 2): 96-7.

33. Cordero, E., Driggers, E., Kessler, M. et al. *Activation of intestinal epithelial surface receptors as a novel approach for the treatment of C-IBS and chronic constipation*. Gastroenterology 2004, 126(4, Suppl. 2): Abst 746.

34. Bueno, L., Beaufraud, C., Mahajan-Miklos, S., Bryant, A.P., Currie, M.G. *Antinociceptive actions of MD-1100, a novel therapeutic agent for C-IBS, in animal models of visceral pain*. Am J Gastroenterol [69th Annu Sci Meet Am Coll Gastroenterol (Oct 29-Nov 3, Orlando) 2004] 2004, 99(10, Suppl.): Abst 867.

35. Bryant, A., Cordero, E., Tobin, J., Rivers, S., Kurtz, C., Currie, M. *Linaclotide significantly improves post-operative ileus and opiate-induced constipation in rats*. Am J Gastroenterol [71st Annu Sci Meet Am Coll Gastroenterol (Oct 20-25, Las Vegas) 2006] 2006, 101(Suppl. 2): Abst 1257.

36. Currie, M.G., Kurtz, C.B., Mahajan-Miklos, S., Busby, R., Fretzen, A., Geis, S. *Effects of a single dose administration of MD-1100 on safety, tolerability, exposure, and stool consistency in healthy subjects*. Am J Gastroenterol [70th Annu Sci Meet Am Coll Gastroenterol (Oct 28-Nov 2, Honolulu) 2005] 2005, 100(9, Suppl.): Abst 894.

37. Kurtz, C.B., Fitch, D., Busby, R.W., Fretzen, A., Geis, S., Currie, M.G. *Effects of multidose administration of MD-1100 on safety, tolerability, exposure, and pharmacodynamics in healthy subjects*. Gastroenterology [Dig Dis Week (May 20-25, Los Angeles) 2006] 2006, 130(4, Suppl. 2): Abst 132.

38. Johnston, J.M., Drossman, D.A., Lembo, A., Kurtz, C.B., Currie, M.G. *Linaclotide improves bowel habits and patient reported outcomes in subjects with chronic constipation*. Am J Gastroenterol, In press.

39. Andresen, V., Camilleri, M., Busciglio, I. et al. *Effects of linaclotide, a novel guanylate cyclase-C agonist, on gastrointestinal transit and bowel function in patients with constipation-predominant irritable bowel syndrome*. Gastroenterology 2007, 133(3): 761-8.

40. Cremonini, F., Mullan, B.P., Camilleri, M., Burton, D.D., Rank, M.R. *Performance characteristics of scintigraphic transit measurements for studies of experimental therapies*. Aliment Pharmacol Ther 2002, 16(10): 1781-90.

41. Heaton, K.W., Radvan, J., Cripps, H., Mountford, R.A., Braddon, F.E., Hughes, A.O. *Defecation frequency and timing, and stool form in the general population: A prospective study*. Gut 1992, 33(6): 818-24.

42. *Microbia and Forest Laboratories announce preliminary results of linaclotide phase 2b studies*. Microbia, Inc. Press Release March 4, 2008.

43. U.S. *Department of Health and Human Services. Guidance for industry: Patient-reported outcome measures: use in medical product development to support labeling claims: Draft guidance*. Health Qual Life Outcomes 2006, 4: 79.