Linaclotide Inhibits Colonic Nociceptors and Relieves Abdominal Pain via Guanylate Cyclase-C and Extracellular Cyclic Guanosine 3′,5′-Monophosphate

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BACKGROUND & AIMS: Linaclotide is a minimally absorbed agonist of guanylate cyclase-C (GUCY2C or GC-C) that reduces symptoms associated with irritable bowel syndrome with constipation (IBS-C). Little is known about the mechanism by which linaclotide reduces abdominal pain in patients with IBS-C. **METHODS:** We determined the effects of linaclotide on colonic sensory afferents in healthy mice and those with chronic visceral hypersensitivity. We assessed pain transmission by measuring activation of dorsal horn neurons in the spinal cord in response to noxious colorectal distention. Levels of *Gucy2c* messenger RNA were measured in tissues from mice using quantitative reverse transcription polymerase chain reaction and in situ hybridization. We used human intestinal cell lines to measure release of cyclic guanosine-3′,5′-monophosphate (cGMP) by linaclotide. We performed a post-hoc analysis of data from a phase III, double-blind, parallel-group study in which 805 patients with IBS-C were randomly assigned to groups given an oral placebo or 290 μg linaclotide once daily for 26 weeks. We quantified changes in IBS-C symptoms, including abdominal pain. **RESULTS:** In mice, linaclotide inhibited colonic nociceptors with greater efficacy during chronic visceral hypersensitivity. Intra-colonic administration of linaclotide reduced signaling of noxious colorectal distention to the spinal cord. The colonic mucosa, but not neurons, was found to express linaclotide's target, GC-C. The downstream effector of GC-C, cGMP, was released after administration of linaclotide and also inhibited nociceptors. The effects of linaclotide were lost in *Gucy2c*$^{-/-}$ mice and prevented by inhibiting cGMP transporters or removing the mucosa. During 26 weeks of linaclotide administration, a significantly greater percentage of patients (70%) had at least a 30% reduction in abdominal pain compared with patients given placebo (50%). **CONCLUSIONS: We have identified an analgesic mechanism of linaclotide: it activates GC-C expressed on mucosal epithelial cells, resulting in the production and release of cGMP. This extracellular cGMP acts on and inhibits nociceptors, thereby reducing nociception. We also found that linaclotide reduces chronic abdominal pain in patients with IBS-C.**

Keywords: CVH; CRD; Signaling Transduction; Analgesia.

Irritable bowel syndrome (IBS) is a prevalent chronic functional gastrointestinal disorder affecting 7%–14% of the North American population.[1] IBS is characterized by abdominal pain or discomfort associated with altered bowel habits and is subclassified as IBS with constipation (IBS-C), IBS with diarrhea, and alternating/mixed IBS.[2] Up to 33% of IBS patients have IBS-C, which places a considerable financial burden on society[3] and negatively impacts the quality of life of those affected.[4] Abdominal pain is the key clinical feature and the most difficult symptom to treat in patients with IBS.[5] Given the limited treatments currently available for patients with IBS-C, additional therapeutic options for abdominal pain relief are urgently needed.

Linaclotide, a synthetic, minimally absorbed, 14-amino acid peptide, is a guanylate cyclase-C (GC-C) agonist related to guanylin and uroguanylin, members of a family of naturally occurring peptide hormones (Supplementary Figure 1).[6] These hormones regulate intestinal fluid and electrolyte homeostasis and, thereby, bowel function through GC-C–mediated production of cyclic-guanosine-3′,5′-monophosphate (cGMP).[7] Linaclotide acts via the same mechanism as the endogenous hormones, through binding and activating GC-C located on the luminal surface of intestinal epithelial cells. This interaction elevates intracellular and extracellular levels of cGMP, inducing fluid secretion and accelerating intestinal transit in animal models.[8–10] In addition, linaclotide has been shown to elicit anti-hyperalgesic effects in several animal models of visceral pain.[11] These pharmacological effects of linaclotide have

Abbreviations used in this paper: cGMP, cyclic guanosine-3′,5′-monophosphate; CRD, colorectal distention; CVH, chronic visceral hypersensitivity; DH, dorsal horn; GC-C, guanylate cyclase-C; IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IR, immunoreactivity; pERK, phosphorylated MAP kinase ERK 1/2; TNBS, trinitrobenzene sulfonic acid.

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translated into the clinic. In phase II and phase III studies, linaclotide accelerated colonic transit and improved abdominal pain and constipation associated with IBS-C[12–14] and chronic idiopathic constipation.[15–17]

However, the exact mechanism by which linaclotide reduces abdominal pain remains unclear. In preclinical studies, anti-nociceptive actions have not been previously described for either guanylin or uroguanylin, and the anti-hyperalgesic effects of linaclotide exhibited in several distinct models of visceral pain are not attributable to alterations in colonic compliance.[11] Although the pathophysiology of IBS is not completely understood, hallmarks of IBS include allodynia and hyperalgesia to mechanical events within the intestine.[18–20] As mechanical hypersensitivity of colonic afferents is implicated in the development and maintenance of visceral pain in IBS,[19–21] we hypothesized that linaclotide and its downstream effector, intestinal epithelial cell–derived cGMP, might be responsible for the inhibition of colonic nociceptors.

We specifically targeted high-threshold nociceptive afferents in the splanchnic pathway, as we have shown they normally respond to noxious levels of colonic distention/contraction.[22,23] They also become hypersensitive[23] and hyperexcitable[24,25] in models of chronic visceral pain, which translates to increased signaling of noxious colorectal distention (CRD) within the thoracolumbar spinal cord.[26] We have shown that specific functional deficits in these afferents translate to reduced sensory responses to noxious CRD in whole-animal studies.[22,27] Most recently, we have shown that alterations in peripheral blood mononuclear cell supernatants from IBS patients correlate with abdominal pain intensity and frequency, and evoke mechanical hypersensitivity of colonic nociceptors.[21]

Here, our data show that linaclotide inhibits colonic nociceptors in vitro and in vivo, and that the efficacy of this inhibitory effect is greatest during chronic visceral hypersensitivity (CVH). Correspondingly, in a new post-hoc analysis of data from a 26-week phase III clinical trial, we show that oral administration of linaclotide significantly increases the percentage of patients with clinically meaningful improvement in abdominal pain, as specified in the recent US Food and Drug Administration guidance for IBS clinical trials[28] compared with placebo. Overall, our data reveal a unique analgesic mechanism of action that suggests linaclotide is able to exert beneficial effects on abdominal sensory symptoms, independent of improvements in bowel frequency.

Methods

For detailed descriptions of the methodology used, please see the Supplementary Material.

Model of CVH

Intra-colonic trinitrobenzene-sulfonic acid (TNBS; 130 μL/mL in 30% ethanol, 0.1-mL bolus) was administered as described previously.[23] TNBS-treated mice were allowed to recover for 28 days, at which stage inflammation had resolved and chronic colonic afferent mechanical hypersensitivity was evident.[23] These mice are termed *CVH mice*.

In Vitro Electrophysiology and Pharmacology

Splanchnic colonic afferents recordings were made from C57BL/6 healthy, CVH mice or GC-C null (*Gucy2c*$^{-/-}$) mice[29] using standard protocols.[22,23,30] High-threshold splanchnic nociceptors were investigated in intact colonic preparations and in those where the mucosa had been removed.

CRD and Phosphorylated MAP Kinase ERK 1/2 Immunohistochemistry

Mice received an enema of either saline or linaclotide (1000 nM). Five minutes later, under anesthesia, a 4-cm CRD balloon catheter was inserted transanally into healthy or CVH mice.[26] After regaining consciousness, CRD was performed (80 mmHg for 10 seconds, then deflated for 5 seconds and repeated 5 times). After sacrifice via anesthetic overdose, mice underwent fixation by transcardial perfusion and the thoracolumbar (T10–L1) spinal cord was removed and cryoprotected. Frozen sections were cut and incubated with monoclonal rabbit anti–phosphorylated MAP kinase ERK 1/2 (pERK) with AlexaFluorR488 used for visualization.

Determination of GC-C Expression With Quantitative Reverse Transcription Polymerase Chain Reaction and In Situ Hybridization

Quantitative polymerase chain reaction was performed using mouse-specific *Gucy2c* and glyceraldehyde-3-phosphate dehydrogenase Taqman probes on complementary DNAs synthesized from total RNAs extracted from a panel of mouse tissues. For in situ hybridization, sections were hybridized overnight at 55°C with either ^{35}S-labeled complementary RNA anti-sense or sense probes to *Gucy2c*.

cGMP Efflux Studies in Human Intestinal Caco-2 Cells

Cells were grown in monolayers and stimulated for 1 hour with linaclotide (1000 nM) in the presence or absence of the cGMP transporter inhibitor probenecid (0.5 mM or 2 mM). Samples from the basolateral chambers were collected and cGMP concentrations determined by liquid chromatography mass spectrometry.

Contractility Studies

Electrical field stimulation was applied to colonic tissues in the presence and absence of linaclotide or membrane permeable 8-bromo-cGMP. Contraction amplitude was compared between each condition.

Phase III Clinical Trial

The current results are from a post-hoc efficacy analysis of a phase III, double-blind, parallel-group, placebo-controlled trial that randomized 805 IBS-C patients to placebo or 290 μg oral linaclotide once daily for a 26-week treatment period. The current efficacy analysis are based on a responder end point for abdominal pain, specified as part of a co-primary end point recommended in the May 2012 US Food and Drug Administration final guidance for industry on the clinical evaluation of products for IBS,[28] defined as a ≥30% improvement from