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LINACLOTIDE – A SECRETAGOGUE AND ANTI-HYPERALGESIC AGENT – WHAT NEXT?

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Abstract

Ongoing clinical trials suggest that linaclotide, a first in class, 14-amino acid peptide guanylate cyclase-C (GC-C) receptor agonist and intestinal secretagogue is an effective treatment for chronic constipation. A study in this issue of the Journal suggests that linaclotide also has anti-hyperalgesic effects in three common rat models of inflammation- and stress-induced hypersensitivity (i.e., acute TNBS colitis, water-avoidance stress, and restraint-induced stress) but not in naïve animals. In mice, linaclotide at least partly reduces hyperalgesia via GC-C receptors. Dose-effect relationships of linaclotide were complicated and non-linear. This viewpoint discusses human clinical trials with linaclotide and the results of this study. Potential mechanisms and clinical significance of these findings are explored. Collectively, these data suggest that GC-C receptors exert other, as yet poorly understood, effects on gastrointestinal sensitivity in conditions associated with inflammation and/or stress-induced increased intestinal permeability. However the data need to be confirmed in humans and in long-term animal models. Further studies are also necessary to elucidate the mechanisms since these effects cannot be explained by linaclotide's known effects on epithelial GC-C receptors.

Introduction

Linaclotide, which is a first-in-class, 14-amino acid peptide, is a guanylate cyclase-C (GC-C) receptor agonist and intestinal secretagogue that improves bowel symptoms and accelerates colonic transit in chronic constipation. (1) In this issue of the Journal, a study by Eutamene and colleagues suggests that linaclotide also attenuates nociceptive reflexes in response to colonic distention in 3 rodent models of visceral hypersensitivity. (2) These findings are exciting since visceral hypersensitivity may contribute to symptoms, particularly abdominal pain and bloating in functional bowel disorders. (3) This viewpoint will review the clinical trials of linaclotide in chronic constipation, discuss the observations in the accompanying paper, and explore their mechanisms and clinical relevance.

Guanylin peptide family

The guanylin peptide family has 4 members, i.e., guanylin, uroguanylin, lymphoguanylin, and renoguanlyin, which appeared early in evolution since they are found in mammals, birds, and fish. (4) The intestine is the main source of guanylin and uroguanylin, which are primarily produced by intestinal goblet cells and enteroendocrine cells. These peptides, the

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bacterial heat stable enterotoxins (ST peptides) of *E. coli* which cause travelers' diarrhea, and linaclotide, increase intestinal secretion via an action at the GC-C receptor. Activation of the GC-C receptor increases cyclic guanosine monophosphate (cGMP), thereby inducing signaling pathways which stimulate chloride and bicarbonate secretion through CFTR chloride channel-dependent mechanisms and, to a lesser extent, CFTR channel-independent mechanisms, (5) and inhibit luminal sodium absorption by a sodium proton exchanger. (6) Guanylin peptides prevent postprandial hypernatremia and hypervolemia after a salty meal by increasing not only intestinal, but also renal secretion. Their effects partly explain why there is a more prominent natriuretic response to oral than intravenous salt administration. This natriuresis is decreased in uroguanylin deficient mice, but preserved in mice lacking GC-C, suggesting that there are GC-C-independent signaling pathways for uroguanylin, at least in the kidney.

Effects of linaclotide in functional bowel disorders

In the first study of 36 patients with constipation-predominant irritable bowel syndrome (IBS), a 5-day course of linaclotide dose-dependently improved bowel symptoms and accelerated colonic transit by a magnitude comparable to other agents, including prucalopride and tegaserod. (1) The beneficial effects on symptoms were confirmed in a phase 2a trial of 42 patients treated for 2 weeks (7) and a phase 2b trial of 310 patients with chronic constipation, randomized to placebo or one of 4 doses (75, 150, 300, or 600 μg) for 4 weeks. (8) Both studies had identical and standard entry criteria (i.e., < 3 spontaneous bowel movements [SBM] per week) with at least one other symptom (i.e., straining, lumpy or hard stools, or a sense of incomplete evacuation for more than 25% of the time), for at least 12 weeks during the 12 months preceding the study. In the phase 2b study, responder rates, (i.e., ≥ 3 SBM per week and an increase ≥ 1 SBM relative to baseline for 3 of 4 treatment weeks) were similar and ranged from 55.4% to 67.7% for all 4 doses compared to 32.4% for placebo. Press releases reporting limited results from two pivotal phase 3 trials of 12 week duration with 633 and 643 patients who had chronic constipation confirm these benefits. (9) For example, in the larger study, overall responder rates were 21.2% (133 μg dose), 19.4% (266 μg dose) and 3.3% (placebo). In these trials, responders had improved symptoms for 9 of 12 weeks. Linaclotide also improved other symptoms including abdominal bloating and discomfort in these patients.

Similar to another secretagogue, lubiprostone, (10) the secretory effects of linaclotide provided the rationale for these therapeutic trials. However, it is conceivable that linaclotide and the GC-C receptor alter gastrointestinal sensorimotor functions via other, as yet poorly understood mechanisms, as suggested in the study by Eutamene *et al.* (2)

Effects of linaclotide on colonic sensitivity in rats

Eutamene *et al.* observed that linaclotide has anti-hyperalgesic effects during colonic distention in rat models. A major strength of this study is that these anti-hyperalgesic effects were documented in three traditional, albeit mechanistically different, forms of visceral hyperalgesia, i.e., rats with TNBS colitis and after acute restraint stress and water avoidance stress (WAS). However, colonic sensitivity and the effects of linaclotide varied among models. For example, in the acute TNBS colitis model (i.e., 4 days after TNBS was administered), hypersensitivity was only present at low distending pressures. Conversely, rats that were exposed to a single episode of water-avoidance stress (WAS) were hypersensitive to high distension pressures only, while rats that underwent acute restraint stress were hypersensitive throughout the range of distending pressures. In all these models, linaclotide reversed the model-specific hypersensitivity but not responses in the normal range. In contrast, linaclotide did not affect the response to colorectal distension in untreated

rats, suggesting that it acts as an anti-hyperalgesic rather than an anti-nociceptive agent. Since sensitivity was evaluated during acute TNBS colitis, a logical extension of these findings would be to determine if hypersensitivity persists in the post-inflammatory state, which is accompanied by altered enteric neural signaling (11) and extrinsic afferent mechanosensitivity. (12) Similarly, the “chronic” WAS model (i.e., WAS for 10 days) more closely approximates IBS since visceral hyperalgesia persists for more than 40 days thereafter. (13)

Linaclotide had dose-dependent effects on colonic sensitivity, but the dose-effect relationship is complicated and non-linear. In both rats and mice, the lowest dose of linaclotide used (0.01 μg/kg) reduced TNBS-induced hypersensitivity. In rats with TNBS colitis, 0.01, 0.03, and 0.3 μg/kg had similar effects while 3 and 30 μg/kg did not have anti-hyperalgesic effects. After WAS in rats, 3 μg/kg reduced hypersensitivity at all pressures, 0.3 μg/kg only reduced hypersensitivity to the highest distending pressure, and 10 μg/kg significantly *increased* stress-induced hypersensitivity. In the restraint stress model, 3 μg/kg reduced colonic hypersensitivity while 0.3 and 30 μg/kg were ineffective. Together, these observations suggest multiple mechanisms of action and potentially nonspecific, poorly understood effects. Linaclotide did not significantly affect colonic pressure-volume relationships suggesting that its effects on colonic sensitivity were not mediated by altered tone. In focusing on the single known action of linaclotide, the authors suggest that increasing doses may increase intracellular cGMP concentrations to levels where it might activate not only cGMP but also cAMP-dependent pathways. Alternatively, linaclotide may act at multiple sites.

The use of GC-C/GC-C^{null} mice in this study provides interesting insights into the action of linaclotide and the role of the GC-C receptor. First, colonic sensitivity was *lower* in GC-C^{null} mutant mice than wildtype mice at baseline, suggesting that GC-C receptors normally contribute to mechanosensitivity in mice. GC-C^{null} mutant mice have normal natriuretic responses but not increased secretion or diarrhea in response to enterotoxic ST peptides. (14,15) This suggests that fluid-ion homeostasis is unlikely to contribute to altered visceral sensitivity. GC-C^{null} mutant mice have enhanced epithelial proliferation, (16) which likely alters the mucosal architecture and may contribute to the observed reduction in basal sensitivity. Second, TNBS augmented colonic sensitivity in both wild type and GC-C^{null} mutant mice suggesting that the GC-C receptor does not contribute to mechanisms of hypersensitivity. Therefore, it is somewhat counterintuitive that while GC-C receptors do not contribute to TNBS-induced hypersensitivity, a GC-C agonist reduces colonic sensitivity. In our current conceptual framework, linaclotide is neither an anti-hyperalgesic agent, i.e., one that targets the molecular mechanisms responsible for enhanced sensitivity nor an anti-nociceptive agent (e.g., opiates), which targets physiologically competing processes to reduce both basal and enhanced sensitivity. Perhaps another receptor might explain the effects of linaclotide. Indeed, natriuretic responses to guanylin and uroguanylin sodium excretion in wildtype and GC-C^{null} mutant mice are similar (17) and low affinity receptors for the enterotoxin ST peptides remain in GC-C^{null} mutant mice, (14) suggesting that other unidentified receptors for these peptides exist. As the causative mechanisms are likely different between inflammation- and stress-induced hypersensitivity, it would also be of considerable interest to learn whether linaclotide ameliorates stress-induced hypersensitivity in GC-C^{null} mutant mice. While the mechanisms that contribute to TNBS-induced hypersensitivity are numerous and not well understood, (12,18–20) TRPV1, endocannabinoid (CB_1), neurokinin-1, corticotrophin-releasing factor-1 (CRF1) and vasopressin receptors have been implicated in explanations of visceral hypersensitivity in the WAS model. (21–23)

Further questions

These exciting data raise several further questions. Since linaclotide reduced colonic sensitivity in diseased but not naïve rats, the first step is to evaluate the effects of linaclotide on colonic and rectal perception in health and disease using established techniques. (24,25) These studies are particularly important since not all agents (e.g., CRF1 antagonists) with gastrointestinal sensorimotor effects in animal models have effects in humans. (26)

Perhaps the most important mechanistic question is to identify the location of the GC-C receptors mediating the anti-hyperalgesic effects of linaclotide. In the non-inflamed gut, linaclotide has very limited bioavailability, i.e., less than 0.1% in rats and not quantifiable in humans. (1) Since GC-C receptors are expressed on the apical surface of enterocytes but not the neuromuscular apparatus, its effects in humans with normal intestinal permeability are attributed to increased secretion alone. The bioavailablity of orally administered peptides is generally low because gastrointestinal proteases metabolize proteins, including 14-amino acid peptides, into absorbable amino acids. (27) That said, linaclotide has biological effects, perhaps because it is protected by a proprietary delivery system. In rodent models of hypersensitivity, which are associated with a leaky intestinal barrier, it is conceivable that a 14-amino acid peptide, which survives degradation could cross into the systemic circulation. Perhaps, this is more likely at higher doses and explains the paradoxical effects on colonic sensitivity at the highest dose (10 μg/kg). Although not documented in other mammals, there is evidence that GC-C receptors exist in nervous tissue of the North American opossum. (28) Thus, it is possible that systemic linaclotide can increase cGMP levels in neurons of pain pathways, which have known anti-nociceptive effects. (29,30)

Alternatively, linaclotide's effects on visceral hypersensitivity may be secondary to its pro-secretory effects since epithelial secretion plays an important role in maintaining the epithelial barrier. However, not all secretagogues have anti-nociceptive effects. In that case, what signaling mechanisms of GC-C receptors might mediate this effect? Might linaclotide activate a kinase domain of unknown function in GC-C receptors (31) and what might be its cellular effects? Cyclic GMP works via cGMP-dependent protein kinase (PKG), cyclic nucleotide gated (CNG) ion channels or cyclic nucleotide phosphodiesterases (PDE). Calcium is another potential signaling mechanism since GC-C receptors enhance intestinal epithelial proliferation by trafficking calcium sensing receptors (CaRs) to the surface of enterocytes and calcium chelaters or (CNG) ion channel blockers block the proliferative effects of the enterotoxin ST peptides. (32) Yet another possibility is that linaclotide may have other potential effects not known for guanylin, uroguanylin or enterotoxin ST peptides.

Conclusions

Linaclotide is a promising agent for treating chronic constipation. Thus far, its effects have been attributed to increased intestinal secretion. However, this new exciting study that suggests that linaclotide also reduces visceral hypersensitivity in animal models. Like most good studies, Eutamene and colleagues have opened the door to several questions, which can be evaluated in human and animal studies. To paraphrase Robert Frost, we have miles to go before we sleep.

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