The Uroguanylin Analog Plecanatide Activates Guanylate Cyclase C Predominantly in the Lumen of the Proximal Small Intestine to Stimulate Chloride and Fluid Secretion and Increase GI Transit in Animal Models

Su1531

Apoorva Joshi,[1] Viren Patwa,[1] Anusha Thadi,[1] John A Foss,[2] Stephen J. Comiskey,[2] Vaseem A. Palejwala,[2] Kunwar Shailubhai[2]

[1]Baruch S. Blumberg Institute, Doylestown, PA, USA; [2]Synergy Pharmaceuticals Inc., Doylestown, PA, USA

Background

- Plecanatide is a guanylate cyclase-C (GC-C) agonist and the first uroguanylin analog clinically tested for the treatment of chronic idiopathic constipation (CIC)[1] and irritable bowel syndrome with constipation (IBS-C).[2] It was recently approved in the United States for the treatment of adults with CIC.
- The primary structure of plecanatide is identical to uroguanylin, an endogenous GI peptide expressed predominantly in the small intestine (the site of normal physiological fluid secretion), with the exception of a single amino acid substitution (Asp^3 with Glu^3 at the N-terminus); thus plecanatide is expected to replicate the activity of uroguanylin.[3]
- Plecanatide activates GC-C receptors in a pH-sensitive manner (slightly acidic pH),[4] stimulating cyclic guanosine monophosphate (cGMP) production to maintain fluid and ion homeostasis in the GI tract.[5]
  - Disruption of fluid and electrolyte homeostasis can result in constipation or diarrhea.
- Stimulation of cGMP production results in increased cystic fibrosis transmembrane conductance regulator (CFTR) activity,[6] resulting in Cl^- and HCO_3^- secretion, inhibition of the Na^+/H^+ exchanger, and passive secretion of fluid into the intestinal lumen to promote bowel movement.[7-11]
- Enhancement of fluid secretion in the intestinal lumen through activation of GC-C signaling is emerging as an attractive approach for treatment of CIC and IBS-C.

Objective

To determine the intestinal sites of action of orally administered plecanatide that results in fluid, cGMP, and electrolyte secretion necessary to facilitate bowel movements

Methods

Animal Care

All procedures for rat studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Charles River Laboratories. Female CD rats aged ~6–7 weeks were obtained and allowed to acclimate for 3–4 days. Charles River Laboratories performed all procedures for the monkey studies.

GI Transit in Rats

Rats were administered an oral gavage of vehicle or plecanatide followed immediately with an oral gavage of 2 mL charcoal meal (n=9–10/treatment group). After 10 minutes, the animals were euthanized, and intestinal transit was assessed by calculating the ratio of the distance between the pyloric sphincter and the leading edge of the charcoal to the distance between the pyloric sphincter and the ileocecal junction.

Fluid Secretion in Ligated Intestinal Loops in Rats

Animals were anesthetized and the duodenum, jejunum, and ileum were ligated to form ~3-cm loops. Subsequently, 0.1 mL of 10 mM phosphate buffer pH 6.0 (vehicle) or vehicle containing 10 µg of plecanatide was injected into the lumen of each loop, the abdominal wall was closed with surgical staples, and animals were placed in a Plexiglas container over a heating pad (n=4–9/segment/treatment). After 30 minutes, animals were euthanized, and the intestinal fluid from each loop (n=4–5/segment) and corresponding loop tissues (n=3/segment) were recovered and levels of cGMP were measured in triplicate using a commercial ELISA kit.

Measurement of cGMP Obtained From Ligated Rat Intestinal Tissues

Total protein from each loop tissue was homogenized in RIPA buffer containing proteinase and phosphatase inhibitors and then incubated on ice for 45 minutes with intermittent mixing followed by centrifugation at 10,000 x g for 45 minutes at 4°C. The supernatant was transferred to a clean tube and protein was estimated using Pierce BCA protein estimation kit. cGMP levels in each supernatant were determined in triplicate using a commercial ELISA kit.

Electrophysiological Studies of Short Circuit Current (*Isc*)

Tissues (harvested from duodenum, jejunum, ileum, or colon) were mounted on an EasyMount Ussing chamber with the apical surface facing upwards (n=5–9 for small intestine segment; n=2–8 for colon); *Isc* measurements were performed with VCCMC8 multichannel current-voltage (I-V) clamp, and data were collected using Acquire & Analyze software.

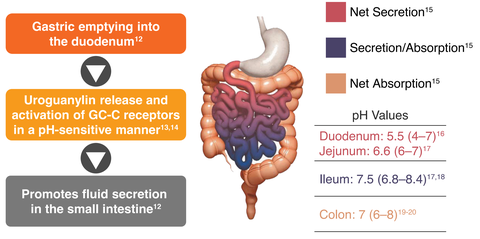
To examine the effect of plecanatide (0.1 µM) on the stimulation of *Isc*, a basolateral to apical chloride gradient was imposed by substituting NaCl in the buffer in contact with the apical membrane with an equal concentration of sodium gluconate and $CaCl_2$.

Statistical Analysis

Data summaries are expressed as mean ± SEM and statistical significance was determined by comparing indicated data sets using a 2-tailed unpaired parametric t-test.

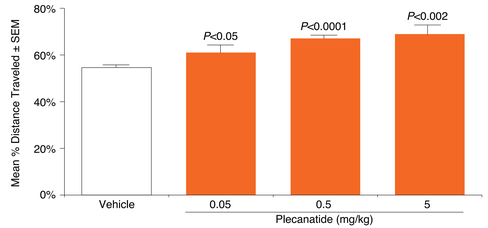
Results

Figure 1. The Intestines Maintain a Compartmentalized Structure



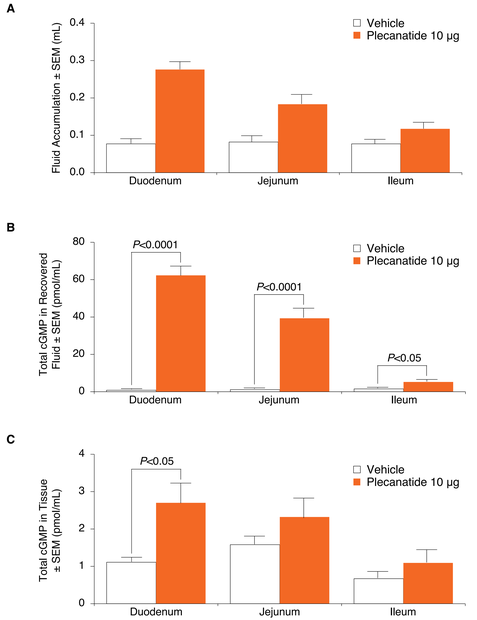
- The intestines can be divided into areas of net secretion (ie, duodenum and jejunum), net absorption (ie, colon), or mixed (ie, ileum)
- A pH gradient exists along the length of the intestinal tract
  - The pH is lowest in the duodenum and gradually increases towards the colon
  - As the pH of the intestinal tract becomes more basic (ie, ileum, colon), uroguanylin activity is decreased[14,19-20]

Figure 2. Oral Administration of Plecanatide Increases GI Transit in Rats



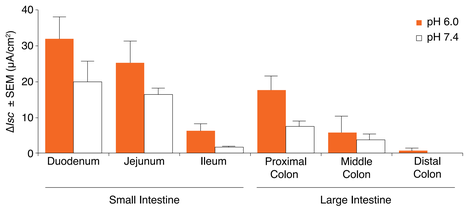
- Plecanatide significantly increased intestinal GI transit (increases of 11.9%, 23.1%, and 24.7% for 0.05 mg/kg, 0.5 mg/kg, and 5 mg/kg, respectively) compared with vehicle.

Figure 3. Plecanatide Administration Stimulates Fluid Secretion and cGMP Production in Rat Intestinal Segments



- Plecanatide stimulated fluid secretion to a greater extent in the duodenum and jejunum compared with the ileum (**Figure 2A**).
- Total cGMP levels in fluid obtained from each compartment were significantly greater compared with vehicle and were highest in the duodenum (**Figure 2B**).
- Total cGMP levels were significantly greater in tissue samples obtained from the duodenum (**Figure 2C**).
- Taken together, these data indicate that plecanatide administration stimulates fluid secretion and significantly increases secretion of cGMP in the proximal small intestine, with a gradation of secretion when moving distally through the small intestine.

Figure 4. Plecanatide Stimulates a Short Circuit Current (*Isc*) Primarily Across Tissues From the Small Intestine



- Plecanatide stimulated *Isc* to a greater extent in the duodenum, jejunum, and proximal colon segments compared with the ileum and more distal segments of the colon.
- Data indicate that plecanatide has maximal activity in the duodenum and jejunum, and to a lesser extent, demonstrates activity in the proximal colon.

Conclusions

- **These studies indicate that orally administered plecanatide acts locally in the lumen of the proximal small intestine, stimulating cGMP secretion, electrolyte movement, and fluid secretion to promote bowel movements. This effect is seen to a greater degree in the duodenum and jejunum and is present, to a lesser extent, in the ileum.**
- **The short circuit experiments indicate that plecanatide has activity in the proximal small intestine but also, to a lesser degree, in the proximal large intestine and that this activity is pH-sensitive (more active at lower pH).**

References

1. Miner PB Jr, Koltun WD, Wiener GJ, et al. *Am J Gastroenterol.* 2017;112:613–621.
2. Fogel R, Dorn S, Krause R, et al. Oral presentation at: Digestive Disease Week (DDW) 2017. Chicago, IL; May 6–9, 2017.
3. Shailubhai K, Palejwala V, Arjunan KP, et al. *World J Gastrointest Pharmacol Ther.* 2015;6:213–222.
4. Harms FK, Eber SL, Chin DT, et al. *Proc Natl Acad Sci U S A.* 1997;94:2705–2710.
5. Forte LR Jr. *Pharmacol Ther.* 2004;104:137–162.
6. Vaandrager AB, Smolenski A, Tilly BC, et al. *Proc Natl Acad Sci U S A.* 1998;95:1466–1471.
7. Basu N, Arshad N, Visweswariah SS. *Mol Cell Biochem.* 2010;334:67–80.
8. Forte LR, Freeman RH, Krause WJ, London RM. *Braz J Med Biol Res.* 1999;32:1329–1336.
9. Shailubhai K. *Curr Opin Drug Discov Devel.* 2002;5:261–268.
10. Sindic A. *ISRN Nephrol.* 2013;2013:813648.
11. Toriano R, Ozu M, Politi MT, et al. *Cell Physiol Biochem.* 2011;28:733–742.
12. Arshad N, Visweswariah SS. *FEBS Lett.* 2012;586:2835–2840.
13. Harms FK, Forte LR, Eber SL, et al. *Proc Natl Acad Sci U S A.* 1993;90:10464–10468.
14. Patwa V, Joshi A, Thadi A, et al. [Poster Abstract 967]. Presented at: Digestive Disease Week Meeting; May 21-24, 2016; San Diego, CA.
15. Seither J, Ratner A, Sloane D. *Concepts in Medical Physiology.* 1st ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2005:427–451.
16. Rune SJ, Viskum K. *Gut.* 1969;10:569–571.
17. Evans DF, Pye G, Bramley R, et al. *Gut.* 1988;29:1035–1041.
18. Bown RL, Gibson JA, Sladen GE, et al. *Gut.* 1974;15:999–1004.
19. Karari TT. *Biopharm Drug Dispos.* 1995;16:351–380.
20. McDougall CJ, Wong R, Scudera P, et al. *Dig Dis Sci.* 1993;38:542–545.

Disclosures

Foss JA, Comiskey SJ, and Palejwala VA are employees and stockholder of Synergy Pharmaceuticals. Shailubhai K is an employee of Synergy Pharmaceuticals and one of the inventors of the drug plecanatide. Joshi A, Patwa V, and Thadi A have no conflicts of interest to declare.