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Plecanatide, a superior analog of uroguanylin, as an oral drug candidate for treatment of gastrointestinal functional disorders and diseases

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BACKGOUND

Agonists of guanylate cyclase-C (GC-C) are emerging as a new class of drug candidates for treatment of gastrointestinal (GI) disorders and diseases. Uroguanylin (UG) and guanylin (GN) are physiological natriuretic peptides that bind and activate GC-C receptors expressed on the epithelial cells lining the GI mucosa, leading to production of cyclic guanosine monophosphate and promoting electrolyte and water secretion needed for normal bowel movements. While UG cooperatively regulate GC-C receptors in a pH-dependent physiological mechanism to regulate water secretion, the *E. coli* enterotoxin ST peptide activates GC-C in an uncontrolled and pH-independent manner, resulting in excessive fluid secretion to cause Traveler's diarrhea. Spatial confirmation of UG assumes several distinct topoisomers in aqueous solution and only one of those are biologically active. Consequently, large scale manufacturing and purification of UG becomes difficult and cost prohibitive. Thus, functional homolog of UG may have the advantage as drugs for normalizing bowel movements with less likelihood of causing severe diarrhea. **Objective of this study was to identify an analog of UG with superior physiochemical properties for the treatments of chronic constipation (CC) and irritable bowel syndrome-constipation (IBS-c).**

Fig 1 Primary structures of plecanatide, uroguanylin and other agonists of GC-C receptors

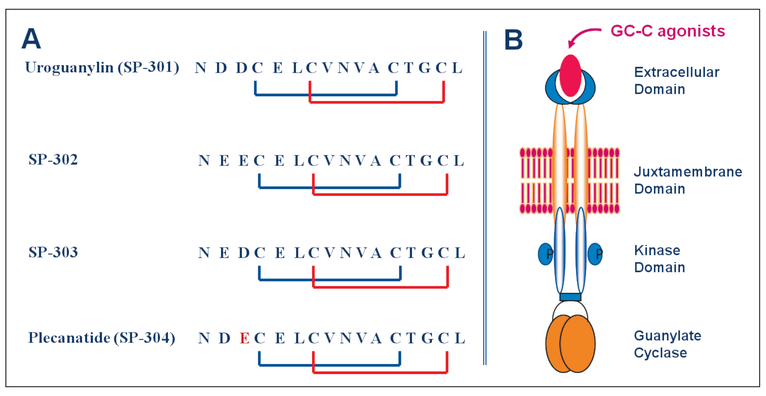
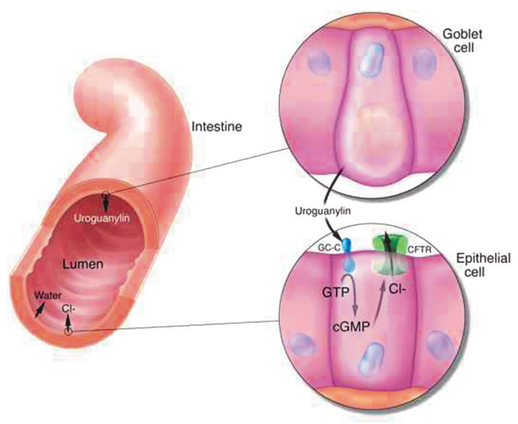


Fig 2 Plecanatide and uroguanylin stimulate water secretion in the lumen of the proximal intestine to normalize bowel movement



An optimal volume of water secretion into the intestinal lumen is essential for normal bowel movement.

METHODS

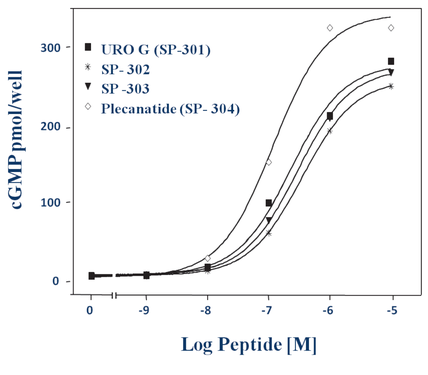
Strategies used to design new analogs: Number of strategies such as thermal bond energy calculations, 3-D structure modeling, structural activity relationships and molecular dynamics were utilized to design new analogs of UG. New analogs were selected based on their potencies to stimulate cGMP synthesis and affinities to bind GC-C receptors expressed on T84 cells. Plecanatide (SP-304), being the most potent analog, was further evaluated for its stability against digestion in simulated gastric and intestinal fluids.

Molecular Dynamics studies: Simulation experiments were performed using the GROMACS 4.5 package on an isothermal-isobaric (NpT) ensemble, keeping the temperature at 300K and pressure at 1 bar; the all-atom OPLS force field was used for peptide structures. Analysis of all the trajectories obtained were performed with the VMD package and distance graphs were analyzed with Grace software

Animal studies: Six male and 6 female CD-1 mice were administered plecanatide, daily by oral gavage for 5 days. Thirty minutes following the last dose, mice were sacrificed and their intestinal tracts were examined for fluid distention. In formulation studies, female monkeys were dosed with plecanatide (11 mg/kg/day) either as uncoated capsules or enteric-coated (pH 7) to bypass the proximal intestine. Fecal consistency and quantity were recorded.

RESULTS

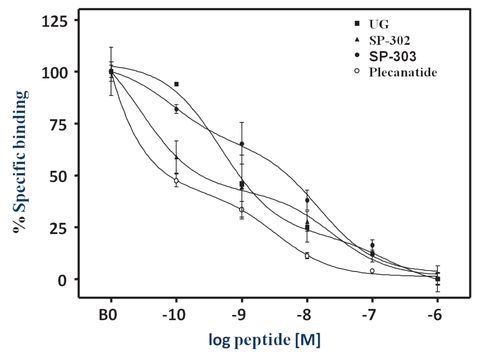
Fig 3 Stimulation of cyclic GMP in T84 cells by plecanatide uroguanylin and other GC-C agonists



Plecanatide was found to be the most potent analog of UG to stimulate cyclic GMP synthesis in T84 cells

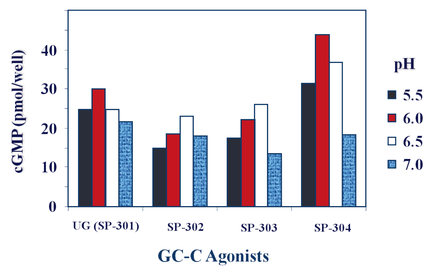
RESULTS

Fig 4 Plecanatide specifically binds to GC-C receptors expressed on T84 cells



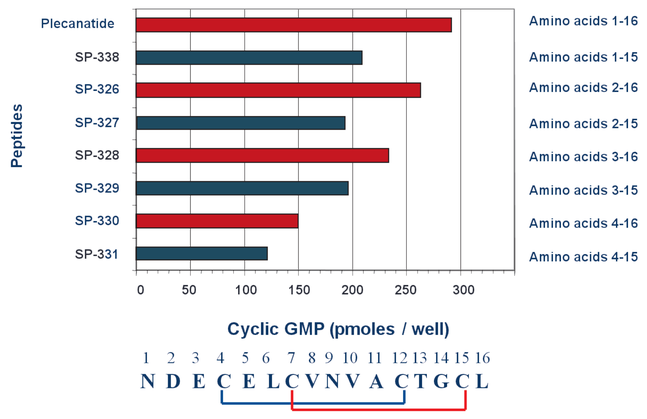
T84 cells appear to express heterogeneous population of 'high-affinity' and 'low-affinity' receptors. Plecanatide exhibits higher affinity for both types of GC-C receptors than UG

Fig 5 Activity of plecanatide, like UG, to stimulate cGMP synthesis is also modulated by acidity



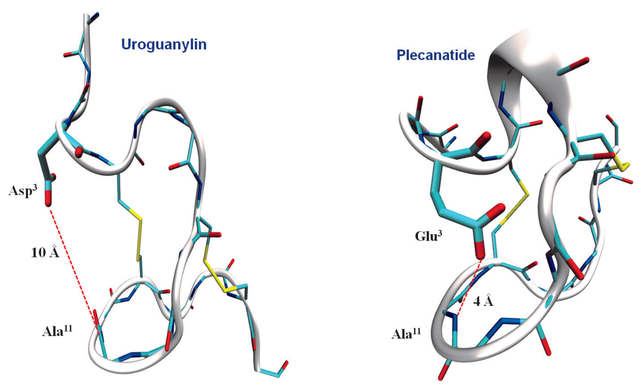
Like UG, activity of plecanatide might also be regulated by the mucosal acidity of the GI tract

Fig. 6 Potencies of truncated analogs of plecanatide for stimulate cGMP synthesis in T84 cells



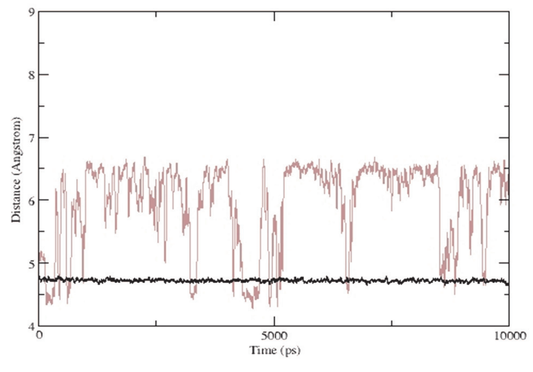
First three amino acids (Asn-Asp-Glu) at the N-terminal and Leu at the C-terminal of plecanatide are crucial for its activity and stability

Fig 7a Molecular dynamics analyses of plecanatide and uroguanylin



The distance between Asp[3] and Ala[11] is larger (10Å) in uroguanylin as compared to that in plecanatide (4Å), suggesting that the a-helical structure of plecanatide is more rigid and stable

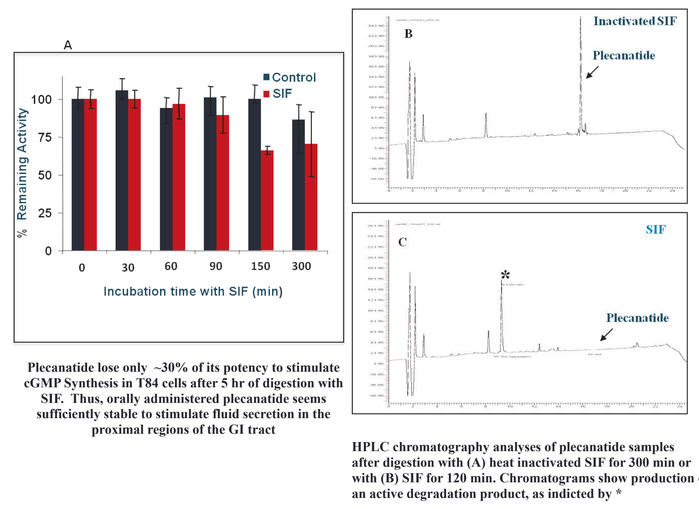
Fig 7b Molecular dynamics analyses of plecanatide and uroguanylin



From the distance plot analysis, it is possible to observe how the carboxylic acid of ASP3 in uroguanylin is very flexible and during the dynamics it often moves away form the interaction loop (e.g. ALA11). However, GLU3 of plecanatide remains closely associated with the loop trough a network of hydrogen bonds for the entire duration of the simulation. This enhanced rigidity provide a structural justification for the increased topological stability of plecanatide vs uroguanylin

RESULTS

Fig 8 Incubation of plecanatide in simulated intestinal fluid (SIF) produces biologically active degradation products



Plecanatide lose only ~30% of its potency to stimulate cGMP Synthesis in T84 cells after 5 hr of digestion with SIF. Thus, orally administered plecanatide seems sufficiently stable to stimulate fluid secretion in the proximal regions of the GI tract

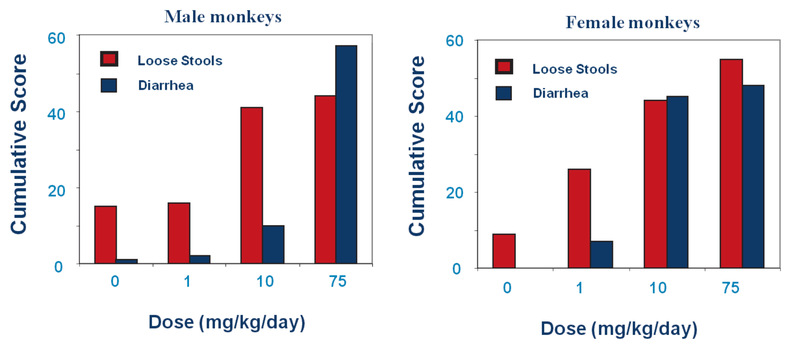
HPLC chromatography analyses of plecanatide samples after digestion with (A) heat inactivated SIF for 300 min or with (B) SIF for 120 min. Chromatograms show production of an active degradation product, as indicted by *

Table 1 Oral dose of plecanatide produced fluid distension only in the proximal intestine

GI Segments	Fluid Distension in Mice	
	Males (%)	Females (%)
Stomach	50	33
Duodenum	33	33
Jejunum	100	100
Ileum	0	0
Cecum	0	0
Colon	0	0

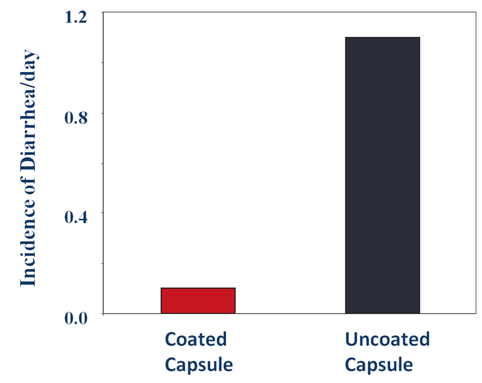
Data suggest that the site of action of plecanatide is likely to be in the proximal region of the gastrointestinal tract

Fig 9 Oral administration with plecanatide increased stool consistency in monkeys



Repeated oral doses of plecanatide increased stool consistency in monkeys, suggesting that the drug is pharmacologically active

Fig 10 The pharmacological activity of plecanatide is reduced when it is formulated for delivery bypassing the proximal intestine



Data clearly demonstrate that orally administered plecanatide acts in the proximal intestine to stimulate fluid secretion to normalize bowel movement

CONCLUSIONS

- **Plecanatide is by far the most potent and stable analog of uroguanylin to stimulate cGMP synthesis in T84 cells**
- **Like uroguanylin, the activity of plecanatide to stimulate cGMP synthesis is modulated by pH. First three amino acids (NDE) at the N-terminal are important for this pH-mediated regulation**
- **Molecular dynamics studies suggest that the side chain of Glu at 3rd position strongly interacts with the residues in the interaction loop, imparting higher stability. Thus, substitution of Asp3 in uroguanylin with Glu3 as in plecanatide minimizes topoisomer formation.**
- **Oral administered plecanatide acts primarily in the proximal intestine to stimulate water secretion needed to improve stool consistency in monkeys**
- **Plecanatide is currently being evaluated in Phase II/III clinical trials in CC patients. Phase II clinical trial with plecanatide in IBS-c patients will start shortly**