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Guanylate Cyclase-C Agonists as a New Class of Drug Candidates to Delay Progression of Colitis to Colonic Tumors in Apc$^{Min/+}$Mice

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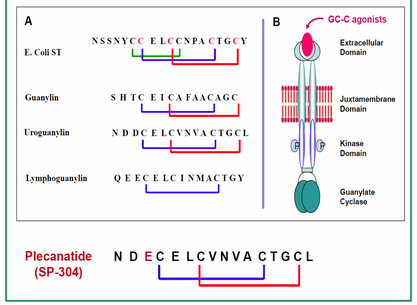
Background

ABSTRACT

Purpose: Uroguanylin (UG) is a peptide hormone produced in the GI tract lumen where it binds to Guanylate Cyclase C (GC-C), which results in enhanced production of cyclic guanosine monophosphate (cGMP) and in activation of cystic fibrosis transmembrane conductance regulator (CFTR). Earlier we reported that UG expression is suppressed in human colon tumors. Oral treatment with UG inhibited not only the formation of adenomas but also their progression to colon tumors in Apc$^{Min/+}$ mice. Plecanatide, a highly potent analog of UG, ameliorated GI inflammation in the DSS- and TNBS-induced colitis models via downregulation of pro-inflammatory cytokines. Here we report that GC-C agonists inhibit formation of inflammation-induced colonic tumors in Apc$^{Min/+}$ mice.

Results: Oral treatment with plecanatide (2.5 mg/kg) reduced the multiplicity of tumors within the colon. Expression of UG was reduced following DSS treatment but it was partially restored following treatment with plecanatide. The treatment increased expression of protein kinase G-II (PKG-II), decreased levels of Ki-67 and of cytoplasmic β-catenin. As expected, treatment with plecanatide also induced apoptosis in colonic epithelial cells. In explant cultures, plecanatide reduced production of the pro-inflammatory cytokines, such as IL-6, IL-17 & TNF-α.

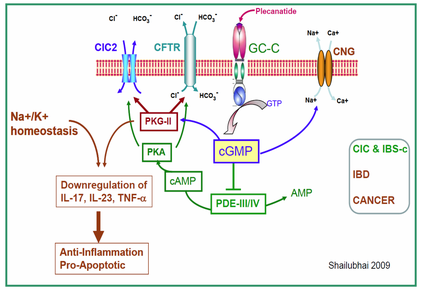
Structures of the known mammalian GC-C ligands



Objectives

Patients with both ulcerative colitis (UC) and Crohn's disease (CD) are at an increased risk of developing colorectal cancer (CRC). This increased risk might be due the persistent inflammation of the colon. However, the precise mechanism as to how chronic inflammation results in carcinogenesis is unclear, but it is possible that the same genetic mutations that result in sporadic CRC are also responsible for its development in inflammatory bowel disease (IBD). **Our continued research efforts to explore GC-C agonists as drugs may increase our arsenal in attempting to reduce the incidence of IBD-associated CRC.**

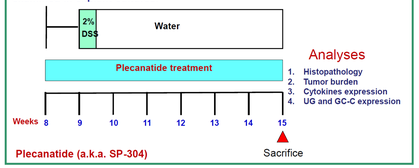
Mechanism of Action



Methods

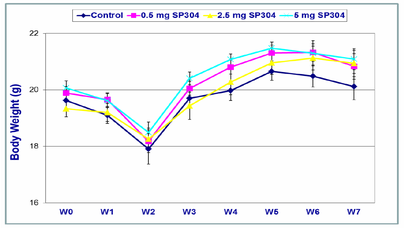
Experimental Design

Apc $^{Min/+}$ mice (8 wks of age) were randomized to receive powdered diet or the same diet supplemented with plecanatide. At 9 weeks of age, animals were administered 2% DSS in the drinking water for 4 days and untreated water for the remainder of the study. Mice were euthanized at 15 wks of age. The number of tumors per animal was counted for each segment of the colon. The level of UG, Ki-67 (cell proliferation) and active caspase-3 (apoptosis) were measured. Expression of β-catenin and PKG-II were measured by immunoblotting. The effect of plecanatide on the expression of inflammatory cytokines was examined in explant cultures.

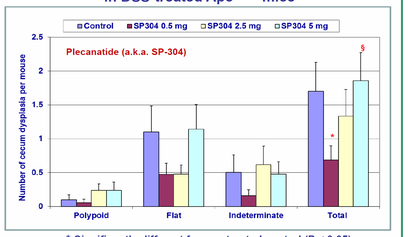


Results

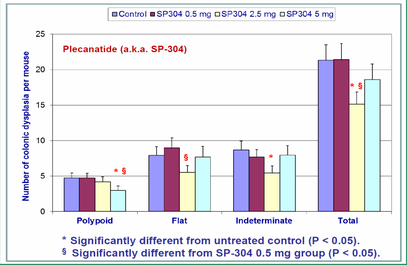
Effect of plecanatide treatment on the body weight of mice treated with DSS



Effect of plecanatide on the multiplicity of cecum dysplasia in DSS-treated Apc$^{min/+}$ mice

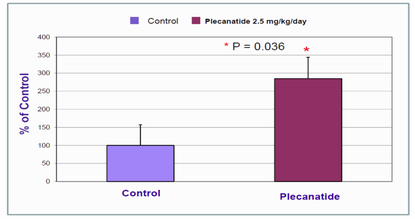


Effect of plecanatide on the multiplicity of colonic dysplasias in DSS-treated Apc$^{min/+}$ mice

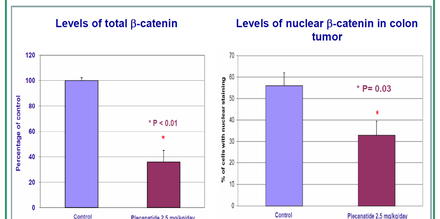


Results

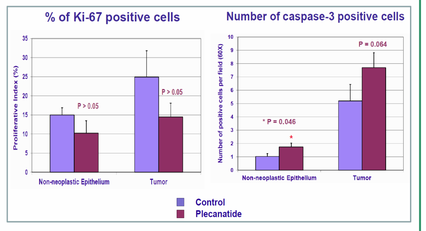
Effect of plecanatide on activation of PKG-II in colon tissues



Oral treatment with plecanatide reduces β-catenin levels in colon

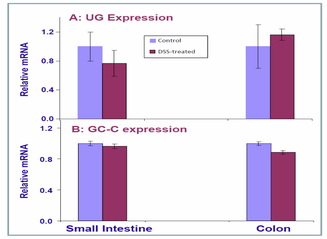


Effect of treatment with plecanatide on levels of Ki-67 and caspase-3 in the colon tissue

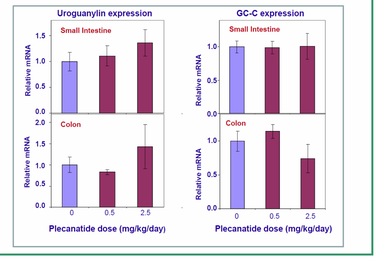


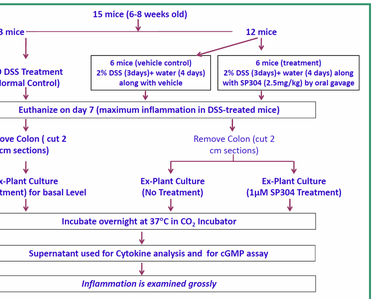
Results

Expression of UG is reduced in small intestine following DSS-induced inflammation

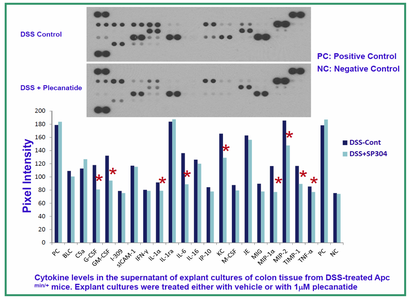


Treatment with plecanatide partially restored expression of UG, which is reduced following DSS-induced inflammation



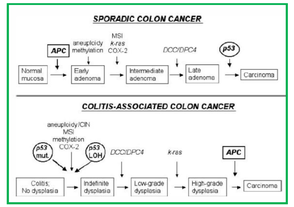


Results



Cytokine levels in the supernatant of explant cultures of colon tissue from DSS-treated Apc$^{min/+}$ mice. Explant cultures were treated either with vehicle or with 1μM plecanatide

Molecular pathogenesis of sporadic colon cancer and colitis-induced colon cancer



COX-2, cyclooxygenase-2; CIN, chromosomal instability; MSI, microsatellite instability; mut, mutation; LOH, loss of heterozygosity; DCC, deleted in colon cancer; DPC, deleted in pancreatic cancer; APC, adenomatous polyposis coli

(Source Reference: Itzkowitz and Yio (2004), Am. J. Physiol. Gastrointest Liver Physiol. 287:G7-G17.

Conclusions

- Oral treatment with plecanatide inhibits formation of inflammation-induced colon tumors in Apc$^{min/+}$ mice.
- Treatment with plecanatide not only ameliorated GI inflammation but also reduced the multiplicity of tumors in the colon of Apc$^{min/+}$ mice.
- Plecanatide activated PKG-II, reduced expression of β-catenin and Ki-67, and induced apoptosis in colonic epithelial cells.
- Our results suggest a novel cGMP-mediated mechanism for the anti-neoplastic activity of GC-C agonists.
- This study opens a new avenue for the development of GC-C agonists as a new class of orally delivered, mucosally active, drug candidates for the treatment of IBD and IBD-associated colorectal cancer.

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