

concentration of OXZ on day 7. OXZ colitis was assessed with the disease activity score (DAS) on weight loss, diarrhea and hemorrhage until day 10. Macroscopic examination of the colitis with colonic damage score (CDS) was performed, and then the colon was used for myeloperoxidase (MPO) activity analysis. **RESULT:** Exposure of the primed mice to intrarectal OXZ challenge developed a colitis marked by weight loss, hemorrhagic diarrhea, ulceration, erosion etc. associated with about 13-fold increased MPO activities in the colon. However, OXZ failed to induce the colitis in C57BL/6 mice which are used for Th1-mediated disease models. In addition, pretreatment with calcineurin inhibitor to suppress Th1 responses deteriorated OXZ colitis. Therefore, oxazolone colitis is Th2-mediated and has similar histologic features to UC. Although oral administration of 5-ASA (100mg/kg) had no significant therapeutic effect on OXZ colitis, prednisolone (10mg/kg p.o.) significantly alleviate the disease state. Notably, the central stimulation of vagus nerves with 2-deoxy-D-glucose (200mg/kg i.p.) significantly ($P<0.05$) improved OXZ colitis [DAS: 5.9 ± 1.8 vs 2.3 ± 0.8 ; CDS: 4.4 ± 1.3 vs 1.5 ± 0.3 ; MPO: 3562.5 ± 1034.2 vs 1399.0 ± 353.4 units/g wwt (OXZ colitis control vs mice pretreated with 2-deoxy-D-glucose)]. Furthermore, subcutaneous administration of nicotine (3.2 mg/kg) significantly ($P<0.05$) suppressed OXZ colitis [DAS: 10.5 ± 1.6 vs 5.1 ± 1.8 ; CDS: 7.6 ± 1.3 vs 3.7 ± 1.3 ; MPO: 5258.6 ± 1466.9 vs 2585.7 ± 234.7 units/g wwt (OXZ colitis control vs mice pretreated with nicotine)]. **CONCLUSION:** Nicotinic acetylcholine receptors work on the cholinergic anti-inflammatory and immune pathway to alleviate Th2-mediated OXZ colitis.

M1696

Sp304, An Analog of Uroguanylin, Ameliorates Inflammation in a Model of Experimental Colitis

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Introduction: Guanylyl cyclase C (GC-C) agonists (uroguanylin (UG) and guanylin), regulate water and ion homeostasis in a variety of tissues and organs, including the gastrointestinal (GI) tract, via cyclic GMP (cGMP). GC-C and its agonists are expressed by intestinal epithelial cells (IEC). The cGMP pathway mediates anti-inflammatory effects of cellular molecules such as nitric oxide and heme oxygenase-1, and therapies that induce cGMP (phosphodiesterase-4 inhibitors) demonstrate efficacy in murine models of IBD. Accordingly, we reasoned that agonists of GC-C, when orally administered may demonstrate anti-inflammatory effects in murine IBD. **Aim:** The present study investigates the immunomodulatory effects of a GC-C agonist, SP304 (Callisto Pharmaceuticals, Inc., NY) in TNBS-induced murine colitis. **Methods:** GC-C, guanylin and UG mRNA expression was studied by RT-PCR in intestinal tissue from 12-week old IL-10 deficient (-/-) mice. To induce colitis, 0.1 ml of 2.5% trinitro benzene sulfonic acid (TNBS) in 50% ethanol was administered via catheter into the colonic lumen of 8 week old female in BALB-c mice (day 0). Mice were administered the GC-C agonist SP304 at 10 (n = 6), 50 µg/day (n = 5) or vehicle PBS for 7 days (n = 8 each) by oral gavage starting at day 0. On day 7, mice were sacrificed. H&E stained colonic tissue sections were assessed for colitis by a pathologist blinded to treatment group. Intestinal explants were cultured for 24 hours and levels of IL-12 p40, IL-12 p70, IL-23 and TNF protein secretion were measured in culture supernatants by ELISA. **Results:** GC-C, guanylin and UG mRNA are expressed in intestinal tissue of IL-10^{-/-} mice. Intestinal guanylin and UG mRNA expression is induced during interventions in IL-10^{-/-} mice that ameliorate colitis. In TNBS-induced colitis, histological assessment of colonic tissue demonstrated significant improvement of the colitis in the SP304-treated mice compared with the vehicle treated mice (Table). Intestinal explant cultures from SP304 treated mice express less IL-12 p40, IL-12 p70, IL-23 and TNF than the vehicle treated control group. **Conclusions:** Histological improvement in IL-10^{-/-} mice correlates with upregulation of UG and guanylin mRNA. Treatment with SP304 exhibited anti-inflammatory effects in TNBS-induced colitis in mice. Importantly, amelioration of colitis was associated with downregulation of proinflammatory cytokines such as TNF.

	PBS (n = 8)	SP304 (10 µg/d) (n = 6)	SP304 (50 µg/d) (n = 5)
Average colitis score	14.13	6.67*	0.8*
SD	4.39	3.27	1.79

* $P<0.05$

M1697

Lansoprazole, a Proton Pump Inhibitor, Suppresses Induction of TNF-α in Experimental Colitis in Rats-New Molecular Implication for Therapy for Intestinal Mucosal Inflammation Beyond Acid Suppression

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Background and Aim: Enteric bacteria play a crucial role in the pathogenesis of inflammatory bowel diseases which are characterized by leukocytic infiltration and overexpression of cytokines in intestinal mucosa. Lansoprazole (LAN), a proton pump inhibitor, has been shown to exert anti-inflammatory effect other than inhibitory effect on acid secretion. We have previously demonstrated that LAN inhibits development of dextran sodium sulfate (DSS)-induced experimental colitis in rats. In this study we investigated molecular mechanisms underlying preventive effect of LAN on colitis. **Methods:** Experiment (1) Experimental colitis was induced in male Wistar rats by administration of 3% DSS solution for 3 days. The rats were also orally given LAN (30 mg/kg BW) or vehicle from day 1 of experiment for 3 days. Colonic tissue was subjected to measurement of myeloperoxidase (MPO) activity (a marker of neutrophil infiltration), assay of mRNA level of tumor necrosis factor-α (TNFα) by real-time RT-PCR, and double-immunostaining with antibodies against TNFα and monocytes/macrophages. To clarify the uptake sites of LAN in the colonic tissues, 3H-labeled LAN (0.5 mCi/100g BW) was given through intraaortic catheter and localization of 3H-LAN

pretreated with LAN (1-100 µM) and then incubated with lipopolysaccharide (LPS). Production of TNFα was determined by ELISA and phosphorylation and degradation of IκBα and phosphorylation of ERK were evaluated by Western blotting. **Results:** (1) Administration of DSS caused inflammation and damage in the colonic mucosa and increased MPO activity and expression of TNFα mRNA. Treatment with LAN inhibited increase in MPO activity and overexpression of TNFα mRNA induced by DSS by 70% and 49%, respectively. Double-immunostaining demonstrated that monocytes/macrophages were main source of TNFα. The uptake site of 3H-LAN in the normal colon mucosa were few, while in inflamed colonic mucosa, 3H-LAN was extensively accumulated in inflammatory cells including macrophages and polymorphonuclear cells. (2) LPS induced production of TNFα, which was inhibited by pretreatment with LAN. LPS induced phosphorylation and degradation of IκBα and phosphorylation of ERK within 60 min. LAN inhibited phosphorylation and degradation of IκBα and phosphorylation of ERK induced by LPS. **Conclusion:** These results suggest that LAN suppresses colonic mucosal inflammation induced by DSS via reduction of TNFα expression in inflammatory cells and this reduction by LAN is due to inhibition of activation of NFκB and ERK signaling pathways.

M1698

Anti-Inflammation and Repair Induced By Bone Marrow-Derived Mesenchymal Stem Cells for Dextran Sulfate Sodium-Induced Colitis in Rats

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Background: Bone marrow-derived cells including a small amount of mesenchymal stem cells (MSCs) had therapeutic effects for clinical human and experimental animal colitis. Its detailed mechanism(s) may be partly mediated by mucosal regeneration, since MSCs have potential for differentiation to several parts of cells. But MSCs were thought to have other functions such as anti-inflammation as well as mucosal regeneration, because anti-inflammatory system is involved in the repair of colitis. We examined the therapeutic efficacy and anti-inflammatory effects of bone marrow-derived MSCs for dextran sulfate sodium (DSS)-induced acute colitis in rats. **Materials & Methods:** Experimental colitis was induced by orally administration of 0, 1, 2, or 4% DSS in drinking water for 7 days in inbred male Lewis rats. Bone marrow was extruded from tibias and femurs. Then, its mononuclear cells were isolated and cultured in low-glucose DMEM containing 10% fetal calf serum for MSCs outgrowth. On 0, 2, and 4 days after the administration of DSS, MSCs (5×10^6 cells) were injected via tail vein. We checked the volumes of food and water intake, stool condition, and body weight everyday. On day 7, total colon was excised and each colonic mRNA expression of inflammatory cytokines such as TNF-α, IL-1β, IL-10, and COX2 was measured by real time RT-PCR method. **Results:** We confirmed the MSC's characterization by both the immunostaining for vimentin and α-smooth muscle actin and the cell surface markers such as CD90, the bone marrow progenitor cell marker, but not CD45, HLA-DR, CD11b, nor CD31 using flow cytometric technique. Optimal dose of DSS for the rats used was confirmed at 4% by the assessing for loss of body weight and appetite, bloody fluid stool, and the shortening of colon length. MSC treatment improved the bloody stool and body weight loss, and significantly inhibited the shortening of colon length. At the rectum of MSC-treated rats, expressions of local inflammatory cytokines such as TNF-α and IL-1β were markedly decreased to about 40 and 15%. Local COX2 expression was also suppressed to 15%. IL-10, an anti-inflammatory cytokine, expression was also decreased to 25%. At the distal colon site (slightly oral side of rectum), similar tendency was observed about the expressions of cytokines in the MSC-treated colons. **Conclusion:** These findings suggested that MSC could have the therapeutic efficacy for the experimental colitis via anti-inflammatory functions.

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Induction of Regulatory T Cell Capacities by the Sphingosine-1-Phosphate Analogue FTY720 in TNBS-Colitis

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Background & Aims: The sphingosine-1-phosphate analogue FTY720 is known to alter migration and homing of lymphocytes via sphingosine-1-phosphate receptor interactions. We studied the effect of FTY720 in acute and established trinitrobenzene sulfonic acid (TNBS)-colitis focused on the induction of regulatory T cell capacities. **Methods:** A rectal enema of TNBS [100 mg/kg body weight (BW)] was applied to male Balb/c mice, and FTY720 [1 or 3 mg/kg] was administered intraperitoneally from day 0-3 or from day 3-5 following the instillation of the haptening agent. The study is conforming to the Guiding principles in the care and use of animals and was performed under approval of the ethical committee of Darmstadt/Germany (F134/03). Colon tissue was analyzed macroscopically and microscopically, and IL-10, transforming growth factor β (TGFβ) and FoxP3 expression were determined in colon protein extracts. **Results:** Treatment with FTY720 reduced the histopathologic severity of TNBS-colitis abrogating macroscopic and microscopic intestinal inflammation. Additionally, treatment with FTY720 resulted in a significant induction of IL-10, TGFβ and FoxP3 (see Table). **Conclusion:** FTY720 exhibits beneficial prophylactic as well as therapeutic effects in TNBS-colitis. Moreover, the induction of IL-10, TGFβ and FoxP3 raised evidence for a tolerance inducing activity also contributing to the beneficial capacities of FTY720 offering new auspicious therapeutic strategies for the treatment of inflammatory bowel disease. **Results FTY720 TNBS-colitis**