# LX1606 (aka LX1032), a Novel Inhibitor of Serotonin Synthesis, Alleviates Development of Inflammatory Bowel Disease in a Preclinical Model

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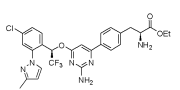
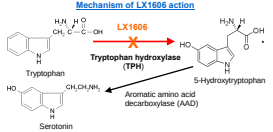
## BACKGROUND

Dysfunctional signaling by the immunoendocrine mediator serotonin (5-HT) may play an important role in the pathophysiology of inflammatory bowel disease (IBD).[1,2] Mucosal inflammatory lesions are accompanied by alterations in 5-HT producing enterochromaffin cells (EC). The first step of 5-HT synthesis in the gut is catalyzed by tryptophan hydroxylase 1 (TPH1), prominently expressed in EC. Experience with both TPH1 knockout mice and the TPH inhibitor para-chlorophenylalanine illustrates that reduction in 5-HT synthesis alleviates inflammatory damage in mouse models of IBD.[1,2] These data, along with the reported association between 5-HT production and various gastrointestinal (GI) diseases, support the hypothesis that lowering 5-HT production could provide a benefit for IBD patients. We have developed TPH inhibitors that deplete serotonin in the periphery but not in the central nervous system (CNS).[3,4] Such agents have potential therapeutic applications where elevated serotonin activity is thought to play a role. We reported the discovery of the TPH inhibitor LX1606 (aka LX1032), which reduced peripheral 5-HT production in preclinical and human studies. We now evaluate the efficacy of LX1606 in an experimental IBD model with the goal of developing a new therapeutic approach for IBD.

**Structure of LX1606**



**Mechanism of LX1606 action**

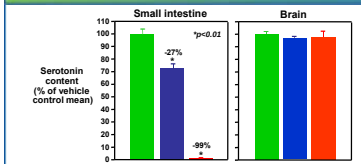



## METHODS

**Animal information:** C57Bl/6brd x 129SvEv F1 hybrid mice were used in all experiments. The studies were carried out with protocols approved by the Institutional Animal Care and Use Committee of Lexicon Pharmaceuticals, Inc.
**5-HT measurement:** Blood was mixed in buffer containing 56 mM sodium ascorbate and 600 mM trichloroacetic acid, and jejunum tissues were homogenized in a buffer containing 300 mM trichloroacetic acid, 100 mM sodium acetate, pH 3.5, 0.01 mM EDTA, and 20 mM sodium bisulfate. The resulting cell lysates were centrifuged and the supernatants analyzed for 5-HT content using reverse phase HPLC with a C18 column and an in-line fluorescence detector.
**TNBS-IBD model:** Animals were challenged via intra-rectal administration with 2% TNBS or left untreated as naïve controls. LX1606 and sulfasalazine were formulated in 0.25% methylcellulose and given to mice once daily via oral gavage starting 6 days before TNBS challenge and continuing during challenge.
**Blood neutrophil count:** Blood was collected in EDTA by retro-orbital bleeding and complete cell counts were measured on a Veterinary cell counter (Hemavet, Drew Scientific, Dallas, TX).
**Histological analysis:** Proximal and distal colon and cecum were collected, fixed in formalin, and sections were stained with hematoxylin and eosin. The sections were scored using a modified TJL (The Jackson Laboratory) system.
**Quantitative polymerase chain reaction (qPCR) analysis of cytokine expression:** Total RNA was extracted from distal colon. Interested genes were analyzed by standard qPCR methods.
**Statistics:** Data are presented as mean ± SEM and analyzed by student's *t* test and Repeated Measures ANOVA. Comparisons are between treatment groups and the vehicle control.

## RESULTS

### TPH1 knockouts reveal a new mechanism to target the serotonin pathway.




Most parameters studied during evaluation of the TPH1 mouse knockout yielded no discernible phenotype. An important initial observation was the lack of neurobehavioral phenotypes. Consistent with this observation, homozygous mutant mice showed normal levels of 5-HT in the brain but virtually undetectable levels of this amine in the gastrointestinal tract.

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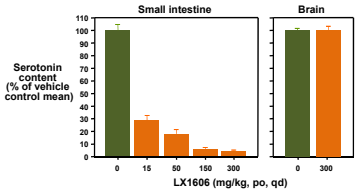
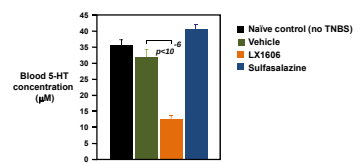
### LX1606 reduces serotonin content in the periphery, but not in the brain.



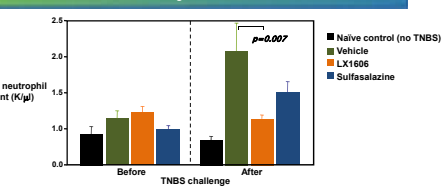

Mice (n=5 per group) were treated with the indicated doses of LX1606 for 6 days before analysis.

### Treatment with LX1606 decreases 5-HT concentration in blood.



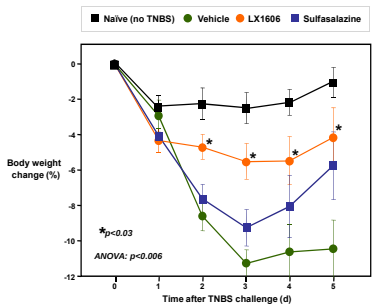

Concentration of 5-HT in whole blood was measured after 6 days treatment with LX1606 (200 mg/kg po, qd), sulfasalazine (100 mg/kg po, qd), or vehicle before TNBS challenge of mice in the IBD study presented below. N = 10 per cohort.

### Treatment with LX1606 prevents the increase in blood neutrophil counts that is observed after TNBS challenge.



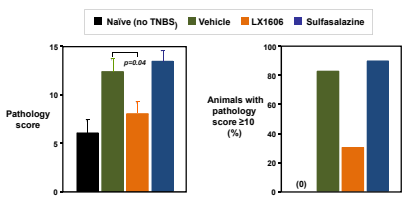

LX1606 (200 mg/kg po, qd), sulfasalazine (100 mg/kg po, qd), and vehicle treatment started 6 days before TNBS challenge and continued throughout the experiment. Blood neutrophil counts were measured 1 day before and on the last day of the TNBS challenge. The study started with 10 mice per group. 4 mice died by the end of the assay in the vehicle group and none in the other groups.

### Treatment with LX1606 provides significant protection in a mouse model of inflammatory bowel disease.



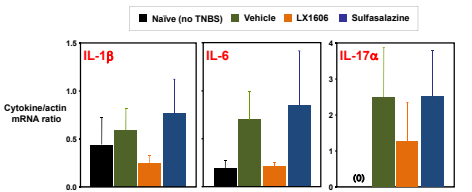

LX1606 (200 mg/kg po, qd), sulfasalazine (100 mg/kg po, qd), and vehicle treatment started 6 days before TNBS challenge and continued throughout the experiment. The study started with 10 mice per group; 4 mice died by the end of the assay in the vehicle group and none in the other groups.

### Histopathology evaluation confirms significant protective effect of LX1606 in the mouse IBD model.




LX1606 (200 mg/kg po, qd), sulfasalazine (100 mg/kg po, qd), and vehicle treatment started 6 days before TNBS challenge and continued throughout the experiment. The study started with 10 mice per group; 4 mice died by the end of the assay in the vehicle group and none in the other groups. Data are expressed as cumulative scores combining inflammation, ulceration, hyperplasia, and area involved in the disease process in the proximal and distal colon including the cecum.

### LX1606 treatment results in a trend toward decreased expression of proinflammatory cytokines after TNBS challenge.




Total RNA was extracted from distal colon tissues of mice from the experiment presented in the previous Figures. Expression levels of the indicated cytokines were measured by qPCR.

## CONCLUSIONS

- LX1606 is a novel, orally-delivered inhibitor of tryptophan hydroxylase that reduces serotonin production:
  - Absorbed into peripheral circulation
  - Does not cross the blood-brain barrier
- LX1606 consistently reduced 5-HT levels in the periphery but not in the brain.
- Treatment with LX1606 showed a strong positive effect in ameliorating TNBS-induced IBD in mice as assessed by various parameters of disease development.
- These preclinical data demonstrate that inhibition of TPH activity by LX1606 may provide a new approach for the treatment of IBD and its serotonin-mediated symptoms.

## REFERENCES

1. Margolis KG, et al. Serotonin has a critical role in the pathogenesis of experimental colitis. *Gastroenterology.* 137:1562-6, 2009.
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4. Brown PM, et al. The tryptophan hydroxylase inhibitor LX1031 shows clinical benefit in patients with nonconstipating, irritable bowel syndrome. *Gastroenterology.* 141:507-16, 2011.

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Disclosure: Authors are employees of Lexicon Pharmaceuticals , Inc., and own stock.