LX1033, a Second Generation Locally-acting Serotonin Synthesis Inhibitor, Demonstrates Increased Potency and Favorable Safety Profile in Preclinical Studies

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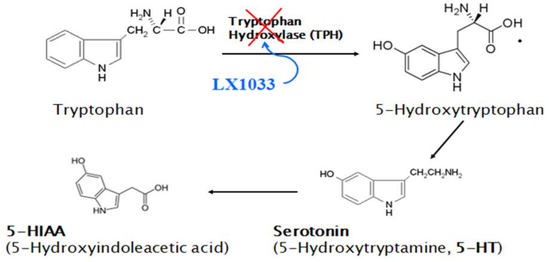
BACKGROUND

- Serotonin (5-HT) mediates gastrointestinal (GI) motility, secretion, inflammation and sensation and may play an important role in irritable bowel syndrome (IBS).
- Serotonin synthesis inhibition with LX1031 has been shown to improve symptoms in non-constipating IBS patients in correlation with decreases in urinary 5-hydroxyindoleacetic acid (5-HIAA).[1]
- We now report that a second generation, locally-acting, novel serotonin synthesis inhibitor (SSI), LX1033 has exhibited improved in vitro inhibition of tryptophan hydroxylase (TPH1) and greater pharmacodynamic effects on 5-HT synthesis in rodents.



Tryptophan hydroxylase (TPH) catalyzes the first step of serotonin synthesis.

OBJECTIVES

These exploratory studies were designed to:

- delineate the in vitro and in vivo characteristics of LX1033, and
- compare its potency and safety profile to the first generation of serotonin synthesis inhibitor, LX1031.

METHODS

In vitro, comparisons were made for LX1033 vs. LX1031 for the half maximum inhibitory concentration (IC50); human TPH1 inhibition was assayed spectrographically and cellular TPH1 inhibition was assessed by measuring 5-HT or 5-hydroxytryptophan (5-HTP) by HPLC. Inhibition of TPH activity in intact cells was measured using 2 tumor cell lines: 1) in RBL-2H3 cells, SSI was incubated at different concentrations with the cells for 3 days and the remaining levels of 5-HT in the cells were measured by HPLC; 2) in BON cells, SSI was incubated with the cells overnight and the remaining 5-HTP levels in the media were determined by HPLC.

In the Sprague Dawley rat study, LX1033 was formulated in 0.25% methylcellulose plus 0.1% Tween 80 and orally administered at 0, 5, 15, 50, or 150 mg/kg twice daily (total daily dose of 0, 10, 30, 100, or 300 mg/kg/day) for 4 consecutive days. Animals were sacrificed approximately 16-18 hours after their last dose. Whole blood and various intestinal segments (mesentery fat removed, gut lumen opened and blotted dry) were collected, snap frozen and stored at -80ºC. Tissues weights were taken prior to 5-HT analysis.

In the LX1033 mouse dietary study, female C57BL/6J mice were acclimatized to single housing and purified low fat diet (LFD: D12450B, Research Diet Inc.), which provides 10% kcal as fat (lard). LX1033 was mixed in LFD and fed to the treated mice for 10 consecutive days to achieve dose levels of 30 and 100 mg/kg/day. Whole blood, brain, and GI tissues were collected and stored frozen prior to 5-HT analysis.

RESULTS

LX1033 proved to be 16x more potent than LX1031 in the human recombinant TPH1 enzyme IC50 assay, and was 16x and 5x more potent than LX1031 in rat RBL and human BON cells, respectively.

Table 1: Comparison of in vitro potency of LX1033 and LX1031: TPH inhibition

	Human recombinant enzyme IC_{50} (μM)	Rat mastocytoma cells: RBL IC_{50} (μM)	Human carcinoid cell line: BON IC_{50} (μM)
LX1033	0.019 0.002	0.010 0.001	0.352 0.051
LX1031	0.309 0.077	0.156 0.011	1.92 0.86[a]
Fold difference	16x	16x	5x

RBL: Rat Basophilic Leukemia cell line
BON: a human pancreatic carcinoid cell line
[a] Based on the 2006 IND filing report

Table 2: Comparison of LX1033 vs LX1031 exposure in rats on Day 1

	10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		300 mg/kg/day	
	C_{max} (ng/mL)	$AUC_{(0-9hr)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-9hr)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-9hr)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-9hr)}$ (ng*hr/mL)
LX1033	5	23	9	45	59	335	ND	ND
LX1031	15	68	38	181	59	339	93	617

ND: Not determined due to an instrument error; all but the first 2 time points from this group were lost.

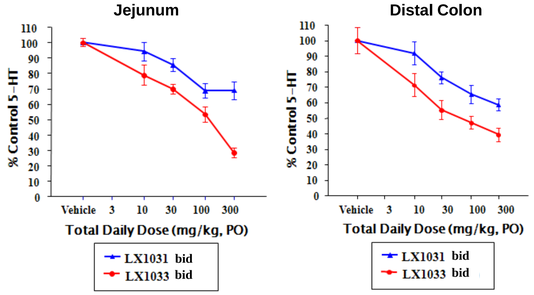
RESULTS (continued)

In a head-to-head study in rats, vehicle, LX1031 and LX1033 were orally administered for 4 consecutive days. LX1033 showed a dose-dependent reduction of 5-HT in the jejunum, ileum, proximal colon, and distal colon, with the greatest reduction at 300 mg/kg/day: 71% (jejunum), 76% (ileum), 65% (proximal colon), and 61% (distal colon). LX1033 demonstrated better reduction of 5-HT than LX1031 in these various GI segments in rats.

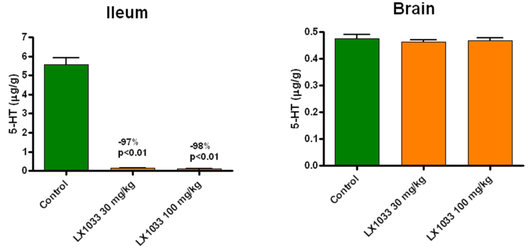
Table 3: Comparison of LX1033 and LX1031 Efficacies in Rats: Reduction in Gut 5-HT Contents (n=6/group)

	Jejunum	Ileum	proximal colon	distal colon
LX1033 (300 mg/kg/day)	71%	76%	65%	61%
LX1031 (300 mg/kg/day)	31%	51%	24%	41%
Fold Difference	2.3x	1.5x	2.7x	1.5x

Comparison of LX1033 vs. LX1031 in jejunum and distal colon 5-HT reduction in rats



Ileum and brain 5-HT levels in mice treated with LX1033 via diet administration for 10 consecutive days (N=9-10/group)



RESULTS (continued)

- After being administered in the diet for 10 consecutive days, LX1033 significantly reduced 5-HT levels in whole blood, duodenum, jejunum, ileum, and colon at 30 and 100 mg/kg/day dose levels.
- LX1033 caused 33% decrease of whole blood 5-HT at 30 mg/kg/day and 40% decrease at 100 mg/kg/day.
- In duodenum, jejunum, and ileum, the extent of decrease in 5-HT ranged from 73% to 97% at 30 mg/kg/day and 88% to 98% at 100 mg/kg/day ($p<0.01$).
- In the proximal colon, LX1033 decreased 5-HT by 52% ($p<0.01$) and 70% ($p<0.01$) at 30 and 100 mg/kg/day, respectively.
- Despite the significant decrease of peripheral 5-HT, brain 5-HT levels did not change with either 30 mg/kg/day or 100 mg/kg/day dose levels when compared to the control.

DISCUSSION

- In a head-to-head study, LX1033 demonstrated better reduction of tissue 5-HT than LX1031 in various regions of the GI tract in rats, while maintaining a low-systemic exposure profile.
- Studies in mice also demonstrated that LX1033 was effective in reducing peripheral 5-HT, while showing no evidence of crossing the blood-brain barrier.
- Results from genetic and animal toxicology studies confirmed that LX1033 was safe and well-tolerated (data not shown).

CONCLUSIONS

- LX1033 proved to be 5-16x more potent than LX1031 based on in vitro enzyme and cell-based assays.
- Preclinical in vitro and in vivo studies in rodents demonstrate that LX1033 inhibits 5- HT synthesis to a greater extent than LX1031.
- LX1031 was previously shown to offer clinical benefit to patients with IBS in 4-week placebo, controlled study.
- LX1033 has demonstrated a favorable safety profile in preclinical studies and may be a novel therapeutic agent for IBS-diarrhea predominant (IBS-D) and other GI disorders.
- Enrollment is ongoing in a placebo-controlled, 4-week Phase 2 trial of LX1033 in 360 patients with IBS-D.

Reference:

1) Brown PM, Drossman DA, Wood AJJ, et al. *Gastroenterology.* 2011;141:507-516

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

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