Lubiprostone, a Chloride Channel Activator, Induces Intestinal Fluid Secretion in Rats at Clinically Relevant Doses

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Abstract
Lubiprostone is an activator of type-2 chloride channels (ClC-2) currently marketed in the US for the treatment of chronic idiopathic constipation in adults and for irritable bowel syndrome with constipation in adults and women. Lubiprostone treatment at 10 and 100 mcg/kg results in significant increases in intestinal fluid volume in rats (Ueno et al., Gastroenterology, 2004;126 [4 Suppl 2, Abstract M1109]).

A study was performed to determine the effect of lower doses of lubiprostone (0.1 mcg/kg) on intestinal fluid volume in Wistar rats. Thirty minutes after oral administration of vehicle, 0.1, 0.5, or 1.0 mcg/kg lubiprostone, each animal was sacrificed. The pyloric end of the duodenum to the terminal portion of the ileum was ligated, and the intestine was removed. The volume of fluid in each intestinal segment was calculated, weighed, and measured for radioactivity. Results showed that both the intestinal fluid weight and the level of radioactivity were statistically significantly increased (P<0.01) in animals treated with lubiprostone when compared with the control group. These results show evidence of intestinal fluid secretion induced by the administration of lubiprostone.

It is noteworthy that the minimum dose at which diarrhea is observed in rat toxicity studies is 1000 mcg/kg, which is 2000 times greater than the lowest lubiprostone dose that significantly increases intestinal fluid secretion in rats. Furthermore, the secretory effects of lubiprostone occur at doses that are within the range of the recommended clinical dose (24 mcg BID). The results of these two rat studies indicate that lubiprostone significantly increases intestinal fluid secretion. Furthermore, this result is achieved without induction of diarrhea, suggesting that colonic fluid absorption is kept intact with lubiprostone treatment at clinical dose levels.

Introduction
• Lubiprostone, a novel activator of type-2 chloride channels (ClC-2) in the apical membrane of intestinal epithelial cells, enhances intestinal fluid secretion and intestinal mobility.1,2
• Lubiprostone is FDA-approved for the treatment of chronic idiopathic constipation in adults without regard to age or gender.3
• A previous study demonstrated that lubiprostone treatment at 10 and 100 mcg/kg resulted in significant increases in intestinal fluid volume in rats.4

Objective
• To determine whether lubiprostone induces a dose-dependent increase in intestinal fluid volume and to explore the mode by which intestinal fluid secretion occurs.

Methods
• Study 1: Determination of Intestinal Fluid Volume With Lubiprostone Treatment
  – Six-week-old male Wistar rats were fasted for 24 hours but allowed drinking water ad libitum.
  – Six rats received oral doses of 0.1, 0.5, or 1.0 mcg/kg lubiprostone in an administration volume of 5 mL/kg. The vehicle consisted of distilled water containing 0.01% Tween 80.
  – Animals were sacrificed 30 minutes after dosing, and the abdomen was opened. The intestine was removed and weighed.
  – Intestinal fluid was collected and its volume measured. Fluid volumes are expressed as the mean ± standard error (SE).

• Study 2: Determination of Intestinal Fluid Secretion in Rats
  – Six-week-old male Wistar rats were fasted for 24 hours but allowed drinking water ad libitum.
  – Six animals in the control group, and five in the treatment group, received 1 mL saline containing tritiated water (1H2O; 9,300,000 dpm/mL) via intravenous injection into the tail vein.
  – One minute after injection, rats in the treatment group received lubiprostone 10 mcg/kg orally in a volume of 5 mL/kg. The vehicle consisted of distilled water containing 0.01% Tween 80.
  – The same method described above was used to measure the fluid volume, weight, and radioactivity level.
  – Using the same method described above, the animals were sacrificed and the intestines were secured and removed. Intestinal fluid was collected and weighed, and aliquot of intestinal fluid was retained for radioactivity measurement.

• Total radioactivity in the intestinal fluid was calculated using the following formula:

\[ \text{Total radioactivity in the intestinal fluid (dpm)} = \text{Radioactivity in the intestinal fluid} \times \left( \frac{\text{Weight of the intestinal fluid specimen}}{\text{Total weight of the intestinal fluid specimen}} \right) \]

• The dose of test substance necessary to induce a 50% increase in intestinal fluid volume was calculated as the ED50 value.
• Student’s t-test was used to compare intestinal fluid volume and average radioactivity values between the group treated with lubiprostone and the control group. P-values <0.05 were considered statistically significant.

Results
Effect of varying doses of lubiprostone on intestinal fluid volume
• Lubiprostone (0.1, 0.5, and 1.0 mcg/kg) increased intestinal fluid volume in a dose-dependent manner (Figure 1).

Figure 1. Stimulating Effect of Lubiprostone on Intestinal Fluid Secretion in Rats

– ED50, for lubiprostone-induced intestinal fluid secretion was calculated to be 0.6 mcg/kg (Figure 2).

Figure 2. Determination of ED50

Effect of higher dose of lubiprostone on intestinal fluid weight and demonstration of mode of lubiprostone-induced intestinal fluid secretion
• Animals receiving 10 mcg/kg lubiprostone demonstrated a 2.6-fold increase in intestinal fluid weight compared with the animals receiving vehicle control, P<0.01 (Figure 3).

Figure 3. Water Excreting Effects of Lubiprostone into the Bowel in Rats

– Concomitant with the increase in intestinal fluid weight was a 3-fold increase in total radioactivity (dpm) in intestinal fluid in the lubiprostone-treated animals vs those given distilled water alone; P<0.01 (Figure 3).

Summary and Conclusions
• Lubiprostone treatment resulted in significant dose-dependent increases in intestinal fluid volume at doses of 0.1 and 1.0 mcg/kg.
• Administration of lubiprostone at 10 mcg/kg stimulated a significant increase in weight of secreted intestinal fluid, further suggesting a dose-dependence effect.
• In animals receiving 10 mcg/kg lubiprostone vs those receiving vehicle alone, total radioactivity levels in the intestinal fluid were significantly increased.
• Based on body surface area conversions, the secretory effects of lubiprostone in rats occur at doses that are within the range of the recommended human clinical dose (24 mcg BID).
• Doses below 1000 mcg/kg did not induce loose stools in rat toxicity studies.5 This dose is 2000 times greater than the lowest lubiprostone dose that significantly increases intestinal fluid secretion in rats.
• Lubiprostone significantly increases intestinal fluid secretion without induction of diarrhea, suggesting that colonic fluid absorption is kept intact with lubiprostone treatment at clinical dose levels.

References