응용약물학회지, The Journal of Applied Pharmacology, **10**, 236-239(2002)

Antidiarrheal Effect of Lacteol^{TM}-Loperamide Combination on Castor oil-induced Mice Model

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(Received November 28, 2002 ; accepted December 13, 2002)

Abstract – The goal of this study was to evaluate the antidiarrheal efficacy of Lacteol^{TM}-loperamide combination against the mouse model of secretory diarrhea. Secretory diarrhea was induced in mice by *p.o.* administration of castor oil (0.3 ml). Antidiarrheal effects of Lacteol^{TM}-loperamide combination were compared with each individual component. Lacteol^{TM}-loperamide combination was the most potent among these agents, eliminating diarrhea in 100% of mice at a dose 1360/4 mg/kg (Lacteol/loperamide, respectively). In this study, we also measured changes of bodyweight as another indicator of the diarrhea, based on the assumption that lower bodyweight loss represented reduced fecal passage. The bodyweight loss of Lacteol^{TM}-loperamide combination administered group was 4 times lower than that of vehicle control. These findings indicate that Lacteol^{TM}-loperamide combination may be more potent than individual component in its antidiarrheal action, so we are going to challenge this combination for further study and clinical evaluation.

Key words □ Lacteol^{TM}-loperamide, secretory diarrhea, castor oil

I. Introduction

Diarrhea can be classified as osmotic, secretory, exudative, postresection, and motor (Schiller, 1995). Some authors distinguish only between osmotic diarrhea and secretory diarrhea (Barrett and Dharmsathaphorn, 1991). Osmotic diarrhea results from the ingestion of poorly absorbed substances that retard fluid absorption. Both postresection and exudative diarrheas result from loss of functional intestinal mucosa. Inhibition of mucosal absorption or stimulation of secretion of fluid and electrolytes results in secretory diarrhea. Abnormally rapid transit time can result in motor diarrhea (Schiller, 1995).

Acute diarrhea is a common, usually self-limited disorder that occurs in a majority of adults at least once a year (Brownlee, 1990). Loperamide hydrochloride is an effective, safe antidiarrheal agent that was originally approved for prescription use in Europe in 1973 and in the United States in 1977; it has been available as an over-the-counter medication in the United States since 1988 (Duke, 1990). Loperamide can improve treatment of diarrhea, including decreased frequency of bowel movements and improved stool consistency, but no improvement in abdominal pain and distention.

The term probiotic refers to live microoganisms that survive passage through the gastrointestinal tract and have beneficial effects on the host (Fuller, 1989). Probiotics can prevent or ameliorate diarrhea through their effects on the immune system. Moreover, probiotics might prevent infection because they complete with pathogenic viruses or bacteria for binding sites on epithelial cells (Perdigon *et al.*, 1992). Lyophilized heat-killed *Lactobacillus acidophilus* has been tested for its therapeutic effects on acute diarrhea mainly caused by rotavirus. The oral rehydration solution containing lyophilized heat-killed *L. acidophilus* decreased the diarrheal period by ≈20 hour particularly marked in children with no antibiotic therapy (Simakachorn *et al.*, 2000).

A safe and effective antidiarrheal agent would be very useful in therapy of acute and chronic diarrheal conditions.

So, the aim of this study is to compare the efficacy of a lyophilized heat-killed *Lactobacillus acidophilus* (Lacteol^{TM})-loperamide combination with those of Lacteol^{TM} alone, loperamide alone, and vechicle control in secretory diarrhea model.

2. Materials and method

Chemicals

Lacteol^{TM} (Lyophilized heat-killed *Lactobacillus acidophilus*) was supplied by pharmaceutical development laboratory of Dong Wha Pharm. Ind. Co. Ltd., Korea. Loperamide hydro-

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chloride and castor oil were obtained from commercial supplier (Sigma, USA).

Experimental animal

Male SPF CD-1 (25-30 g of body weight) mice were purchased from Biogenomics (Korea) for this experiment. These experimental animals were housed under standard environmental conditions including a 12/12 hr light/dark cycle. Food and water were given *ad libitum*.

Method

After a week acclimatization animals were fasted 12 hr before the experiments. The animals were divided into six groups. Secretory diarrhea was induced by modification of previous method (Shook *et al.*, 1989). After *p.o.* administration of 0.3 mL of castor oil, mice were placed in stainless steel wired polycarbonate cages. Three hours after oil challenge, mouse and cages were inspected for the presence of the characteristic diarrhea dropping; their absence was recorded as a protection from diarrhea (Izzo *et al.*, 1994). Results were analyzed using an all or none criterion, with ano-genital staining and soft stools counting as diarrhea. Results are provided as percentage of mice with diarrhea at each time and the cumulative number of positives over the 3-hour period. Inhibition percent was calculated by comparison to the vehicle control. All drugs and vehicle were given 30 min before administration of castor oil. The proper dosage of Lacteol^{TM} and loperamide was determined by preliminary studies. Control mice received vehicle used for suspending compounds (5% Tween20/salinc, 0.1 mL/10 g of body weight).

Statistics

Antidiarrheal activity was evaluated using the chi-square test. The rate of bodyweight loss was analyzed for statistical differences by ANOVA, followed by student's t-test when significant differences were indicated; a $P<0.05$ was considered significant.

3. Results

Data are presented as the number of mice showing diarrhea and as a percentage of inhibition compared to vehicle control. Castor oil induced diarrhea in 100% of vehicle-treated control mice. The results from this study are shown in Table 1. Lyophilized heat-killed *L. acidophilus* (Lacteol^{TM})-loperamide combination produced a statistically significant inhibition of castor oil induced diarrhea (100% of inhibition rate). 4 and 8 mg/kg of loperamide significantly blocked castor oil induced diarrhea at the 3 hr observation period when compared with vehicle control group (inhibition rate: 70% and 100%, respectively). No significant inhibitions were observed in Lacteol^{TM}-treated groups. But pretreatment of Lacteol^{TM} produced dose-related inhibition of diarrhea.

We also measured the rate of bodyweight loss as another indicator of antidiarrheal effect. Based on the assumption that lower bodyweight loss represented reduced fecal passage. Lac-

Table 1. Antidiarrheal effects of Lacteol^{TM} and loperamide on castor oil-induced diarrhea model (n=10)

Treatment	Dose (mg/kg)	No. of mice with diarrhea/ No. of mice with tested	Antidiarrheal effect (%)
Vehicle control		10/10	
Loperamide	4	3/10[†]	70
	8	0/10[†*]	100
Lacteol^{TM}	1360	10/10	0
	2720	7/10	30
Lacteol^{TM} with loperamide	1360 4	0/10**	100

Secretory diarrhea was induced by administration of castor oil (0.3 ml). Mice with ano-genital staining and soft stools counting as diarrhea during 3 hours after oil challenge. Statistical analysis was done using the chi-square test.
*$P<0.05$ compared with vehicle control group.
**$P<0.01$ compared with vehicle control group.

Table 2. Effect of Lacteol^{TM}-loperamide on the loss of body weight in castor oil-induced mice at 180 min

Treatment	Dose (mg/kg)	Rate of bodyweight loss (%)[a]
Vehicle control		1.44±0.283
Loperamide	4	0.62±0.216[*]
	8	−0.02±0.248[b,**]
Lacteol^{TM}	1360	1.06±0.268
	2720	0.44±0.107[†]
Lacteol^{TM} with loperamide	1360 4	0.36±0.190[**,†]

Secretory diarrhea was induced by administration of castor oil (0.3 ml). All drugs and vehicle were given 30 min before administration of castor oil. The rate of bodyweight loss was measured as another indicator of antidiarrheal effect. Results were analyzed by student's t-test.
[a]Data are represented Mean±SEM (n=9 or 10).
[b]The 'minus' indicates bodyweight gain.
*P<0.05 compared with vehicle control group.
**P<0.01 compared with vehicle control group.
[†]P<0.05 compared with Lacteol^{TM}-treated group (1360 mg/kg).

teol^{TM}-loperamide combination reduced the rate of bodyweight loss, which was 1.7, 3 and 4 times lower than those of loperamide, Lacteol^{TM} and vehicle control, respectively (Table 2).

4. Discussion

The castor oil induced diarrhea model has been used extensively as a basic pharmacological test to study the role of endogenous substances involved in diarrhea and to screen antidiarrheal drugs (Diurno *et al.*, 1996). The mechanism of the diarrheogenic activity of castor oil is complex. At first, castor oil is metabolized to ricinoleic acid in the lumen of the intestinal tract. Ricinoleic acid then produces a marked increase in net secretion of fluid and electrolytes in the intestine resulting in diarrhea. When castor oil was administered by oral gavage to rats, the duodenum and jejunum, but not the stomach, produced large amounts of Platelet Activating Factor (PAF) 3-7 hours after oil challenge with a peak at 3 hours (Mascolo *et al.*, 1996). PAF is one of the most potent inflammatory phospholipid mediators secreted by proinflammatory cells.

Diarrhea is a major cause of infant death worldwide and can be incapacitating in adults, the widespread use of probiotics could be an important, non-invasive means to prevent and treat these diseases, particularly in developing countries. Probiotic bacteria have also been shown to preserve intestinal integrity and mediate the effects of inflammatory bowel diseases, irritable bowel syndrome. Especially, Lactobacillus is safe and effective as a treatment for children with acute infectious diarrhea (Van Niel *et al.*, 2002).

Loperamide is at present one of the most efficacious and widely employed antidiarrheal drugs; loperamide antagonizes diarrhea induced by castor oil, prostaglandins or cholera toxin. The therapeutic effect of loperamide is believed to be due to its antimotility and antisecretory properties (Coupar, 1987). But it should be noted that antimotility agents are not recommended for patients with acute diarrhea with high fever or blood or mucus in the stool.

In this study, Lacteol^{TM}-loperamide combination product has been shown to be a highly effective antidiarrheal activity in a castor oil-induced model of secretory diarrhea. This Lacteol^{TM}-loperamide combination product decreased the incidence of diarrhea in mice (100% inhibition). Moreover, this compound reduced the rate of bodyweight loss.

We also estimated the effect of these compounds on the gastrointestinal motility, assessed as the distance traveled by the carmine dye expressed as a percentage of the length of the whole small intestinal tract (data are not shown). At 3 hours after oil challenge, Lacteol^{TM}, loperamide and this combination reduced the gastrointestinal motility. The mean percentage of distance traveled by carmine dye of Lacteol^{TM}, loperamide and this combination was 96.4, 72.2 and 63.7%, respectively. But it couldn't be calculated in vehicle control group, because the carmine dye was observed in the feces of diarrhea dropping already.

The results obtained from this study clearly indicate that use of the Lacteol^{TM}-loperamide combination product leads to an enhancement in secretory diarrhea beyond that seen with each individual component. The exact mechanism of this observed response is not well known. But, loperamide's ability to decrease gut motility, increase fluid reabsorption, and decrease intestinal secretion could conceivably enhance Lacteol's anti-inflammatory activity by increasing the contact time on a reduced volume of intestinal fluid. Antonopoulou *et al.* (1996) studied lipids with PAF and anti-PAF activity in cow's milk and yogurt and found that *Streptococcus thermophilus* and *Lactobacillus bulgaricus* biosynthesized important quantities of PAF inhibitors.

In summary, these data indicate that Lacteol^{TM}-loperamide combination product exhibits enhanced antidiarrheal activity in a castor oil-induced model of secretory diarrhea. We are going to evaluate this combination product for another animal model of diarrhea and clinical research.

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