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Basic nutritional investigation

Enteral feeding with low-methoxyl pectin accelerates colonic anastomosis healing in rats

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ABSTRACT

Objective: Enteral feeding with pectin has proven beneficial for anastomosis healing in rats. The aim of this study was to investigate the effects of low-methoxyl pectin (LMP) or high-methoxyl pectin (HMP), on colonic anastomosis healing in rats.
Methods: Male Sprague-Dawley rats (age 7 wk) were fed liquid diets containing LMP, HMP, or no pectin (pectin-free [PF]) for 14 d (n = 10/group). The rats underwent colonic anastomosis surgery on day 7 and were sacrificed on day 14. Bursting pressure, breaking strength, and salt-soluble hydroxyproline at the anastomosis site were used as indices of anastomosis healing. Short-chain fatty acids (SCFAs) in the cecal contents were analyzed.
Results: Breaking strength was higher in the LMP group than in the other two groups ($P < 0.001$). The salt-soluble hydroxyproline content was higher in LMP group than in the PF group ($P < 0.01$). Bursting pressure did not differ among the three groups. The LMP group produced normal, formed stools, whereas watery stools were observed in HMP and PF groups throughout the experimental period. Cecal SCFAs were highest in LMP group.
Conclusions: These results suggest that LMP promotes healing of colonic anastomosis more effectively than HMP, which may be explained by the mechanical stresses generated by the movement of normally formed stool though the colon.
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Introduction

Pectin feeding activates a novel nutrient sensing mechanism by stimulating a short-chain fatty acid (SCFA) sensor [1]. Previous studies have reported that the intake of dietary fibers that increase SCFA production in the gut by intestinal bacteria have beneficial effects on anastomosis healing [2,3]. At the same time, mechanical loading to the intestine from early postoperative feeding also has been positively associated with the promotion of anastomosis healing [4]. Thus, the combination of SCFA production and mechanical loading around the anastomosis site may constitute an effective approach to promote anastomosis healing. Although the beneficial effects of pectin in short bowel syndrome are known [5,6], to our knowledge there are few reports in which the pectin properties have been distinguished.

Pectic polysaccharides are characterized by a high content of D-galacturonic acid (GA) [7]. Physical and biochemical characteristics of pectins are determined by the degree of esterification (DE) at the carboxyl groups in GA. Pectins with DE ≥50% are called high-methoxyl pectin (HMP) and those with DE <50% are called low-methoxyl pectin (LMP).

HMP and LMP are different in gelation mechanism [8]. Pectin is well fermented in the colon, but recent reports show different fermentability between HMP and LMP [9–11], suggesting different physiological effects.

Considering possible physiological differences of HMP and LMP, it may be possible that HMP and LMP have different effects on anastomosis healing. In the present study, the effect of early enteral feeding with a liquid diet containing LMP or HMP on colonic anastomosis healing in rats was investigated.

All authors are employed Otsuka Pharmaceutical Factory, Inc. F.Y. and K.H. contributed equally to this article and wrote the manuscript. F.Y., K.H., and G. Ebisu designed the research. F.Y. conducted the research. N.E. provided essential reagents. F.Y. and S.M. analyzed the data. All authors read and approved the final manuscript.

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Materials and methods

Animals and housing

All animals were treated in accordance with the guidelines established by the Animal Care and Use Committee of Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). The experimental protocol was approved by the Animal Experimentation Committee of Otsuka Pharmaceutical Factory, Inc.

Thirty 7-wk-old male Sprague-Dawley rats were purchased from Charles River Japan (Yokohama, Japan) and housed in a conventional animal room under controlled conditions (temperature, 23 ± 3°C; 12/12-h light/dark cycle, lights on at 0700). Rats were acclimated for 1 wk before the start of the experiment, under ad libitum access to water and AIN-93 G rodent diet (Oriental Yeast Co., Ltd., Tokyo, Japan). During the experimental period, the rats were individually housed in metabolic cages.

Preparation of liquid diets

Pectins used in this experiment (LMP and HMP) were obtained from CP Kelco Japan (Tokyo, Japan). Liquid diets containing 0.9 g of LMP (HINE E-GEL; Otsuka Pharmaceutical Factory, Inc.) or HMP per 100 kcal of feed, or containing no pectin (pectin-free [PF]) were provided during the experimental period. The HMP-containing and the PF diets were provided by Otsuka Pharmaceutical Factory, Inc. Those liquid diets were isocaloric and isonitrogenous except for the pectin products added. The nutritional compositions of the liquid diets were based on the Japanese required dietary allowances (for humans). Although dietary requirements for rats differ from humans, free feeding of liquid diet containing LMP for 2 wk maintained the body weight gain and nutritional status of rats in this study.

Experimental design

Thirty rats were assigned to three groups according to body weight on day 1 and fed a liquid diet (80 kcal/d) containing LMP, HMP, or no pectin (n = 10 rats/group) for the experimental period. On day 6, the preoperative day, the liquid diets were removed for 24 h. On day 7, the day of surgery, the rats were fed a dose of 40 kcal/d, instead of 80 kcal/d. Thereafter, the rats were fed a dose of 80 kcal/d. The rats underwent anastomosis surgery on day 7 and were sacrificed on day 14.

Anastomosis: surgical procedure

Rats were fasted for 24 h preoperatively, and anesthetized by isoflurane inhalation (Intervet Inc., Tokyo, Japan) immediately before the anastomosis surgery. An ~4-cm incision was made downward from slightly below the sternum along the central line of the abdomen. The ileocecal region was moved to the right side of the abdominal cavity, exposing the descending colon. The area 3 cm from the peritoneal reflection of the descending colon was removed and the ends were stitched back together with a single-layer, end-to-end anastomosis using eight interrupted sutures with 7-0 Prolene sutures (Ethicon; Johnson & Johnson K.K., Tokyo, Japan) in an inverted interrupted fashion. The anastomosis surgeries were carried out by a designated person to control for technical differences. The fascial layer of the abdominal wall and the abdominal skin were closed in a continuous pattern of 3-0 Perma-Hand Silk (Ethicon).

Anastomosis bursting pressure and breaking strength

On day 14, the animals were sacrificed using isoflurane. The skirted suture site of the lower abdomen was opened, and anastomotic bursting pressure was measured in situ. A polytetrafluoroethylene disposable oral sonde (Fuchigami, Kyoto, Japan), which connected to the transducer via a catheter, was inserted 1.5 cm proximal to the site of the anastomosis, and ligations were made at both 1.5 cm proximal and 1.5 cm distal from the anastomosis site by using 3-0 silk sutures. Normal saline solution was infused continuously through the catheter at a rate of 1 mL/min using a syringe pump. Intra-luminal pressure was monitored using a transducer (MLT0670 BP; AD Instruments, Inc., Tokyo, Japan) and recorded on a chart recorder (PowerLab; AD Instruments). When anastomotic leakage was confirmed, saline infusion was stopped immediately, and bursting pressure was recorded as the peak pressure attained immediately before rupture of anastomosis.

After bursting pressure test, a 3-cm section of the colon extending from 1.5 cm proximal to 1.5 cm distal from the anastomosis and mesenteric fat were excised. Both ends of the tissue were fixed to grips of a digital force gauge (ZP-500 N; Imada Inc., Aichi, Japan) for the tensile force test. During measurement of breaking strength, one grip moved at a speed of 30 mm/min away from the other grip, so that the tissue section was continuously pulled until the anastomosis site was completely ruptured. Breaking strength was defined as the maximal tension before the breaking of anastomosis.

Biochemical analysis of anastomosis tissue

After breaking strength test, the colon tissue was weighed, and its hydroxyproline concentration was determined as an index of collagen content using a modified method based on previous reports [12]. Briefly, colon tissue was homogenized in 2 mL of normal saline and centrifuged at 3000*g* for 10 min. The supernatant was collected as salt-soluble collagen fraction, and pellet, which was subsequently hydrolyzed with 6 N hydrochloric acid at 110°C for 24 h, was collected as the insoluble collagen fraction. The hydroxyproline content in the fractions was determined by a liquid chromatograph-mass spectrometer (LC-MS2020; Shimadzu, Kyoto, Japan) and nitrogen content was determined using a nitrogen analyzer (TN-100; Mitsubishi Chemical Co., Ltd., Tokyo, Japan).

Nutritional parameters

The body weight of each rat was measured on days 1, 7, and 14. Diet intake was recorded for each rat from days 7 to 14. Blood samples were collected from abdominal aorta after the bursting pressure test, and plasma levels of total protein, albumin, glucose, and blood urea nitrogen were determined using an automatic biochemistry analyzer (7180; Hitachi High-Technologies Co., Ltd., Tokyo, Japan).

Cecal contents

The cecal content was obtained on day 14. The pH of cecal content was measured using a spear tip pH electrode (Oakton®; Nikko Hansen & Co., Ltd., Osaka, Japan). The content of several organic acids in cecal section (i.e., formate, acetate, propionate, isobutyrate, n-butyrate, isovalerate, n-valerate, succinate, and lactate) was determined by using an internal standard method, a high-performance liquid chromatography equipped with a Shim-pack SCR-102 H column (Shimadzu) and an electroconductivity detector (CDD-6 A; Shimadzu).

Statistical analysis

Data are presented as mean ± SD. Data were statistically analyzed by Tukey's multiple comparison test (SAS, CAC Croit Corporation, Japan); differences were considered statistically significant at $P < 0.05$.

Results

Analysis groups

Six rats (four in the LMP group and two in the HMP group) died of bleeding or obstruction of the intestine within 24 h of the anastomosis surgery, likely resulting from unsuccessful surgical procedures, and were excluded from the analysis.

The remaining rats tolerated the surgery and had no anastomotic leakage. The number of animals subjected to analysis was 6, 8, and 10 for the LMP, HMP, and PF groups, respectively.

Anastomosis bursting pressure and breaking strength

The bursting pressure value did not significantly differ among the three groups (Table 1). However, breaking strength was significantly higher ($P < 0.001$) in the LMP group than in the HMP and PF groups (Table 1).

Table 1
Anastomosis bursting pressure and breaking strength

	LMP (n = 6)	HMP (n = 8)	PF (n = 10)
Bursting pressure, mm Hg	170.44 ± 22.77	157.78 ± 19.07	167.36 ± 18.34
Breaking strength, N	2.77 ± 0.48	1.36 ± 0.20*	1.59 ± 0.30*

HMP, high-methoxyl pectin; LMP, low-methoxyl pectin; PF, pectin free
Rats were fed an LMP, HMP, or PF diet. Data are presented as mean ± SD
* $P < 0.001$ vs LMP group.

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