The Effect of Probiotics on Improving Intestinal Mucosal Permeability in the Postoperative Ileus Model

Min-Jae Kim

The Graduate School
Yonsei University
Department of Medicine

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Min-Jae Kim

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This certifies that the Dissertation of Min-Jae Kim is approved.

Thesis Supervisor	Hyojin Park
Thesis Committee Member	Sungsoon Fang
Thesis Committee Member	Jae Jun Park
Thesis Committee Member	Eun Jung Park
Thesis Committee Member	Chang Hwan Choi

The Graduate School
Yonsei University
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ABSTRACT

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Background: This study aimed to explore the mechanisms underlying the improvement of postoperative ileus (POI) through probiotic pretreatment. We assessed intestinal permeability, inflammation, tight junction (TJ) protein expressions in the gut epithelium, and plasma interleukin (IL)-17 levels in a guinea pig model of POI.

Methods: Guinea pigs were divided into control, POI, and probiotic groups. Animals in the POI and probiotic groups underwent surgery, with the probiotic group receiving probiotics before the procedure. Harvested tissues included the ileum and proximal colon. Intestinal permeability was measured by horseradish peroxidase absorbance. Inflammation was evaluated by leukocyte count in the intestinal wall muscle layer, and calprotectin expression in each intestinal wall layer was analyzed immunohistochemically. TJ proteins were analyzed through immunohistochemical staining, and plasma IL-17 levels were measured using enzyme-linked immunosorbent assay (ELISA).

Key results: The POI group exhibited increased intestinal permeability and inflammation, whereas probiotic pretreatment reduced these POI-induced changes. Probiotics restored the expression of TJ proteins occludin and zonula occludens-1 in the proximal colon, which were increased in the POI group. Calprotectin expression significantly increased in the muscle layer of the POI group and was downregulated in the probiotic group; however, no clear differences were observed between the mucosal and submucosal layers. Plasma IL-17 levels did not significantly differ among the groups.

Conclusions: Probiotics pretreatment improves POI by reducing intestinal permeability and inflammation and by restoring TJ protein expressions in the gut epithelium. These findings suggest a potential therapeutic approach for POI management.

Key words: postoperative complications, ileus, probiotics, intestinal permeability, tight junction protein

I. INTRODUCTION

Postoperative ileus (POI) refers to impaired gastrointestinal (GI) transit as a response to surgical stress.[1,2] It commonly occurs after GI surgery, affecting up to 30% of patients undergoing such procedure, although it can also manifest after other surgeries.[3] POI contributes to patient discomfort, complications, increased morbidity, and prolonged hospital stays, leading to significant healthcare costs, particularly in patients undergoing surgery.[4-6] Since knowledge of effective therapies for POI is still limited, it represents a major complication in surgery, especially abdominal surgery.[1]

Numerous studies have investigated the underlying mechanisms of POI, revealing the involvement of inflammatory, pharmacological, hormonal, and neurogenic factors. However, the precise pathophysiology of POI is still not completely understood.[7]

Intestinal permeability, closely associated with intestinal inflammation, is regulated by tight junction (TJ) proteins in the GI epithelium. Alterations in intestinal permeability and TJ proteins have been observed in various diseases, including inflammatory bowel disease (IBD), tumoral disease, irritable bowel syndrome (IBS), metabolic diseases, and autoimmune diseases.[8-11] In a previous study on an animal model of POI, increased intestinal inflammation and permeability were accompanied by changes in TJ proteins such as claudin-1 and claudin-2.[12,13]

The gut microbiota plays a crucial role in influencing bowel diseases and various aspects of host physiology, including nutrient, xenobiotic, and drug metabolism, maintenance of the gut mucosal barrier's structural integrity, immunomodulation, and protection against pathogens.[14,15] Dysbiosis of the gut microbiota, a major concern for multiple diseases, is associated with the pathogenesis of both intestinal and extra-intestinal conditions.[16] Surgical interventions, particularly GI surgery, induce physiological stress, which can transform intestinal bacteria into a more virulent phenotype.[17-19] A previous study on an animal model of POI demonstrated the induction of gut bacterial dysbiosis following surgery. Administration of probiotics before surgical intervention prevented a decrease in beneficial intestinal bacteria, butyrate production, and bowel movement.[20] However, the mechanisms connecting the improvement in colonic transit time and gut microbiota dysbiosis through probiotic pretreatment have not been investigated yet.

Therefore, the objective of this study was to explore the mechanisms by which probiotic pretreatment improves POI. We measured intestinal permeability, intestinal inflammation, TJ protein

expression in the GI epithelium, and plasma interleukin (IL)-17 levels in a guinea pig model of POI.

II. MATERIALS AND METHODS

2.1. Preparation of animals

Adult male Hartley guinea pigs (Orient Bio Inc., Seoul, Korea) weighing 250–350 g were acclimatized to controlled breeding conditions for at least 1 week prior to the surgical intervention. The conditions included including a temperature of 20–22 °C, humidity of 50 ± 10%, and a 12-hour light/dark cycle commencing at 7 AM. The guinea pigs had ad libitum access to edible water and a diet consisting of ≥16% crude protein, ≥2.0% crude fat, ≤20% crude fiber, ≥0.8% calcium, and ≥0.52% phosphorus. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee, Department of Laboratory Animal Resources, Yonsei Biomedical Research Institute, Yonsei University College of Medicine, with an Institutional Review Board (IRB) protocol number 2020-0271.

2.2. Group setting

The guinea pigs were randomly assigned to control, POI, and probiotic groups with 8–9 animals per group. The control group did not receive any manipulation or drugs before tissues or serum collection. The POI and probiotic groups underwent the following surgical procedure: after a 24-hour fast (except for water) prior to the procedure, a mixture of Zoletil, Rompun, and saline was injected into the abdominal cavity. After 15 min, the abdomen was shaved and disinfected with an alcohol swab. A minimal peritoneal incision was made after incising the abdominal skin and muscle layers. The cecum was extracted, gently rubbed with wet gauze for 1 min using the fingers, and then sutured.

The probiotic group guinea pigs received 50 mg kg^{-1} probiotics (5 mg 2.5×10^5 CFU *Enterococcus faecalis*, 25 mg 2.5×10^5 CFU *Bacillus mesentericus*, and 25 mg 5×10^5 CFU *Clostridium butyricum*) mixed with buffered saline via an intragastric tube once daily for 5 days before the surgical procedure. The POI group guinea pigs received buffered saline for 5 days before the surgical procedure. The purpose was to determine the effect of the buffer on the efficacy of probiotics.

The ileum and proximal colon tissues were harvested from each guinea pig. The tissues were harvested from the POI and probiotic group animals 6 h post-operation based on previous reports evidencing high inflammatory cell counts and intestinal permeability in the ileum and proximal colon 6 h post-operation.[12,13]

2.3. Intestinal permeability

To evaluate the intestinal permeability, the harvested tissues were placed in a modified Ussing chamber. Each half of the tissue was bathed with 2 mL Krebs–Ringer bicarbonate (KRB) solution to cover the mucosal and serosal sides of the specimens. A gas mixture of 95% O_2 and 5% CO_2 was provided to both sides at 37 °C. After a 30-minute equilibration period, the KRB solution on the mucosal side was replaced with KRB solution containing horseradish peroxidase (HRP) at a final concentration of 0.4 mg mL^{-1}. The KRB solution on the serosal side was replaced with fresh KRB solution, and a 0.3 mL sample was collected from the serosal side and replaced with 0.3 mL KRB. The serosal samples were enzymatically analyzed using the modified Worthington method with o-dianisidine dihydrochloride (OPD; Sigma Chemical Co., St Louis, MO, USA) as the substrate. Samples (50 μL) were transferred to microtiter plates, and 100 μL of OPD working solution (diluted 1:10 in OPD) as a stable peroxide buffer was added to each well. Subsequently, the plates were incubated with shaking at 300 rpm at room temperature. After 30 min, 100 μL of 2.5 M sulfuric acid was added. After 10 min, the absorbance of the decolorized products was measured at 492 nm using a microplate reader (Model 680; Bio-Rad Laboratories Inc., Hercules, CA, USA). All samples were analyzed in duplicate, and the concentrations were calculated using a standard curve. The HRP flux was represented as ng 2 h^{-1} mm^{-2} during steady-state permeation. Intestinal permeability via the Ussing chamber was expressed as a percentage change compared to the mean flux of the control group animals.

2.4. Expression of Occludin, Zonula occludens (ZO)-1

Occludin and ZO-1 expression was determined immunohistochemically. The ileum and proximal colon tissues collected 6 h post-operation were fixed in 4% paraformaldehyde, embedded in paraffin, and sliced into 4 μm thick sections. The sections were deparaffinized, rehydrated, and rinsed using standard methods. Subsequently, they were incubated overnight with the primary antibodies for

occludin (1:100; Invitrogen, South San Francisco, CA, USA) or ZO-1 (1:500; Invitrogen) at 4 °C, followed by washing and incubation with the secondary anti-rabbit IgG antibody (1:200; Santa Cruz Biotechnology) for 30 min at 37 °C. The stained samples were incubated with streptavidin-HRP for 30 min, treated with AB peroxidase solution, and counterstained with hematoxylin. Images were analyzed using MetaMorph (MDS Analytical Technologies, Sunnyvale, CA, USA) microscopy automation and ImageJ (NIH and LOCI, University of Wisconsin, USA) software.

2.5. Intestinal inflammation

The harvested ileum and proximal colon muscle layers were sectioned, fixed in 10% neutral-buffered formalin, and embedded in paraffin. The embedded sections were sliced into 4 μm thickness and stained with hematoxylin and eosin. Leukocyte counts were compared between the control and POI groups, as well as the POI and probiotic groups, using a semi-quantitative scoring system.

2.6. Expression of Calprotectin

Calprotectin expression was determined using immunohistochemical analysis. The paraffin-embedded ileum and proximal colon sections were deparaffinized and incubated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. The tissue sections were incubated overnight at 4 °C with the primary antibody anti-calprotectin (1:250; ThermoFisher, Waltham, MA, USA). After three washes with phosphate-buffered saline, they were incubated with the secondary antibody anti-mouse IgG (1:200; Vector Laboratories). Next, the sections were incubated with streptavidin-HRP for 30 min, treated with AB peroxidase solution, and counterstained with hematoxylin. The images were analyzed using MetaMorph and ImageJ software. When analyzing slide images, each intestinal layer was manually designated and separately analyzed.

2.7. Plasma interleukin (IL)-17

Blood samples were collected via cardiac puncture before euthanizing the animals. The plasma was separated by centrifugation and then stored at -70 °C until the assay. Plasma IL-17 levels were determined using an ELISA kit (MyBioSource, San Diego, CA, USA) according to the manufacturer’s instructions.

2.8. Statistical methods

The data are expressed as the mean ± SE, and statistical analysis was performed using non-parametric tests (Mann–Whitney U test for two groups and Kruskal–Wallis H test for multiple comparisons). SPSS version 26.0 (IBM Corp, Armonk, NY, USA) was used for statistical analysis. A two-tailed $p<0.05$ indicated statistical significance.

III. RESULTS

3.1. Intestinal permeability

Intestinal permeability was assessed by measuring HRP absorbance in the ileal and proximal colonic samples of the control, POI, and probiotic groups (eight, nine, and eight samples, respectively) (Figure 1).

The HRP absorbance in both the ileal and proximal colonic tissues of the POI group animals was significantly higher than that in the control group animals ($p=0.046$ and 0.13 in the ileum and proximal colon, respectively). However, pre-administration of probiotics decreased the permeability ($p=0.021$ and 0.236 in the ileum and proximal colon, respectively).

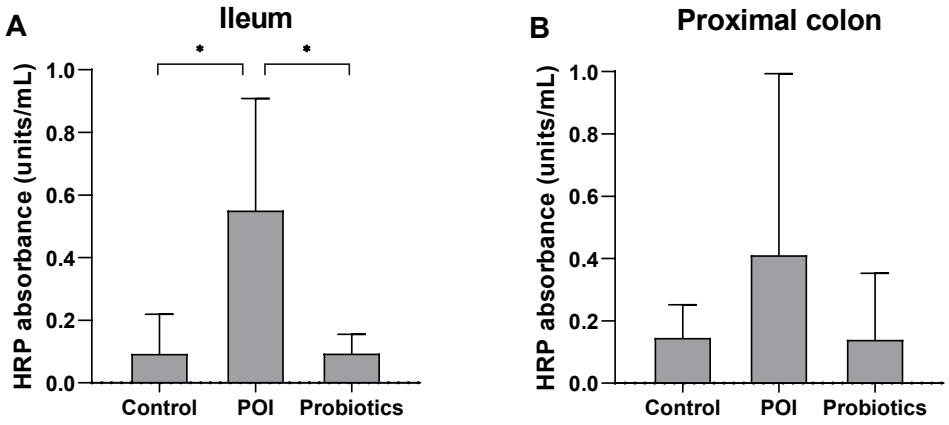


Figure 1. Intestinal permeability in the ileum and proximal colon of the control, postoperative ileus (POI), and probiotic group animals. HRP absorbance was measured 6 h post-operation. Bars indicate the mean ± SEM (control group, n=8; POI group, n=9; probiotic group, n=8). (*p<0.05 with respect to that in the POI group animals).

3.2. Expression of Occludin, ZO-1

Occludin and ZO-1 expression levels were measured by analyzing images of the immunohistochemically-stained ileum and proximal colon tissues. In the proximal colon, occludin expression was lower in the POI group animals than in the control group animals (p=0.431); however, no significant differences were observed in the ileal tissue (Figure 2A). Administration of probiotics improved the expression of occludin in the proximal colon (p=0.064), while no significant differences were observed in the ileum.

Similar results were observed for ZO-1 expression levels. No significant differences were observed in the ileal tissue among the three groups. However, ZO-1 expression in the proximal colon of the POI group animals was significantly lower than that in the control group animals (p=0.047). ZO-1 expression increased in the probiotic group animals than that in the POI group animals (p=0.002; Figure 3A).

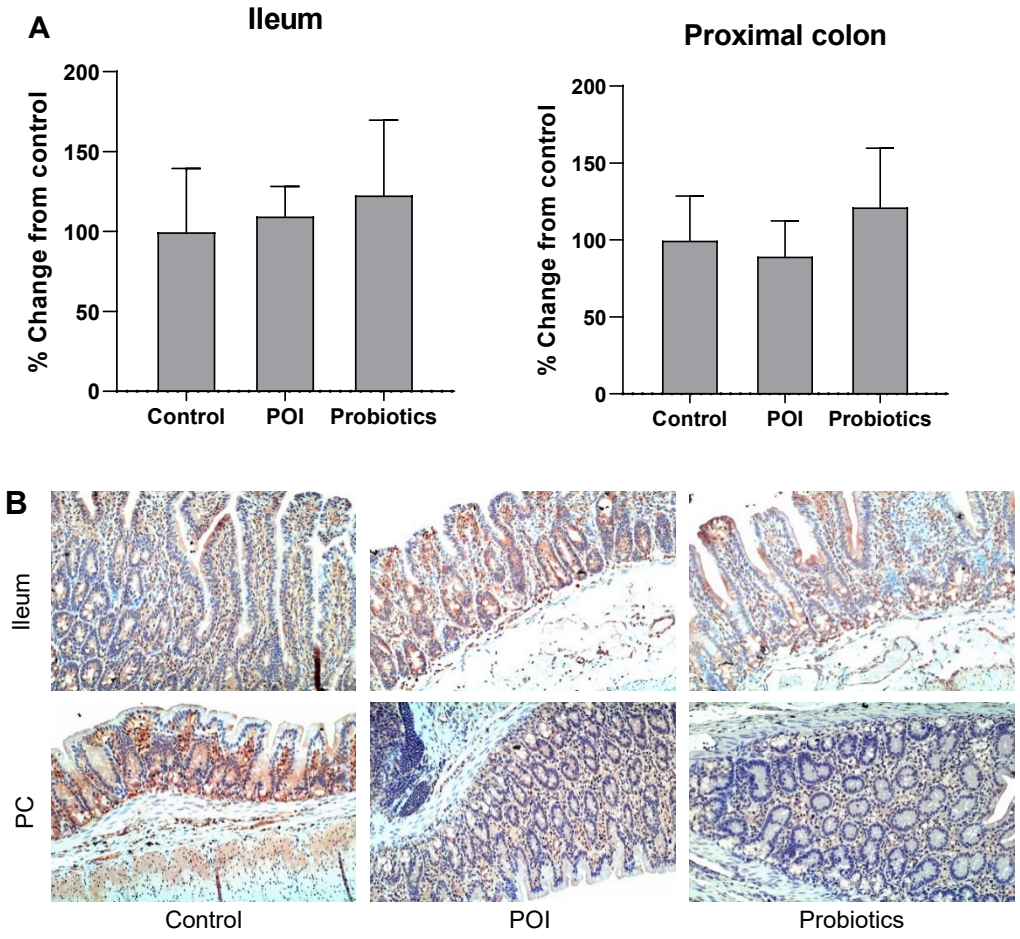


Figure 2. (A) Occludin expression in the ileum and proximal colon of the control, postoperative ileus (POI), and probiotic group animals. (B) Representative immunohistochemical occludin staining in the ileum and proximal colon of the control, POI, and probiotic group animals 6 h post-operation. Bars indicate the mean ± SEM (control group, n=8; POI group, n=8; probiotic group, n=8). PC, proximal colon.

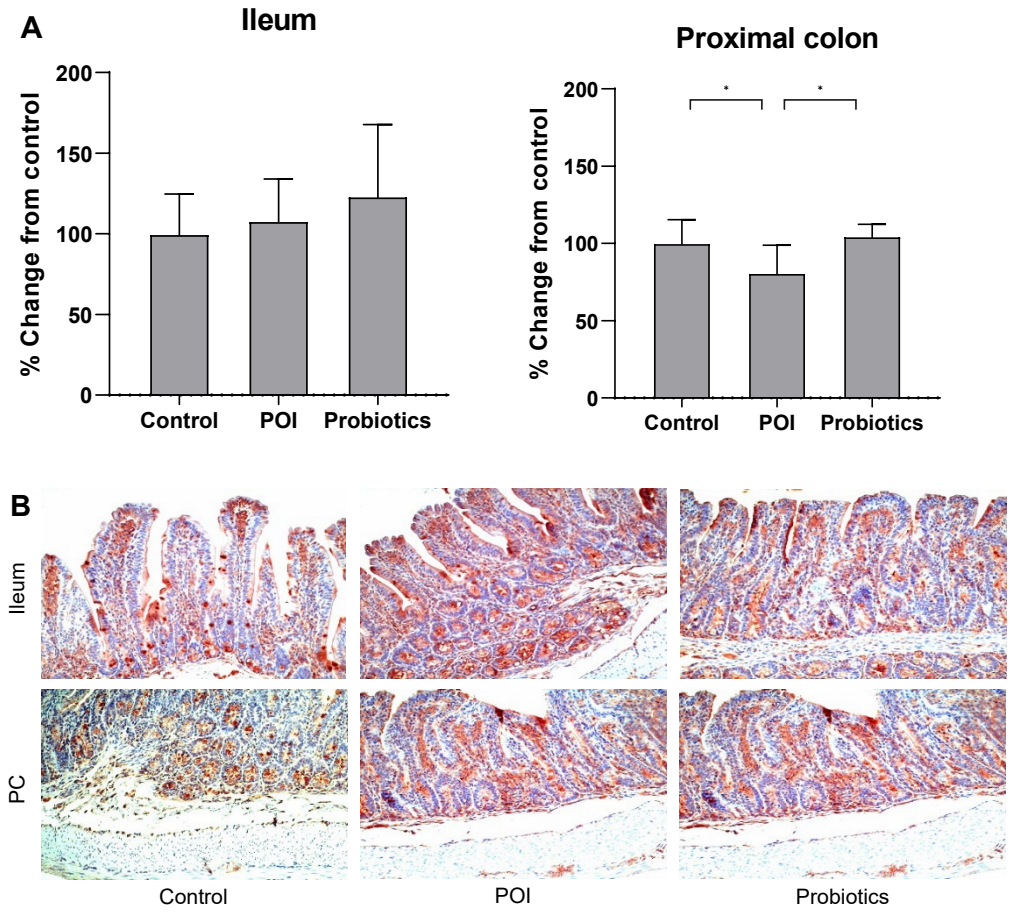


Figure 3. (A) Expression of zonula occludens (ZO)-1 in the control, postoperative ileus (POI), and probiotics group. (B) Representative immunohistochemical ZO-1 staining in the ileum and proximal colon of the control, POI, and probiotic group animals 6 h post-operation. Bars indicate the mean ± SEM (control group, n=7; POI group, n=7: probiotics group, n=8). (*p<0.05 with respect to that in the POI group animals) PC, proximal colon.

3.3. Intestinal inflammation

Analysis of leukocyte counts evidenced that intestinal inflammation in both the ileal and proximal colonic muscle layers of the POI group was significantly higher compared to the control group (p=0.001). In contrast, the leukocyte count in both the ileal and proximal colonic muscle layers of the probiotic group was significantly lower than that in the POI group (p=0.001 and 0.028 in the ileum and proximal colon, respectively; Figure 4).

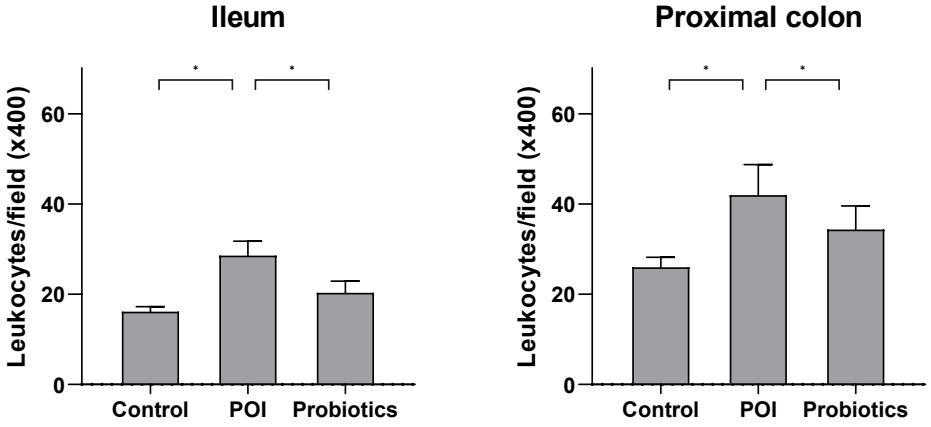
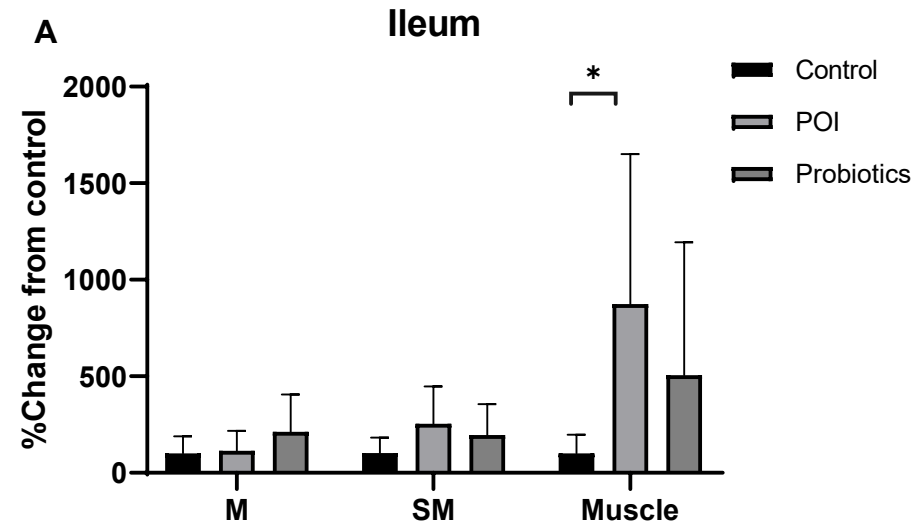
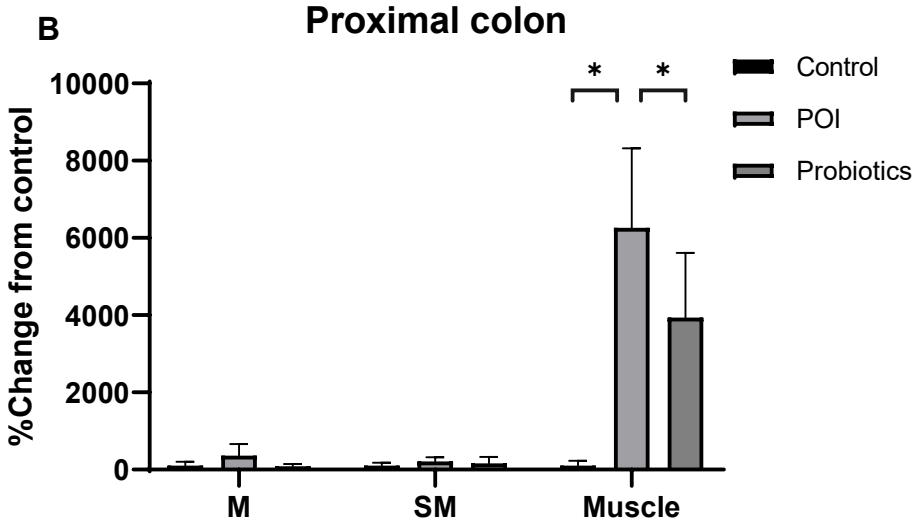


Figure 4. Leukocyte count field^{-1} (400x in the ileum and proximal colon of the control, postoperative ileus (POI), and probiotic group animals. Bars indicate the mean ± SEM (control group, n=8; POI group, n=8; probiotic group, n=8). (*p<0.05 with respect to that in the POI group).

3.4. Expression of Calprotectin

Calprotectin expression in each intestinal wall layer was analyzed immunohistochemically. We classified and designated the mucosal, submucosal, and muscle layers in the images for separate analysis. Calprotectin expression in the ileal and proximal colonic mucosal and submucosal layers of the control, POI, and probiotic groups were not significantly different. However, calprotectin expression in the ileal or proximal colonic muscle layer of the POI group was significantly higher than that in the control group (p=0.001) and probiotic group (p=0.001 and 0.028 in the ileum and proximal colon, respectively; Figure 5A and B).





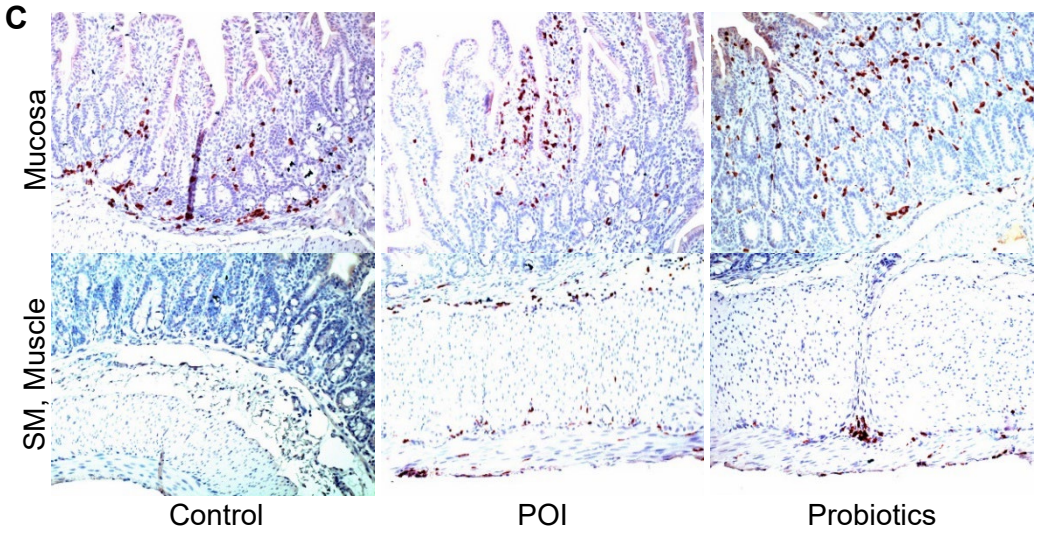


Figure 5. Calprotectin expression in the intestinal wall mucosal, submucosal, and muscle layers of the ileum (A) and proximal colon (B) of the control, postoperative ileus (POI), and probiotics groups. Bars indicate the mean ± SEM (control group, n=8; POI group, n=8; probiotic group, n=8). M, mucosal layer; SM, submucosal layer; Muscle, muscle layer.

3.5. Plasma IL-17

The plasma IL-17 levels did not differ significantly among the three groups (Figure 6).

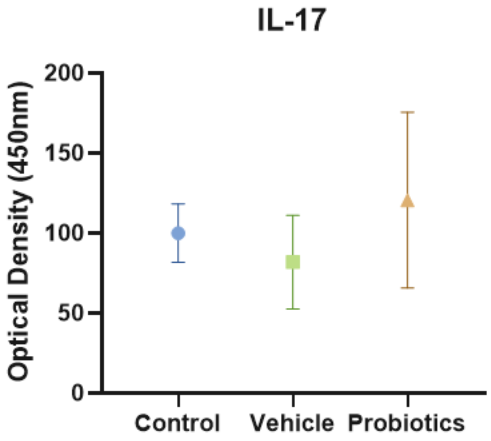


Figure 6. Plasma interleukin (IL)-17 levels in the control, postoperative ileus (POI), and probiotic group animals. Bars indicate the mean ± SEM (control group, n=8; POI group, n=8; probiotic group, n=8).

IV. DISCUSSION

Probiotics are used to rectify gut microbiota dysbiosis in various diseases.[21] In this study, we found that intestinal permeability and inflammation were significantly downregulated in the probiotic group animals compared to those in the POI group animals (Figure 1). A previous study has evidenced that gut microbiota composition significantly changed before and after surgical intervention in guinea pigs, as the count of *Bifidobacterium* and *Lactobacillus*, known as lactic acid-producing bacteria, decreased and *Bacteroides* and *Blautia* counts increased, and pretreatment with probiotics prevented these changes in microbiota components and delay in colonic transit time.[20] It had determined that intestinal manipulation in a mouse model increases intestinal permeability and significantly increases translocation of aerobes and anaerobes into the tissue than laparotomy alone, which may be an important factor influencing the development and persistence of POIs.[22] In addition,

gut microbiome dysbiosis increases intestinal permeability in various chronic diseases, which in turn causes secondary inflammation.[23] We have previously demonstrated that increased gut paracellular permeability is strongly associated with the typical features of POI, particularly delayed contractile activity recovery and increased inflammation.[13] Our finding suggest that probiotic pretreatment reduces the incidence and degree of POI by preventing increased intestinal permeability.

Increased intestinal permeability after surgical stimulation enhance the movement of pathogen-associated molecules from the intestinal lumen to tissues, producing an inflammatory response in the intestinal muscle layer.[24,25] In this study, the increased leukocyte count in the muscle layer of the POI group animals compared to that in the control group animals supports this hypothesis. Moreover, the decreased leukocyte count in the probiotic group animals compared to that in the POI group animals seems to be caused by preventing the increase in intestinal permeability. Several studies already have evidenced that gut microbiota dysregulation is closely associated with the increased intestinal permeability in chronic diseases like obesity, diabetes, IBD, IBS, cirrhosis, autoimmune diseases, and even prolonged psychological stress.[26-31] Moreover, intestinal permeability increases in some acute diseases such as colitis and acute pancreatitis.[32,33] Probiotics are expected to improve altered intestinal permeability by modifying gut microflora, dietary proteins, and bacterial enzyme activity but most studies have evidenced that probiotics usually did not reduce the already increased intestinal permeability in chronic diseases, colitis, and acute pancreatitis.[34-38] Only few studies have reported that probiotic consumption improved intestinal permeability. Ait-Belgnaoui et al. reported that probiotic pretreatment prevented an increase in intestinal permeability in an acute psychological stress rat model but did not evidence a positive effect on the increase of intestinal permeability in a chronic psychological stress rat model.[39,40] Liu et al. reported occludin and ZO-1 restoration in an autoimmune hepatitis mouse model after compound probiotic treatment.[41] Few animal studies, including ours, have confirmed the effect of probiotics on improving intestinal permeability. Although the reason for this improvement remains unclear, it may be related to the differences in the pathophysiology of each disease, intestinal permeability measurement methods, probiotic strains used, and probiotic intake method or dose.

TJ proteins are integral transmembrane proteins found in the tight junctions of all epithelia and endothelia, which mediate cell-to-cell adhesion and seal the paracellular space between epithelial cells.[42] TJ structure regulation is influenced by various physiological and pathological stimuli, and its disruption increases intestinal permeability, which is closely related to various diseases.[43]

Occludin and ZO-1 are TJ proteins localized at endothelial cell junctions, which associate with each other to create a complex compound.[44] Occludin maintains the integrity and barrier function of the TJ, and ZO-1 is an important liker protein in TJ, binding to C-terminal sequences of occludin and beta-actin and acting as a bridge between the plasma membrane and cytoskeleton proteins.[45] Increased occludin and ZO-1 expression accompanies reduced intestinal permeability.[46] In this study, occludin and ZO-1 expression decreased and increased in the colonic tissues of POI and probiotic group animals, respectively (Figures 2, 3). This supports the hypothesis that probiotics prevent an increase in intestinal permeability by preventing TJ protein downregulation, thereby preventing POI. However, TJ protein expression in the ileal tissue was not significantly different among the three groups. It is unclear why there were differences in the changes in TJ protein expression in each organ. In a previous study, the expression of claudin-1, another TJ protein that regulates intestinal permeability, was statistically significantly reduced in both the ileum and proximal colon of the guinea pigs POI group compared to that in the control group.[13] This suggests that the TJ proteins affected by the pathophysiology of POI in the proximal colon and ileum are different, but further experiments are required to confirm this.

Leukocyte infiltration in the intestinal muscular layer increased in the POI group (Figure 4), indicating intestinal wall inflammation. According to several studies, POI pathophysiology consists of several steps involving various factors.[1,3,25] Neural dysfunction is predominant in the early stages, and intestinal inflammation is considered an important contributor in the late stages.[1,47] In a POI animal model, intestinal manipulation induced inflammation-mediated impaired smooth muscle contraction, which is one of the main mechanisms of POI.[47] In our study, probiotics prevented intestinal muscle layer inflammation followed by intestinal manipulation. Intestinal smooth muscle inflammation is associated with reduced smooth muscle contraction in multiple diseases and clearly connected to the intestinal microbiota. Several studies have reported a crosstalk between microbiota, intestinal wall adipose tissue, and muscle in intestinal inflammation,[48] and probiotics should improve intestinal inflammation in various diseases.[49] In fact, several animal and human studies have reported that probiotics improve intestinal inflammation. However, no clear mechanism for suppressing the incidence or degree of inflammation in the intestinal muscle layer is known.

We evaluated calprotectin expression in each layer, which was not significantly different in the mucosal and submucosal layers; however, calprotectin expression was markedly increased in the muscle layer of the POI animal model (Figure 5). Calprotectin, an abundant calcium-binding protein

belonging to the S100 family, is derived predominantly from neutrophils, monocytes, and macrophages. It has direct antimicrobial effects and plays a role in innate immune responses. Clinically, fecal calprotectin is a useful surrogate marker of gastrointestinal inflammation.[50] We analyzed calprotectin expression in each layer to determine whether the mechanism by which probiotics inhibit POI occurs through changes in the mucosal layer, where intestinal microorganisms and their products directly contact, but any noticeable changes was not confirmed in the mucosal layer. If toxic substances or bacteria penetrated the intestinal wall during POI and caused an inflammatory reaction in the muscle layer, an increase in calprotectin accompanied by an inflammatory reaction would have been confirmed in the mucosal and submucosal layers. However, this hypothesis was rejected by the results. The increased calprotectin expression in the muscle layer was attributed to increased macrophage activity. Macrophages residing in the muscularis externa of the GI tract are highly specialized cells essential for tissue homeostasis during steady-state conditions as well as during disease.[51] They closely communicate with the enteric nervous system and regulate colonic peristalsis by changing the pattern of smooth muscle contractions in both the inflammatory and steady states.[48] Particularly during inflammation, muscularis macrophages secrete inflammatory cytokines and recruit inflammatory cells, which further accelerate the inflammatory process.[52-54] A previous study using a murine POI model reported that vagus nerve stimulation reduced intestinal inflammation by activating cholinergic enteric neurons in close contact with muscularis macrophages.[55] It seems that regulatory function of probiotics in POI indirectly affect muscularis macrophage through the nervous system, but the exact mechanism is still unclear. However, previous studies have evidenced that short-chain fatty acids (SCFAs), such as butyrate, affect the central nervous system by altering the expression of brain-derived neurotrophic factor, and pre-administration of probiotics in the POI guinea pig model inhibited the decrease in fecal butyrate levels after surgery. This suggests that it may be related to the fact that SCFAs produced by pretreated probiotic-regulated gut microbiota inhibited the increased inflammatory response in the muscle layer.[20,56]

IL-17 is a well-known proinflammatory cytokine that increases intestinal inflammation, such as in IBD and colitis. Plasma IL-17 level did not increase in this study, which seems to be due to the small role of systemic cytokine in the acute phase of POI generation, However, since IL-17 is also secreted from muscularis macrophages, it is expected to rise in the POI group in the long term; however, this rise was not observed possibly due to timing of blood sampling, 6 h after surgical

intervention. A recent study has reported that gut microbiota-derived SCFAs regulate IL-17 production by intestinal γδ T cells in mouse and human; however, at least in the early stages of POI development, the effect of IL-17 on pathophysiology is not significant and probiotics are not the direct cause for POI prevention.[57]

Guinea pigs are better models of certain human medical conditions than other rodents. E-cadherin on the intestinal surface of guinea pigs is homologous to that of humans, which serves as the primary receptor interacting with bacteria upon initiation of intestinal invasion.[58] Hildebrand et al. compared the intestinal metagenomes in guinea pigs and humans, which were highly similar at the phylum level.[59] Therefore, we chose guinea pigs as an experimental model because guinea pigs may represent a suitable model for investigating the microbiota-dependent effects.

Our study had several limitations. First, we have previously proved the occurrence of POI in guinea pigs as a delay in colonic transit time;[20] however, since we did not check the occurrence of POI in each individual in this study, we could not accurately confirm the occurrence and extent of POI in the individuals used in this experiment. The preoperative administration of probiotics can inhibit the occurrence of POI by preventing an increase in intestinal permeability; however, we did not identify how probiotics improve intestinal permeability. Probiotics inhibit damage to occludin and ZO-1, particularly in the colon. However, how probiotic administration can have a positive effect on TJ proteins is not known yet. In addition, since the same probiotic dose and strain were administered to all probiotic group animals, which probiotic strain should be administered at what dose for optimum outcomes is still unknown. Owing to the species differences between guinea pigs and humans, further research on the dosage and strains should be conducted when applying them to humans. Despite many human studies, the most appropriate strain, dose, and duration of probiotic use to treat each disease are still not well understood. Several meta-analyses have compared the effectiveness of probiotics under different conditions; however, the strains, doses, and durations of probiotics considered effective for each condition appear to differ. For example, one meta-analysis found that a short duration of probiotic use was effectively improved overall IBS symptoms, but the articles analyzed assumed months of probiotic use.[60] In contrast, 65% of the investigated articles in a meta-analysis of probiotic use in acute infectious diarrhea in pediatric patients reported probiotic use for 5 days.[61] Therefore, even if the conclusions of these animal studies hold true in humans, further research is needed to determine the most effective way to administer probiotics.

V. CONCLUSION

POI increases intestinal mucosal permeability, which seems to be closely related to the altered TJ proteins, occludin, and ZO-1 expression. Even though inflammation of the intestinal muscular layer plays an important role in POI development, the mucosal and submucosal layers were not inflamed. Preoperative probiotic administration prevents both increased intestinal permeability and intestinal muscular layer inflammation; therefore, it can be considered a preventive measure of POI.

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Abstract in Korean

수술 후 장 마비 모델에서 프로바이오틱스의 장관 점막 투과도 개선 효과

수술 후 장 마비는 수술 환자들의 부작용 및 재원기간을 늘리는 원인이다. 본 연구에서는 프로바이오틱스 전처치가 어떻게 수술 후 장 마비를 개선할 수 있는지 그 메커니즘을 규명하기 위해 수술 후 장 마비 기니피그 모델을 대상으로 실험을 진행하였다. 기니피그를 무작위로 대조군, 수술 후 장 마비군, 프로바이오틱스 치료군의 세 그룹으로 나누었다. 수술 후 장 마비군 및 프로바이오틱스 치료군은 외과적 시술을 받았고, 프로바이오틱스 치료군은 수술 전 5일 동안 매일 1회 50mg/kg의 프로바이오틱스 혼합물을 투여했다. 수술 6시간 후 회장과 근위 결장에서 조직을 채취했다. 장 투과성은 horseradish peroxidase 흡광도를 측정하여 평가했다. 장벽의 염증은 장벽 근육층의 백혈구 수를 측정하여 평가하였고, Calprotectin의 발현은 면역조직화학염색 슬라이드의 각 층별 분석을 통해 측정했다. 면역조직화학염색 이미지 분석으로 Tight junction protein (Occludin, Zonula occludens −1)을 분석하고 ELISA를 이용하여 혈장 IL−17 수치를 측정하였다.
장 점막 투과도와 장 근육층의 염증이 수술 후 장 마비군에서 증가했으며 프로바이오틱스로 전처치한 군에서 수술 후 장 마비로 인한 장 투과성 및 염증 증가가 예방되었다. 또한 프로바이오틱스는 수술 후 장 마비군의 근위결장 조직에서 Tight junction protein(Occludin, Zonula occludens −1)이 감소하는 것을 방지했다. Calprotectin 발현 수준은 수술 후 장 마비군의 근육층에서 유의하게 증가했고 프로바이오틱스 치료군에서는 하향 조절되었지만 점막과 점막하층에서는 뚜렷한 차이가 나타나지 않았다. 혈장 IL−17은 두 그룹 간 차이를 보이지 않았다. 프로바이오틱스로 수술 전 전처치하는 경우 장 투과성, 염증을 줄이고 장 상피의 긴밀한 접합 단백질을 복원하여 수술 후 장 마비를 개선할 수 있다. 이 연구는 수술 후 장 마비 관리를 위한

잠재적인 치료 접근법을 제공하며 프로바이오틱스의 치료 메커니즘을 일부 확인하는데에 의의가 있다.

핵심되는 말 : 수술 후 합병증, 장 마비, 프로바이오틱스, 장 투과성, 밀착연접 단백질