An altered composition of fecal microbiota, organic acids, and the effect of probiotics in the guinea pig model of postoperative ileus

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All glory to God.

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ABSTRACT

An Altered Composition of Fecal Microbiota, Organic Acids, and the Effect of Probiotics in the Guinea Pig Model of Postoperative Ileus

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Background: The aim of this study is to investigate the altered composition of fecal microbiota, organic acids, and the effect of probiotics in the guinea pig model of the postoperative ileus (POI).

Methods: A laparotomy with cecal manipulation was performed to induce POI in guinea pigs. Fecal pellets were collected before the operation (the baseline) and 1 day, 3 days, and 5 days after the operation. The extracted fecal DNA was amplified and sequenced using the Illumina MiSeq sequencing system. The same POI procedures were performed after oral pretreatment of the probiotics for 7 days before operation. The effect of the probiotics on the selected taxa and fecal acetate were evaluated, as were the butyrate levels. The colonic transit was assessed by measurement of the fecal pellet output.

Results: The communities of the baseline and POI groups indicated significantly distinct composition. The genera *Bifidobacterium* and *Lactobacillus* were more abundant in the baseline group compared with the POI groups, and *Bacteroides* and *Blautia* were more abundant in the POI groups. Decreased abundances of the species *Bifidobacterium bifidum* and *Bifidobacterium longum* after the POI procedure were

significantly increased in the probiotics group. The decreased fecal butyrate level after the POI procedure was significantly increased, and colonic transit was significantly improved in the probiotics group.

Conclusions: POI induces gut bacterial dysbiosis. Moreover, pretreatment of probiotics before operation restores the beneficial bacterial species, butyrate production, and bowel movement. The modulation of gut microbiota may help the treatment and prevention of POI.

Key words: ileus, postoperative complications, microbiota, butyrates, probiotics

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I. INTRODUCTION

Postoperative ileus (POI) is the disturbance of gastrointestinal (GI) motility in response to surgical stress.[1,2] POI is also defined as the interval from surgery until the passage of flatus or a stool, and tolerance of an oral diet.[3] POI is not only a major concern after surgical operations but also a significant public health problem, because it increases the length of hospitalization, postoperative morbidity, and the socio-economic costs.[4,5] However, the understanding of POI remains insufficient. Numerous strategies have been suggested to prevent and treat POI, but they have also showed conflicting results.[6,7]

Gut microbiota, including approximately 10^{14} prokaryotic organisms with a biomass greater than 1kg, represent a complex and dynamic microbial ecosystem in the human colon.[8,9] They engage in nutrient metabolism, epithelial cell functions and inflammatory signaling, which are closely related to the host's immune system.[9,10] This immune system develops from the interaction of gut microbiota and pathogens, and short chain fatty acids (SCFAs), especially butyrate and acetate, have been suggested as key mediators for this interaction.[11-13] Because SCFAs are microbial fermentation products of dietary fiber, their concentrations are closely affected by the composition of the bacterial community. Although the composition of gut microbiota is influenced

by various factors like lifestyle, including even one day of diet alteration, the overall composition maintains the balance in most healthy individuals.[14,15]

However, previous studies have shown that stressful conditions such as major burn injury and tissue ischemia induce alteration in their community, resulting in a dysbiosis.[13,16,17] Surgical intervention, which is one of the greatest causes of physiologic stress, has also been shown to induce a distinct shift in the composition of gut microbiota.[18,19] Recently, increasing evidence has indicated that gut microbiota play a key role in the development of surgical complications. A substantial alteration in gut microbiota induced by surgical stress has been suggested as a factor in infectious complications after GI surgery.[20] The development of anastomotic leakage has also been found to be associated with gut microbiota.[18,21] Accordingly, much effort has been made to find out about the bacterial shift and to modify the community using probiotics or synbiotics in patients with these surgical complications.[22] Several beneficial effects of probiotics have been shown to occur in infectious complications, including reducing anastomotic leakage, and the length of hospital stay.[23,24] However, to date, little has been known about the relationship between gut microbiota and POI. Trying to identify changes in gut microbiota developed with POI and modulate them might help to understand the complex mechanism of POI and prevent this significant clinical burden.

Human study has several limitations that development of POI is unpredictable, and various uncontrolled situations which affect microbial community can occur before and after surgery**.** Therefore, this study aims to explore the compositional changes of fecal microbiota, acetate, butyrate and the effect of probiotics in the guinea pig model of POI.

II. MATERIALS AND METHODS

1. Preparation of the Animals

Adult male Hartley guinea pigs (Orient Bio Inc., Gyeonggi-do, Korea)

weighing 300-350g were used in the present study. The guinea pigs were acclimated to their holding room (21 ± 1 °C, 50 ± 10% humidity, and 12-hour light/dark cycle commencing at 7 AM), and a standard guinea pig diet and water were provided for at least 1 week prior to surgery. All the experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Department of Laboratory Animal Resources, Yonsei Biomedical Research Institute, Yonsei University College of Medicine with Institutional Review Board (IRB), protocol number 2018-0005.

2. Experimental Design

A. Experiment 1. Compositional Changes of the Fecal Microbiota in the POI Model

(A) Sample Collection and Preparation

In the first series of experiments, the compositional changes of fecal microbiota in the POI model were investigated. Fecal pellets were collected before (baseline) and 1 day (D-1) and 3 days (D-3) after the POI procedure. POI is known to last at least two to four days, and it is suggested that abdominal surgery triggers two different phases; the neural-mediated first phase begins immediately after operation, and the inflammatory second phase starts 3-4 hours after surgery.[25,26] Thus, three days was thought to be enough to investigate changes in microbial community after operation based on the next-generation sequencing.

POI procedure which has been already established through previous animal studies,[27-29] was performed as follows: the guinea pigs were subjected to a 24-hour fast with liberal access to water before being anesthetized by intraperitoneal injection with Zoletil 4ml + Rompun 2ml + Saline 8ml mixture. After that, a laparotomy took place, consisting of an incision of 3-4cm through the abdominal skin and muscle layers, followed by evisceration and gentle manipulation of the cecum using a wet gauze for 60

seconds, closed by suture. After the operation, they allowed to have free access to food and water, and the food was provided with the same kind of feed. The collected fecal samples were immediately placed in a freezer and stored at -80℃.

(B) Fecal Microbiota Analysis

PCR amplification was performed using barcoded primers targeting the V3 to V4 region of the bacterial 16S rRNA gene. For bacterial amplification, primers of 341F (5'-TCGTCGGCAGCGTC-AGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3') and 805R (5'-GTCTCGTGGGCTCGG-AGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3') were used. The amplifications were carried out under the following conditions: initial denaturation at 95 ℃ for 3min, followed by 25 cycles of denaturation at 95 ℃ for 30 sec, primer annealing at 55 ℃ for 30 sec, and extension at 72 ℃ for 30 sec, with a final elongation at 72 ℃ for 5 min. Then, secondary amplification for attaching the Illumina NexTera barcode was performed with an i5 forward primer 5'-AATGATACGGCGACCACCGAGATCTACAC-XXXXXXXX-CGTCGGCAGCGTC-3';X indica tes the barcode region) and an i7 reverse primer 5'-CAAGCAGAAG ACGGCATACGAGAT-XXXXXXXX-GTCTCGTGGGCTCGG-3').

B. Experiment 2. Effect of Probiotics on Selected Taxa and Organic Acids

(A) Probiotics, Placebo, and Control Group

The probiotics used were a mixture of 5mg *Enterococcus faecalis* ($2.5x10^5$ CFU), 25mg *Bacillus mesentericus* ($2.5x10^5$ CFU), and 25mg *Clostridium butyricum* ($5.0x10^5$ CFU). The 50m/kg of probiotics mixed with buffered saline were administrated via an intragastric tube once daily for 1 week before the POI procedure (the "Probiotics group"). The same amount of normal saline was administrated during the same period to other subjects (the "Placebo group"). Subjects without administration of probiotics or the

placebo before the POI procedure were defined as the “Control group”. The POI procedure was performed on three groups, as in experiment 1, and fecal pellets were collected at the baseline and one day (D-1), three days (D-3) and 5 days (D-5) after the POI procedure.

(B) Analysis of the Selected Taxa

DNA from fecal pellets was extracted using a QIAamp DNA stool kit, and the DNA concentration was measured using a nano-drop and was equilibrated to 100ng for the PCR reaction. A dose of 25 μl of PCR reaction mixture was prepared for the PCR (2.5 μl of 10x Ex Taq buffer, 2 μl of dNTPs, 1 μl of template, 0.15 μl of forward primer, 0.15 μl of reverse primer, 0.125 μl of Ex Taq DNA Pol, 19.075 μl of sterilized dH_2O). Initial denaturation was carried out at 98℃ for 2 minutes, followed by 30 cycles of the following denaturation steps: 98℃ for 10 seconds, 55℃ for 30 seconds, and 72℃ for 2 minutes. A final extension was also carried out at 72℃ for 5 minutes. Primers targeting a specific region of the 16S rRNA gene were used in the amplification process as follows:

5’-TGAGGTAACGGCTCACCAAGGCT-3’ and 5’-TCCGAACTGAGACC GGTT-3’ for *B.bifidum,*[30] 5’-CGGTCGTAGAGATACGGCTT-3’ and 5’-AT CCGAACTGAGACCGGT -3 for *B.longum,*[30] 5’-TTGGAGAGTTTGATCC TG GCTC-3’ and 5’-ACGTCATCCCCAC CTTCCTC-3’ for *C.butyricum,*[31] 5’-AGAGTTTGATCATGGCTCAG-3’ and 5’-CGGTATTAGCATCTGTTC C-3’ for *L.plantarum,*[32] and gel electrophoresis was used for the PCR products.

(C) Fecal Organic Acids

Acetate and butyrate assays were performed using an enzyme-linked immunosorbent assay kit. Supernatants prepared from fecal pellets were used, and all samples and standards were run in duplicate. A volume of 10 μl of the 100 mM acetate stock solution was diluted with 990 μl of water, and 10 μl of the 10 mM (10nmole/μl) β-Hydroxybutyrate standard solution was

diluted with a 90μl beta-Hydroxybutyrate buffer, to prepare a 1mM standard solution. Volumes of 0, 2, 4, 6, 8, and 10μl of the 1mM standard solutions were added into a 96 well plate for generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. The reaction mixes were set up and 50 μl of the appropriate reaction mix was added to each the wells. After mixing in a horizontal shaker, the mixes were incubated for 40 minutes at room temperature, and the absorbance was measured at 450nm (A_{450}).

(D) Colonic Transit

The colonic transit was assessed by fecal pellet output. Both the weight and the number of fecal pellets produced by the guinea pigs were measured and recorded for the control and probiotics groups at D-1, D-3 and D-5.

3. Statistical Analysis

For the output data from the Illumina MiSeq sequencing system, the EzBioCloud 16S database and the 16S microbiome pipeline (ChunLab Inc., EzBioCloud 16S-based MTP app, https://www.EZbiocloud.net) were used for data processing, statistical analysis and the graph data. Chao1 estimation and the Shannon diversity index were used for the richness and evenness of the samples. The overall phylogenetic distance among the groups was estimated using Bray-Curtis dissimilarity and was visualized by principal coordinate analysis (PCoA). The differences among the probiotics, placebo, and control groups were calculated by using SPSS version 18.0 (SPSS, Chicago, IL). The statistical analysis was performed using a one-way analysis of variance (ANOVA) for multiple comparisons, and a Mann-Whitney test for comparison between two groups. Data are expressed as the mean ± standard error (SE), and n value indicated the number of subjects. For all comparisons, two-sided p-values < 0.05 were considered statistically significant.

III. RESULTS

1. Microbial Diversity

As shown in Figure 1, analysis of the α-diversity showed that both species' richness (Figure 1A) and evenness (Figure 1B) tended to decrease at D-3 compared with those at baseline, although the differences did not reach statistical significance (p=0.464, p=0.294). Analysis of the β-diversity based on Bray-Curtis distances and PCoA showed that the fecal microbiota communities at the baseline, D-1, and D-3 are significantly separated (Figure 1C, p=0.001).

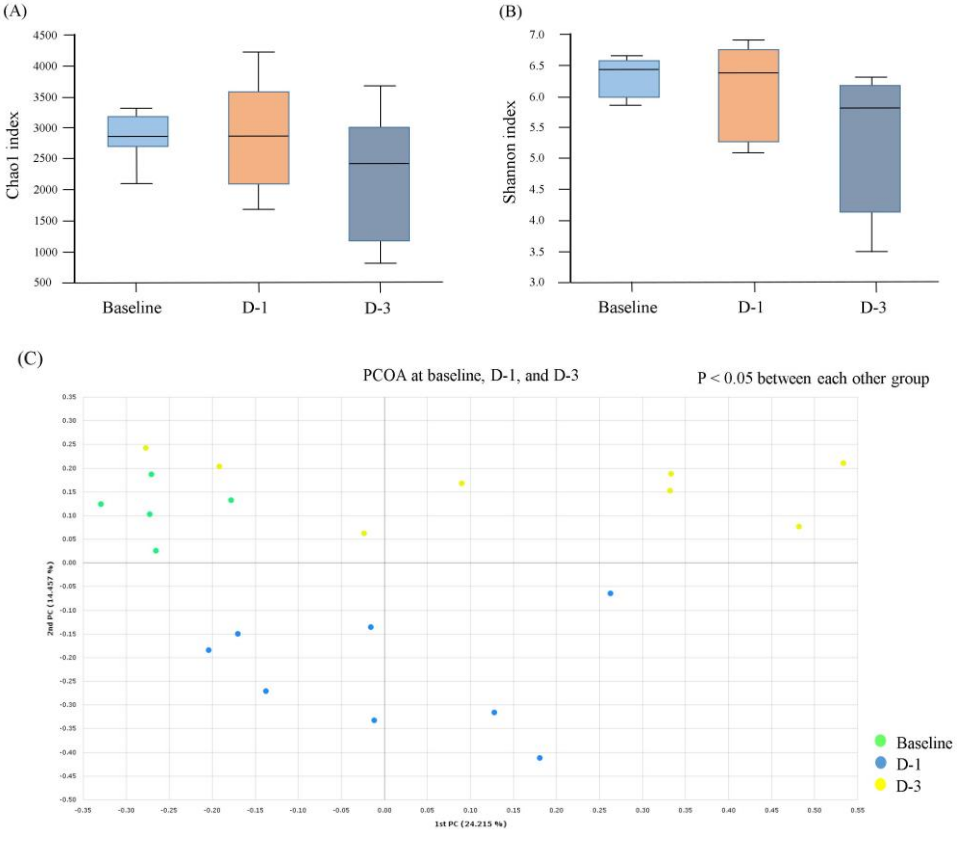

Figure 1. Community diversity in the guinea pig model of the postoperative ileus. The guinea pigs were subjected to laparotomy with cecal manipulation.

(A) Chao 1, richness, (B) Shannon, evenness, and (C) beta diversity (n=5/group). D-1, 1 day after the procedure; D-3, 3 days after the procedure.

2. The Taxonomic Composition of Fecal Microbiota

The four most abundant bacterial phyla of the subject were *Firmicutes* (42.29%), *Bacteroidetes* (41.16%), *Saccharibacteria_TM7* (12.41%) and *Actinobacteria* (4.14%) at the baseline (Figure 2). The abundances of the two major phyla *Firmicutes* and *Bacteroidetes* were not significantly changed, but those of the phyla *Saccharibacteria_TM7* and *Actinobacteria* decreased after the POI procedure. The relative abundances of *lactic acid bacteria*, including the genera *Bifidobacterium* ($P<0.01$) and *Lactobacillus* ($P<0.05$) were significantly decreased at D-1 (Figure 3A, 3B, 3C). The genera *Bacteroides* and *Blautia* and the species *B.cellulyticus* showed a significant increase in abundance at D-1 ($P<0.05$) and D-3 ($P<0.01$) compared with those at the baseline (Figure 3D, 3E, 3F).

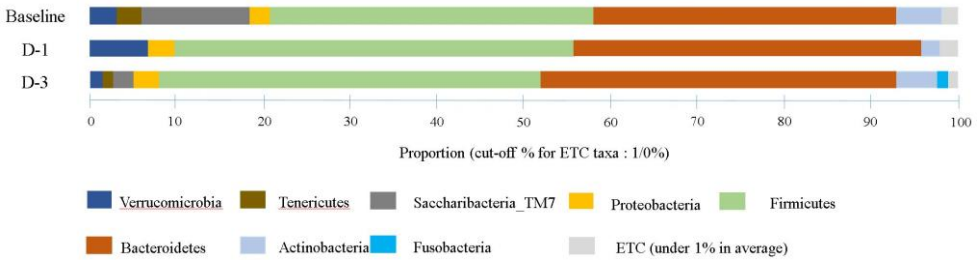

Figure 2. Average taxonomic composition of phylum level in the guinea pig model of the postoperative ileus. Baseline, 1 day after the procedure, and 3 days after the procedure (n = 5/group). D-1, 1 day after the procedure; D-3, 3 days after the procedure.

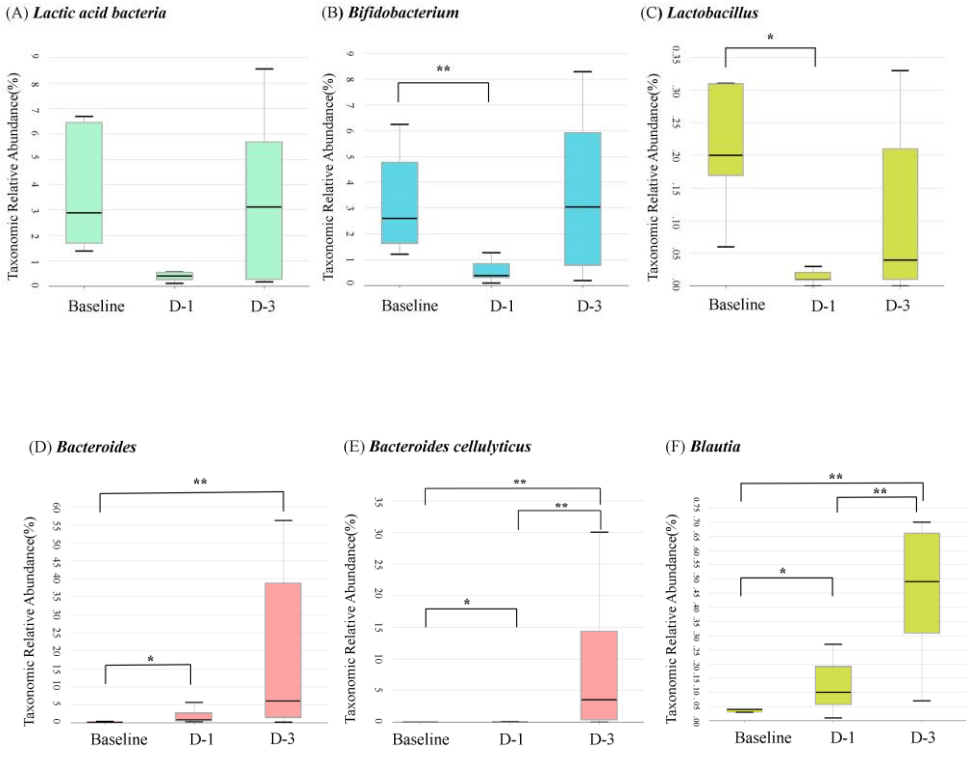

Figure 3. Changes in the taxonomic relative abundance in the guinea pig model of the postoperative ileus. A, Lactic acid bacteria, B, *Bifidobacterium*, C, *Lactobacillus*, D, *Bacteroides*, E, *Bacteroides cellulyticus*, and F, *Blautia* (n = 5/group). The data represent the mean ± SEM (n = 5/group); **P < .01; *, <.05. D-1, 1 day after the procedure; D-3, 3 days after the procedure.

3. The Effect of Probiotics on Selected Taxa

Semi-quantitative PCR analysis strongly showed reduced quantitative expression for the four selected taxa, *B.bifidum, B.longum, C.butyricum,* and *L.plantarum* after the POI procedure in the control group. The changes in the mean integrated density are shown in Figure 4. The abundance of *B.bifidum* significantly decreased at D-1 and D-3 compared with that at the baseline (Figure 4A, P<0.05). The abundances of *B.longum* and *C.butyricum*

significantly decreased at D-1 (Figure 4B, 4C, P<0.05), and the abundance of *L.plantarum* was significantly lower at D-3 compared with that at the baseline (Figure 4D, P<0.05).

In the probiotics group, strongly increased semi-quantitative expressions of *B.bifidum* and *B.longum* were identified at D-3 and D-1, respectively. The changes in the mean integrated density showed significant increase at D-1 and D-3 compared with those of the control and placebo groups (Figure 4A, 4B, P<0.05). There were no significant differences in the abundances of *C.butyricum* and *L.plantarum* compared with those of the control and placebo groups (Figure 4C, 4D).

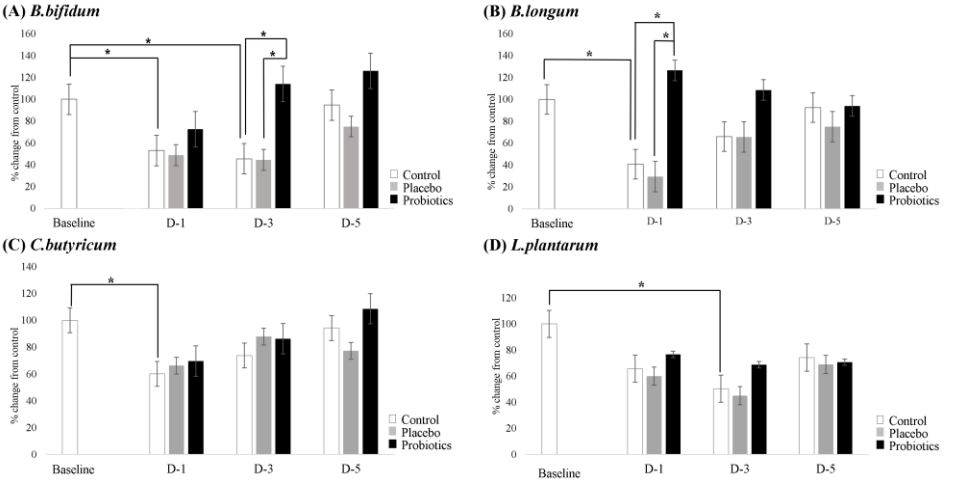

Figure 4. The effects of pretreated probiotics on the abundance of selected taxa in the guinea pig model of the postoperative ileus. The mean integrated density of the PCR analysis. A, *B. bifidum*, B, *B. longum*, C, *C. butyricum*, and D, *L. plantarum*. The data are expressed as the percentage change of the mean intensity compared with that at the baseline. The data represent the mean ± SEM (control group, n = 9; placebo group, n = 7; probiotics group, n = 7); *P < .05. D-1, 1 day after the procedure; D-3, 3 days after the procedure; D-5, 5 days after the

procedure.

4. The Effect of Probiotics on Fecal Organic Acids

The mean of the fecal butyrate level tended to decrease after the operation, compared with that at baseline. However, in the probiotics group, the butyrate level showed a significant increase at D-5 compared with that of the control group (Figure 5A, 1.78 ± 0.38 Mmol/50ul vs. 3.69 ± 1.47 Mmol/50ul, P=0.023). The acetate level showed no significant change in the control or the probiotics group at D-1, D-3, and D-5 (Figure 5B).

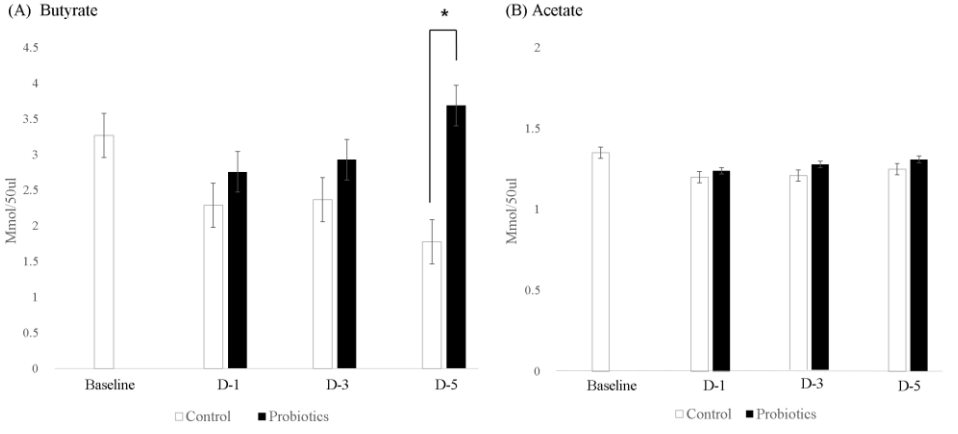

Figure 5. The effect of the pretreated probiotics on the fecal organic acid in the guinea pig model of the postoperative ileus. A, butyrate, and B, acetate. The data represent the mean ± SEM (n = 7/group); *P < .05. D-1, 1 day after the procedure; D-3, 3 days after the procedure; D-5, 5 days after the procedure.

5. Effect of Probiotics on Colonic Transit

As shown in Figure 6, the number of fecal pellets expelled in the probiotics group was significantly higher than that of the control group at D-5 (Figure 6A, 32.71 ± 8.46 vs. 48.00 ± 13.81, p=0.032). The weight of the fecal pellets in the probiotics groups was also significantly higher than that of the control group at

D-5 (Figure 6B, 2.98 ± 0.74 g vs. 4.53 ± 1.44 g, p=0.048).

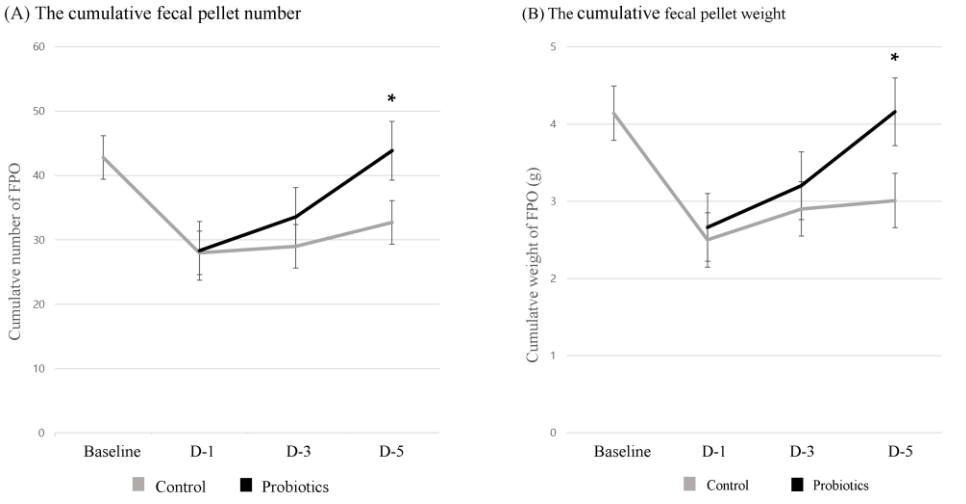

Figure 6. The effect of the pretreated probiotics on the fecal pellet output in the guinea pig model of the postoperative ileus. Data between two groups at each point were compared. A, the cumulative fecal pellet number, and B, the cumulative fecal pellet weight in grams. The data represent the mean ± SEM (n = 7/group). groups; *P < .05. D-1, 1 day after the POI procedure; D-3, 3 days after the POI procedure; D-5, 5 days after the procedure; FPO, fecal pellet output.

IV. DISCUSSION

As in humans, this study has found that *Firmicutes* and *Bacteroidetes* are two major fecal bacterial phyla dominated in guinea pigs (Figure 2). Because previous study which compared the guinea pig microbiome to human data based on Illumina shotgun metagenomics already showed this similarity,[33] we carried out our experiments with guinea pigs. Alpha diversity index denotes a mathematical measure of species diversity in a community, and beta diversity means diversity in microbial community between different environments.

Although there was no statistical significance, decreasing tendency of alpha diversity was identified in POI model. A significant separation between the baseline, POI D-1 and D-3 groups was identified, as shown in the Bray-Curtis distances and PCoA (Figure 1C). These mean that distinct changes of microbial community occur with the development of POI. The composition of fecal microbiota was also altered in the POI groups. The population size of the lactic acid-producing bacteria, including the genera *Bifidobacterium* and *Lactobacillus*, decreased, and the population sizes of *Bacteroides* and *Blautia* increased in the POI groups (Figure 3).

Several studies have reported that various kinds of microbiota undergo numerical and compositional changes after surgery.[34-36] A decrease in *Bifidobacterium and Lactobacillus* are commonly identified findings in patients with infectious complications.[37] *Bifidobacterium* and *Lactobacillus* are two main genera consisting of lactic acid bacteria which produce lactic acid as the end-product of carbohydrate fermentation.[38] The use of *Bifidobacterium* and *Lactobacillus* strains decreased the incidence of postoperative infection in patients who underwent major resection of the GI tract and liver transplantation.[39-41] Promoting anti-inflammatory responses and enhancing the intestinal barrier function have been suggested as the main protective mechanisms.[40,42,43] In line with previous studies, the present study has demonstrated that the population sizes of *Bifidobacterium* and *Lactobacillus* decrease in POI groups (Figure 3B, 3C). Since the inflammatory response and barrier dysfuction have been suggested as the possible main mechanism of POI,[44,45] these findings may aid understanding of the development of POI.

The increased abundances of the genera *Bacteroides* and *Blautia* also partly reflect the inflammatory environment in the GI tract in the POI group (Figure 3D, 3F). *Bacteroides* are components of gut commensal bacteria which affect the host immune regulation and the maintenance of homeostasis.[46] These *Bacteroides* show an increased abundance in several intestinal diseases related

to inflammation, such as ulcerative colitis and celiac disease.[47-49] Species *B. cellulosilyticus,* which was significantly more numerous 3 days after the POI in the present study, was reported to produce bacterial products modulating T cells and inducing anti-inflammatory IL-10-secreting T regulatory cells.[50] *Blautia* also showed anti-inflammatory properties in *in vitro* experiments, and an absence of *Blautia* in fecal samples before surgery was found to be associated with the development of infectious complication.[51]

This study attempted to investigate the compositional changes of the selected bacterial taxa *B.bifidum, B.longum, L.plantarum, and C.butyricum* which are well-known beneficial species for host health, and the effects of probiotics on their populations (Figure 4). All selected taxa showed significant decreases in abundance after the POI procedure. Although the probiotics used in this experiment do not include *Bifidobacterium,* the supplement of this compound promoted and recovered the population of the *Bifidobacterium* species in feces. This finding is consistent with a previous randomized controlled study which analyzed children with acute infectious diarrhea using the same probiotics.[52] *B.mesentericus*, one of the species consisting of these probiotics, has been reported to enhance the growth of the *Bifidobacterium* species via 3,3-dihydroxyazetidine in previous studies.[53,54] As several previous studies have already shown,[55] the interactions with the resident gut microbiota and the modified microbial community are also possible explanations for the increased number of *Bifidobacterium* species. The four selected phyla have something in common that they enhance the intestinal barrier function, although the exact mechanisms are not fully understood.[56-58] Thus, changes in their populations suggest important issue on pathophysiology of POI, and further studies with more focus on barrier function and microbiota in POI are needed.

Short-chain fatty acids (SCFAs) including acetate and butyrate are produced by gut microbial fermentation of indigestible dietary carbohydrates. Much evidence supports the beneficial effects of these SCFAs, especially acetate and

butyrate. Acetate has been found to enhance the intestinal barrier function and protect the host against infectious pathogens.[43] Butyrate is known as an energy source for colon epithelial cells which stimulates the proliferation of epithelial cells. It has been also demonstrated that butyrate plays a key role in the maintenance of the intestinal barrier function and immunomodulation.[59] By stimulating mucin secretion and the mitogen-activated protein kinase signaling pathway, butyrate has modulated bacterial adhesion in a previous *in vitro* study.[60] Butyrate also has a central role in the anti-inflammatory process, inducing the differentiation of colonic regulatory T cells which suppress inflammatory reactions.[61]

In the present study, the fecal butyrate levels decreased after POI procedure compared with that of the baseline in control group. However, the butyrate level in the probiotics group recovered close to baseline and significantly higher than that of the control group at D-5. (Figure 5A). One interesting finding is that the main fermentation product from carbohydrates produced by *Bifidobacteria* is acetate, but the butyrate level rather than acetate was significantly increased. This result is likely to be related to cross-feeding interaction between *Bifidobacteria* and the butyrate-producing bacteria. It has been shown that acetate produced by *Bifidobacteria* is used as a substrate and converted to butyrate by a butyrate-producing species.[62,63] An increased level of fecal butyrate in the probiotics group partly supports these cross-feeding interactions. The enhanced population of *Bifidobacterium* induced by the administration of probiotics may be attributed to the increase in butyrate through this interaction in the probiotics group. Considering the pathway of cross-interaction and microbial fermentation,[64,65] the butyrate level is thought to significantly increase one or two days after the increase of *Bifidobacterium.* The probiotics group showed a significant improvement in colonic transit compared with the control group (Figure 6). This result is explained by the significantly increased butyrate level. Previous animal study showed that

butyrate increase colonic transit and neurally mediated contractile response.[66] Butyrate also regulated the microbial TLR-dependent sensing, which is implicated in the gut motility.[67] The increased SCFAs, including butyrate, have been suggested as another reason for this, because the total fecal SCFA levels were negatively correlated with the colonic transit time but positively correlated with the stool frequency in a previous human study.[68]

In this study, the four taxa appeared to recover close to baseline at D-5, but the colonic transit and butyrate level were significantly improved only in the probiotics group which the composition of microbiota were modulated at D-1 and D-3. As we mentioned at materials and methods section, neural and inflammatory pathways suggested as pathophysiology of POI are found to begin immediately after operation.[26] And, several reports have shown that POI that lasts more than 5 to 6 days increases postoperative morbidity and socio-economic burden.[25] It can thus be suggested that first few days after operation is golden hour to decide whether or not the POI is developed and deteriorated. The early modulation of microbial composition in this period might improve the GI motility.

There are important issues that still need to be addressed. First, although this study has demonstrated that probiotics improve the GI motility in POI model, the exact mechanisms associated with this have not been investigated. Second, the amount of intake for each subject that could affect the microbial community was not identified, although we applied the same diet protocol to all subjects. Finally, this study has also not confirmed the effects of probiotics on other microbiota, except for the selected species. However, this study has demonstrated the positive effects of probiotics on beneficial gut microbiota and an improvement in the GI motility in the POI model. To the best of our knowledge, this is the first study to investigate the compositional changes in gut microbiota and the effects of probiotics focused on the POI. Because not only the established treatment but also the approach regarding gut microbiota have

not existed for patients with POI, findings of this animal study can be used in a clinical setting for treatment and prevention of POI. Further studies on whole genome sequencing and molecular biology would be beneficial to provide a deep understanding of this field.

In summary, the POI induces significant changes in the microbial community and reduces the fecal butyrate level. Pretreatment of probiotics before an operation increases the abundance of several beneficial bacterial species and the butyrate production in the colonic lumen. Additionally, the bowel movement is significantly improved in these conditions.

V. CONCLUSION

The POI induces significant changes in the microbial community and reduces the fecal butyrate level. Pretreatment of probiotics before an operation increases the abundance of several beneficial bacterial species and the butyrate production in the colonic lumen. Additionally, the bowel movement is significantly improved in these conditions. Therefore, we may conclude that the modulation of gut microbiota by probiotics aids the treatment and prevention of the POI.

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ABSTRACT (IN KOREAN)

기니 픽의 수술 후 장마비 모델에서 장내 미생물 무리 및 유기산 변화와 프로바이오틱스의 효과

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신 승 용

수술 후 장 마비는 수술에 의한 물리적인 장관 조작과 자율신경 기능장애, 염증 매개 물질 등 여러 가지 원인으로 장관 운동의 정상적인 협력 체계가 마비되어 위장관 내용물의 운반 장애가 나타나는 것을 의미한다. 이는 환자의 삶의 질 저하는 물론, 재원 기간 연장 및 과다한 사회경제적 비용을 초래한다. 하지만 현재까지 수술 후 장마비에 대한 공인된 예방 혹은 치료 약제는 없는 실정이다. 최근 여러가지 수술 후 합병증이 장내세균의 변화로부터 기인한다는 주장들이 제기되고 있다. 그러나 수술 후 장마비와 장내 세균의 관련성에 대해서는 알려진 바가 거의 없다. 따라서 본 연구는 기니픽의 수술 후 장마비 모델에서 실제 장내 세균 조성의 변화를 확인하고, 수술전 프로바이오틱스 투여가 장내 세균, 아세트산 및 부티르산, 그리고 장관운동의 변화에 미치는 효과에 대해 확인하고자 하였다.

수술 후 장마비를 유발하는 수술을 진행한 기니픽에서 수술 전, 수술 후 1일, 3일, 5일째에 각각 대변을 채집하였고, 장내 세균은

16s rRNA gene sequencing을 통해, 부티르산, 아세트산은 ELISA 기법으로 분석되었다. 동일한 실험을 수술 전 7일간 프로바이오틱스 혹은 대조약을 복용한 기니픽을 대상으로 진행하였다.

연구 결과, 수술 후 장 마비 모델에서 장내 세균 조성이 수술 전에 비해 유의하게 변화되는 것을 확인하였고, 특히 장 점막 투과도를 유지시켜 장벽을 보호 하는 효과를 지닌 것으로 알려진 B. bifidum, B. longum, C.butyricum, L.plantarum 과 부티르산, 아세트 산이 감소하는 것을 확인 할 수 있었다. 그리고 수술 전에 프로바이오틱스를 투여한 군에서 그렇지 않은 군에 비해, B. bifidum, B. longum 과 부티르 산이 유의하게 회복 및 증가되었고, 장관 운동 또한 유의하게 개선되었음을 확인 할 수 있었다.

본 연구를 통해, 수술 후 장마비모델에서 장내 세균의 불균형과 부티르산의 감소를 확인할 수 있었고, 프로바이오틱스의 사용이 이를 효과적으로 개선시키고, 더 나아가 장관 운동을 회복시킴을 확인할 수 있었다. 따라서, 향후 수술 전 프로바이오틱스 사용이 수술 후 장마비의 예방 및 치료에 큰 역할을 할 수 있을 것으로 기대된다

핵심되는 말: 장마비, 수술 후 합병증, 장내 세균, 부티르산, 프로바이오틱스

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