Impact of perioperative probiotic treatment for surgical site infections in patients with colorectal cancer

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Received December 1, 2014; Accepted December 22, 2014

DOI: 10.3892/etm.2015.2640

Abstract. The aim of the present study was to estimate the effect of the perioperative administration of probiotics in patients undergoing colorectal cancer (CRC) surgery. The study focused on a total of 156 consecutive surgeries carried out from among all the elective CRC surgeries performed between April 2009 and March 2013. The patients involved in surgeries undertaken between April 2009 and October 2011 were placed in the non-probiotic group (group A, 81 patients) and those involved in surgeries between November 2011 and March 2013 were placed in the probiotic group (group B, 75 patients). Postoperative infectious complications were recorded, and the immune responses and fecal microbiota were determined. A breakdown of infectious complications showed that 21 (13.5%) patients experienced superficial incisional surgical site infections (SSIs), of which 16 patients were from group A (19.8%), and five patients from group B (6.7%) (P=0.016). The ImmuKnow® adenosine triphosphate values peaked on the first postoperative day (POD) in both groups. In group A, the ImmuKnow value of the first POD was increased significantly compared with the preoperative value (P=0.022). In group B, the value of the first POD did not increase compared with the preoperative value (P=0.28). In conclusion, probiotic treatment can reduce superficial incisional SSIs in patients undergoing CRC surgery. Perioperative probiotic treatment can enhance immune responses and improve the intestinal microbial environment.

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Abbreviations: CRC, colorectal cancer; SSIs, surgical site infections; T-RFLP, terminal-restriction fragment length polymorphism; BMI, body mass index; POD, postoperative day; SCFA, short-chain fatty acid

Key words: probiotics, surgical site infections, colorectal cancer surgery, terminal-restriction fragment length polymorphism, ImmuKnow

Introduction

The occurrence of surgical site infection (SSI) extends the duration of hospitalization, raising the costs of admission and potentially reducing the quality of life of the patients (1). Since the publication of the Guideline for the Prevention of Surgical Site Infection in 1999 by the Center for Disease Control and Prevention (2), there has been a declining trend in SSI. Takesue *et al* (3) reported, based on the results of a multi-center research project, that the implementation of effective infection prevention practices can maintain SSI incidence rates to <15%.

Probiotics that improve the intestinal microbial balance in the host are considered to have beneficial effects on human health (4). By comparing the intestinal environment in patients with colorectal cancer (CRC) and healthy individuals, Wang *et al* (5) found that there is an intestinal microbial imbalance in patients with CRC, represented by a reduction in the number of butyrate producers and an increase in opportunistic pathogens. Disturbance of the intestinal microbiota appears to be an important factor inducing perioperative SSI (6). This disturbance is caused by the stress of invasive surgery, the administration of antibacterial drugs to prevent infection, the weakness of intestinal tract peristalsis and the atrophy of the intestinal mucosa due to the perioperative fasting and intestinal tract ischemia (6).

We hypothesized that the perioperative administration of probiotics should reduce the incidence of SSIs among the patients undergoing elective CRC surgery. In addition, the study was designed to investigate the effect of the perioperative administration of probiotics on the immune response, intestinal microbiota and surgical outcome in the clinical setting.

Materials and methods

Patient enrolment. The present study focused on 156 consecutive surgeries carried out by the same team from among all the elective CRC surgeries performed at Fukuoka University Hospital (Fukuoka, Japan) between April 2009 and March 2013, following the exclusion of inoperable patients and the provision of informed consent from the patients. The patients involved in surgeries conducted between April 2009 and October 2011 were placed in the non-probiotic group (group A, 81 patients) and those involved in surgeries between November 2011 and March 2013 were placed in the probiotic group (group B,

75 patients). This study was approved by the Human Research Review Committee of Fukuoka University Hospital (12-3-08).

Treatment. All surgeries were performed by the same team, which included three surgeons, and perioperative management was performed under the same conditions for all patients (other than the probiotic treatment). For the probiotic treatment, six tablets of BIO-THREE® (Toa Pharmaceutical Co., Ltd., Tokyo, Japan) were administered orally daily. Each BIO-THREE tablet contained 2 mg *Enterococcus faecalis* T110, 10 mg *Clostridium butyricum* TO-A and 10 mg *Bacillus mesentericus* TO-A. All patients received a regular diet preoperatively. The administration of the BIO-THREE (six tablets/day) was started three to 15 days prior to the surgery, and then was restarted the same day the patient started drinking water. All the patients underwent the same intestinal preparation with magnesium citrate (Magcorol P®; Horii Pharmaceutical Co., Ltd., Tokyo, Japan) without per oral administration of antibiotics. Antibiotic prophylaxis was initiated with the administration of 1 g cefmetazole sodium 30 min prior to the surgery, with additional administration every 3 h during the surgery. The intravenous administration of antibiotics continued twice per day until the second postoperative day (POD). Fecal and blood sampling was performed prior to surgery and at 1 week following surgery. The fecal samples were suspended in 4 M guanidinium thiocyanate, 100 mM Tris-HCl (pH 9.0) and 40 mM EDTA following washing three times with sterile distilled water. The mixture was then beaten with glass beads using a mini bead beater (BioSpec Products Inc., Bartlesville, OK, USA).

Fecal terminal-restriction fragment length polymorphism assay. Twenty-four patients (n=10, group A; n=14, group B) were included in the evaluation. Amplification of the 16S rDNA, the digestion of restriction enzymes, size fractionation of T-RFs and analysis of TRFLP data were performed according to the protocol described by Nagashima *et al* (7). Briefly, polymerase chain reaction (PCR) was performed using the total fecal DNA (10 ng/µL) and primers of 516f (5'-TGC CAGCAGCCGCGGTA-3'; *Escherichia coli* positions, 516-532) and 1510r (5'-GGTTACCTTGTTACGACTT-3'; *E. coli* positions, 1510-1492). The 5'-ends of the forward primers were labeled with 6'-carboxyfluorescein, which was synthesized by Applied Biosystems (Tokyo,Japan). The amplified 16S rDNA genes were purified using a GFX PCR DNA and Gel Band Purification kit (GE Healthcare Bio-Sciences, Tokyo, Japan) and redissolved in 30 µl distilled water. The purified PCR products (2 µl) were digested with 10 units of BslI at 55˚C for 3 h. The length of the T-RF was determined using an ABI PRISM 3130x1 genetic analyzer (Applied Biosystems) in GeneScan mode. Standard size markers were used (MapMarker X-Rhodamine Labeled; 50-1,000 bp; BioVentures, TN, USA). The fragment sizes were estimated using the local southern method of the GeneMapper software (Applied Biosystems). The T-RFs were divided into 30 operational taxonomic units (OTUs), according to the protocol described by Nagashima *et al* (7) The OTUs were quantified as the percentage of values of an individual OTU per total OTU area, expressed as the peak percentage area under the curve (%AUC). The cluster analyses were performed using GeneMaths software (Applied Maths, Belgium), based on the *Bsl*I T-RFLP patterns. Pearson similarity coefficient analysis and the unweighted pair-group method with arithmetic means were used to establish the type of dendrogram.

Cylex® ImmuKnow® assay. Peripheral venous blood samples were taken preoperatively and on the first, fourth and eight PODs. Samples from 20 patients (n=10, group A; n=10, group B) were evaluated by the Cylex ImmuKnow assay (Cylex, Columbia, MD, USA). Whole blood samples, which were subjected to sodium heparin anticoagulation treatment, were collected at Fukuoka University Hospital and tested in the laboratory. The immune response was measured using the Food and Drug Administration (FDA)-approved Cylex ImmuKnow assay in accordance with the manufacturer's instructions (Cyclex) (8). Cluster of differentiation (CD4)-(ImmuKnow) T cells were positively selected within the microwells using magnetic particles coated with anti-human CD4 monoclonal antibodies and a strong magnet. The release of adenosine triphosphate (ATP) was measured using luciferin/luciferase and a luminometer (Berthold Technologies, LLC, Knoxville, TN, USA). The level of immune response was assessed based on the amount of ATP, expressed in ng/ml.

Recording of infectious complications. Detailed daily records of the postoperative course were kept for each patient. The infectious complications included SSI (superficial incisional, deep incisional and space/organ), postoperative pneumonia, urinary tract infections and enteritis. These were recorded for up to 30 days after surgery. An SSI was defined as spontaneous or surgically released purulent discharge with positive culture results.

Statistical analysis. The statistical analysis was performed using the χ^2 and t-tests to compare the two groups, and a logistic regression analysis for the multivariable analysis. Significant differences were concluded from results using a value of $P<0.05$ in all cases. The statistical analysis software program used was SPSS for Windows (version 11.5; SPSS, Tokyo, Japan).

Results

Demographic characteristics of study participants. A total of 156 patients were assigned to one of the two treatment arms. The demographic characteristics of the study patients, site of the tumor, use of open/laparoscopic surgery, creation of an ostomy, cancer stage and intraoperative characteristics are shown in Table I. A significant difference was noted in the preoperative body mass index (BMI) and whether or not the surgical procedure was performed by an open or laparoscopic method. With regard to the intraoperative characteristics, no significant difference was noted. With regard to the postoperative course, the length of time prior to the passage of gas and meal intake in group B was significantly shorter than that in group A (Table II).

An SSI was observed in 27 (17.3%) of the 156 patients. A breakdown of the infectious complications showed that 21 (13.4%) patients had a superficial incisional SSI, 16 of who were in group A (19.8%) and five of who were in group B (6.7%) (P=0.016). All superficial incisional SSIs were below grade II

Table I. Baseline characteristics of the patients with colorectal cancer (n=156).

Characteristic	Group A	Group B	P-value
Age[a], years	69.1±11.3	68.0±13.8	0.58
Gender, M/F	44/37	47/28	0.34
BMI[b], kg/cm^2	23.3±3.8	21.7±2.7	0.0034
PNI[a]	48.8±7.5	48.0±7.4	0.47
Diabetes mellitus, n (%)	14 (17.3)	12 (16.0)	0.83
Smoking, n (%)	18 (22.2)	14 (18.7)	0.73
Chronic renal failure, n (%)	9 (11.1)	5 (6.7)	0.33
ASA score, n			0.08
1	29	38	
2	46	29	
3	6	8	
Immunosuppression, n (%)	2 (2.6)	2 (2.7)	0.94
Open/laparoscopic, n	52/29	35/40	0.041
Site of tumor, n			0.86
C	3	5	
A	8	14	
T	6	5	
D	3	0	
S	25	16	
R	22	8	
Creation of an ostomy, n (%)	16 (19.6)	14 (18.7)	0.86
Cancer stage, n			0.34
I	29	31	
II	32	16	
IIIA	11	10	
IIIB	3	3	
IV	6	8	
Surgery time[c], min	209 (100-630)	240 (115-555)	0.09
Blood loss[c], ml	120 (0-2060)	100 (0-1190)	0.08
Intraoperative hypotension, n (%)	19 (23.5)	25 (33.3)	0.26
Intraoperative hypothermia, n (%)	2 (2.5)	2 (2.7)	0.62

[a]Presented as the mean ± SD. [b]Presented as the median ± SD. [c]Presented as the mean (range). Group A, n=81; group B, n=75. M, male; F, female; BMI, body mass index; PNI, prognostic nutrition index; ASA, American Society of Anesthesiologists; SD, standard deviation. C, cecum; A, ascending colon; T, transverse colon; D, descending colon; S, sigmoid colon; R. rectum.

Table II. Postoperative characteristics.

	Group A, n=81	Group B, n=75	P-value
Time of flatus (days)	2.8±2.0	2.0±1.1	0.001
Time of meal intake (days)	4.7±1.8	3.9±1.5	0.002

Data are presented as the mean ± standard deviation.

in the Clavien-Dindo classification. Among the six (3.8%) patients with deep and organ/space SSI, four were in group A (4.9%) and two were in group B (2.7%) (Table III). In group A, two of the patients were grade II and two were grade IIIB in the Clavien-Dindo classification. In group B, the two patients were grade IIIB. Pneumonia, urinary tract infection and enteritis were below grade II.

The rate of SSI incidence by age, gender, BMI, prognostic nutrition index (9), history of diabetes mellitus, smoking, chronic renal failure, American Society of Anesthesiologists

Table III. Infectious complications.

Complications	n	Group A, n (%)	Group B, n (%)	P-value
Surgical site infection	27			
Superficial incisional	21	16 (19.7)	5 (6.7)	0.016
Deep, organ/space	6	4 (4.9)	2 (2.7)	0.40
Pneumonia	1	0 (0)	1 (1.3)	-
Urinary tract infection	1	0 (0)	1 (1.3)	-
Enteritis	8	3 (3.7)	5 (6.7)	0.40
Total	37	23 (28.3)	14 (18.7)	0.15

Group A, n=81; group B, n=75.

Table IV. SSI (univariate analysis).

Characteristics	No superficial incisional SSI	Superficial incisional SSI	P-value
Age[a], years	67.9±12.8	71.7±11.0	0.149
Gender, M/F	75/60	18/9	0.164
BMI[b], kg/cm^2	22.5±3.1	22.8±4.5	0.701
PNI[a]	48.7±7.6	47.1±6.7	0.297
Diabetes mellitus, n	21	5	0.777
Smoking, n	28	4	0.422
Chronic renal failure, n	8	4	0.244
ASA score, n			0.147
1	67	8	
2	75	16	
3	14	3	
Immunosuppression, n	3	1	0.681
Site of tumor, n			0.095
C	8	3	
A	28	4	
T	15	1	
D	49	9	
S	35	10	
R			
Creation of an ostomy, n	18	12	0.030
Open/laparoscopic, n	68/61	19/8	0.094
Surgery time[a], min	247.9±103.9	265.3±93.9	0.425
Estimated blood loss[a], ml	157.8±233.6	437.5±423.7	0.001
Intraoperative hypotension, n	2	2	0.396
Intraoperative hypothermia, n	44	4	0.179
Probiotics, n	68	7	0.003

[a]Presented as the mean ± SD. [b]Presented as the median ± SD. SSI, surgical site infection; M, male; F, female; BMI, body mass index; PNI, prognostic nutrition index; ASA, American Society of Anesthesiologists; SD, standard deviation. C, cecum; A, ascending colon; T, transverse colon; D, descending colon; S, sigmoid colon; R. rectum.

(ASA) score, state of immunosuppression, open/laparoscopic procedure, length of the surgery, volume of blood loss, intraoperative hypotension, hypothermia and oral administration of probiotics was investigated in the univariate analysis. It was found that the patients with SSI had a higher frequency of undergoing an ostomy creation and more blood loss. Probiotic

Table V. SSI (multivariate analysis).

Factor	SSI/total (n)	SSI rate (%)	Risk ratio	95% CI	P-value
Creation of an ostomy				0.087-1.200	0.090
+	12/30	40.0	1.00		
-	15/126	11.9	0.32		
Estimated blood loss			1.00	1.000-1.004	0.140
Probiotics				1.340-12.200	0.013
+	7/75	9.3	1.00		
-	20/81	24.7	4.10		

SSI, surgical site infection; CI, confidence interval.

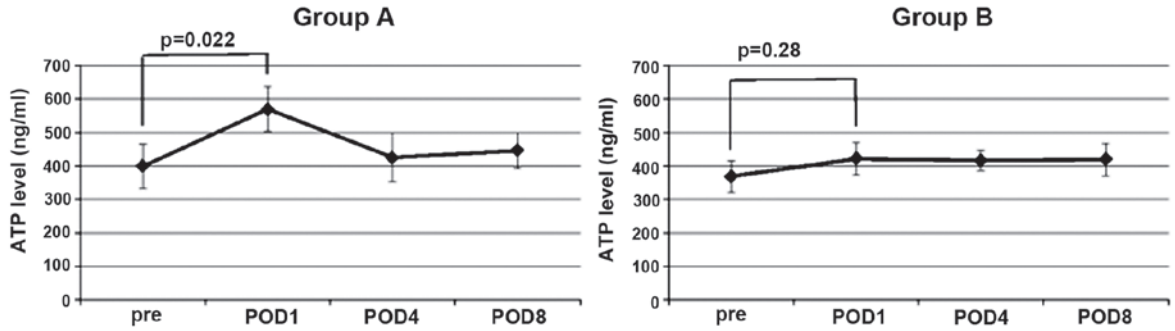

Figure 1. ImmuKnow adenosine triphosphate values for groups A and B. POD, postoperative day.

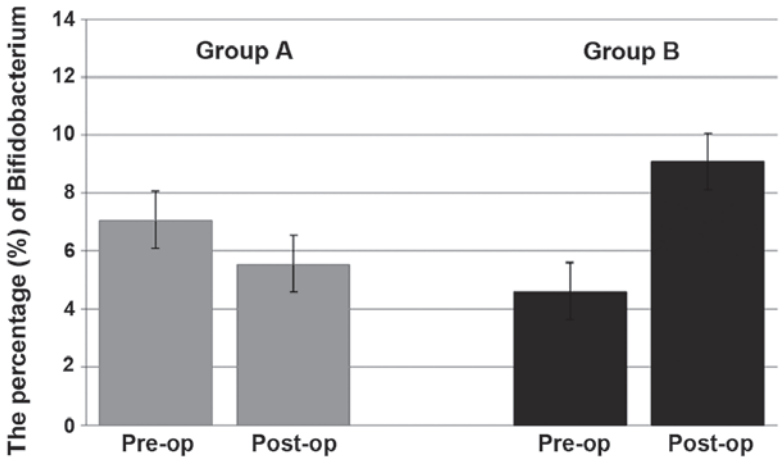

Figure 2. Changes in fecal microbiota (ratio of *Bifidobacterium*) in groups A and B. Pre-op, pre-operative; post-op, post-operative.

administration was higher in the group of patients without SSI (68, vs. 7; Table IV. In the multivariate analysis, only the oral administration of probiotics was identified as an independent risk factors for SSI (Table V).

ImmuKnow ATP assay. In the two groups of patients with CRC, the ImmuKnow ATP values were observed to peak on the first POD. In group A, the ImmuKnow value of the first POD was increased significantly compared with the preoperative value (P=0.022). By contrast, the ImmuKnow value on the first POD in group B was not significantly higher than the preoperative value (P=0.28) (Fig. 1). On the fourth and eighth PODs, the ImmuKnow ATP values were observed to exhibit a decreasing trend, but no significant differences were noted in the two groups.

Changes in fecal microbiota. The changes in the fecal microbiota resulting from the surgery and subsequent to probiotic administration were next investigated. Almost no changes in the number of beneficial bacteria, such as *Bifidobacterium*, were observed in group A; however, the mean proportion of *Bifidobacterium* increased in group B between 4.6 and 9.1% (Fig. 2).

Discussion

The development of various perioperative management techniques has contributed to a decrease in the incidence of SSIs; however, the rate of superficial incisional SSI incidence in elective CRC surgery remains between 2.5 and 20.5% (10). In the present study, the rate of SSI was 17.3%.

Previous studies have reported that incisional SSIs are caused by the imbalance of infectious bacteria, surgical technique and the patient's condition (3,4,6). The factors associated with infectious bacteria are the use of preoperative, non-absorbable, oral antibiotics and prophylactic antibiotic use (11). The factors associated with the surgical technique are the preoperative skin preparation, the length of the surgery, the use of open versus laparoscopic surgery, the creation or closure of an ostomy, the suture material used for fascial closure and the type of skin closure (12). It has been reported that the relevant factors associated with the patient's condition are the gender, BMI, ASA score, immunosuppression, smoking, wound classification, requirement for a blood transfusion, subcutaneous fat thickness and postoperative hyperglycemia (13).

It has recently been reported that perioperative probiotic and synbiotic treatment can reduce infectious complications, such as incisional SSI, in esophageal cancer, biliary cancer and CRC surgery (14-16); however, the evidence in those reports was relatively weak, and neither perioperative probiotic treatment nor synbiotic treatment were found to be independently associated as a risk factor of incisional SSI.

It has been demonstrated that probiotics can improve the intestinal microbial environment and activate host immune function, leading to the prevention of infectious complications (17-19). In the present study, BIO-THREE tablets were selected as the probiotics, as one BIO-THREE tablet contains 2 mg *Enterococcus faecalis* T110, 10 mg *Clostridium butyricum* TO-A and 10 mg *Bacillus mesentericus* TO-A, all of which are well-documented beneficial bacteria, and these tablets can be effectively absorbed to increase the ratio of beneficial bacteria in the body (20). The intestinal microbiota plays an important part in the host immune system (21). The intestinal microbiota includes beneficial, opportunistic and harmful bacteria. Beneficial bacteria in the intestinal microbiota protect the intestinal tract against the invasion of harmful bacteria, while harmful bacteria exploit decreases in the host resistance to exhibit pathogenicity (22). In the present study, the ratio of beneficial bacteria tended to be increased with the perioperative administration of probiotics. Beneficial bacteria increase the concentration of short-chain fatty acids (SCFAs), such as acetic, propionic and butyric acids. These SCFAs have numerous important roles in the colon, including maintaining the acidity of the intestinal environment, stimulating the proliferation of epithelial cells and intestinal motility, enhancing the secretion of mucin by the epithelium and acting as metabolites for energy metabolism in epithelial cells (23). In the present study, intestinal peristalsis was improved earlier in group B than group A.

The clinical validity of the Immune Cell Function Assay (Cylex ImmuKnow assay) as an objective tool for assessing immune function has been validated previously, when the assay was used to tailor immunosuppression to optimize the treatment of rejection and infections, as well as in immunosuppression weaning protocols (24). Prior to its introduction, there had been no available tool for the direct assessment of the immune function of a patient. The FDA approved the use of the Immune Cell Function Assay as a means of quantifying cell-mediated immunity by measuring the concentration of ATP from $CD4^+$ T cells in 2002. The Cylex ImmuKnow assay has since been shown to be clinically useful in the assessment of the relative risk of infection and rejection (24). To the best of our knowledge, this is the first report that has evaluated the perioperative immune function of patients undergoing CRC surgery with the Cylex ImmuKnow assay.

In this study, the extent of systemic inflammation was evaluated using the Cylex ImmuKnow assay. The activation of the cytokine cascade can predict infectious conditions. The ImmuKnow assay is an acute-phase reactant that increases in response to cytokine stimulation, thereby serving as a marker of the magnitude of inflammation (25). During the perioperative treatment of the patients in the present study with probiotics, the inflammation was estimated using the Cylex ImmuKnow assay preoperatively and on the first, fourth and eighth PODs. In group A, the ImmuKnow value of the first POD was increased significantly compared with the preoperative value. By contrast, the value of the first POD in group B did not increase significantly. The administration of probiotics may have reduced the inflammation associated with the surgical stress following the CRC surgery. Perioperative administration of probiotics can reinforce the immune function of the host and increase the resistance to infections. As a result, it could reduce the incidence of superficial incisional SSI (26). Furthermore, since the perioperative administration of probiotics reduced the postoperative inflammatory response, it could lead to a decrease in the length of the hospital stay. The present results suggest that systemic inflammatory responses can be favorably modified by probiotics. The microbial imbalance induced by CRC and surgical stress may be rapidly improved by perioperative probiotic treatment. Increasing the ratio of beneficial bacteria aids in the maintenance of the host defense, and colonic SCFAs may have beneficial effects on the epithelial cell integrity and participate in the local defense of the colon.

In the present study, the administration of probiotics induced a decrease in superficial incisional SSI incidence and an increase in $CD4^+$ ATP activity. Probiotic administration therefore appears to result in the perioperative enhancement of the host immune function. In conclusion, consecutive preoperative and postoperative probiotic treatment could reduce the incidence of superficial incisional SSI, and could increase the ratio of beneficial bacterial in the feces. The ImmuKnow assay indicated that the oral administration of probiotics induced ATP activity in the $CD4^+$ lymphocytes. This reduction in the incidence of superficial incisional SSI may involves the enhancement of immune function through the activity of ATP in $CD4^+$ cells by probiotics. The oral administration of probiotics as a food supplement is simple and safe. We therefore recommend the perioperative use of probiotics in all patients undergoing surgical treatment.

Acknowledgements

The study was partially supported by Toa Pharmaceutical Co., Ltd. (Tokyo, Japan).

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