Impact of Perioperative Probiotic treatment for Surgical Site Infections in Colorectal Cancer Patients

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Abbrevations:

CRC:colorectal cancer

SSI: surgical site infections

T-RFLP: terminal-restriction fragment length polymorphism

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AISU et al: Effects of probiotics to prevent surgical site infections in Colorectal Cancer Patients

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Abstract

The aim of the present study was to estimate the effects of perioperative administration of probiotics in patients undergoing colorectal cancer surgery. A total of 156 consecutive surgeries carried out from among all elective colorectal cancer surgeries between April 2009 and March 2013. The surgeries implemented between April 2009 and October 2011 were placed in the non-probiotic group (Group A: 81 patients) and those between November 2011 and March 2013 were placed in the probiotic group (Group B: 75 patients). Postoperative infectious complications were records. Immune responses and fecal microbiota were determined in this study. A breakdown of infectious complications showed 21 (13.4%) patients of superficial incisional surgical site infections (SSI), of which 16(19.7%) patients were among Group A, and 5 (6.7%) patients among Group B (p=0.016). The Immuknow Adenosine triphosphate (ATP) values peaked on the first postoperative day in both groups. In Group A, the Immuknow value of the first postoperative day was increased significantly compared to the preoperative value (p= 0.022). In Group B, the value of first postoperative day didn't increase compared to preoperative day (p=0.28). The effects of probiotic treatment can reduce superficial incisional SSI in patients undergoing colorectal cancer surgery. Perioperative probiotic treatment can enhance immune responses, improve intestinal microbial environment.

Introduction

The incidence of surgical site infections (SSI) extends the duration of hospitalization, raising the costs of admission, and potentially reducing the quality of life of 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, there has been a declining trend in SSI (2). Takesue reported, based on the results of a multi-center research project, that implementation of best practices with regard to infection prevention can maintain SSI incidence rates below 15% (3).

Probiotics that benefit the host by improving the intestinal microbial balance are considered to have beneficial effects on human health (4). By comparing the intestinal environment in colorectal cancer (CRC) patients and healthy persons, Wang have identified that there is an intestinal microbial imbalance in patients, represented by a reduction of butyrate producers and an increase of opportunistic pathogens (5). Disturbance of the intestinal microbiota seems to be an important factor that induces perioperative SSI (6). That 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.

We hypothesized that the perioperative administration of probiotics should reduce SSI among patients undergoing elective CRC surgery. This study was also designed to investigate the effects of perioperative administration of probiotics on the immune responses, the intestinal microbiota, as well as the surgical outcome in the clinical setting.

Materials and Methods

Patient Enrollment

This study focused on 156 consecutive surgeries carried out by the same team, from among all elective CRC surgeries implemented at Fukuoka University Hospital between April 2009 and March 2013 after excluding inoperable patients. The surgeries implemented between April 2009 and October 2011 were placed in the non-probiotic group (Group A: 81 patients) and those 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 the Fukuoka University Hospital (12-3-08).

Treatment

All surgeries were performed by the same team including three surgeons, and perioperative management was performed under the same conditions for all patients other than the probiotic treatment. For the probiotic treatment, the following agents were administered orally: six tablets of BIO-THREE ® (Toa Pharmaceutical Co, Ltd, Tokyo, Japan). Each BIO-THREE tablet contains 2 mg of *Enterococcus faecalis* T110, 10 mg of *Clostridium butyricum* TO-A and 10 mg of *Bacillus mesentericus* TO-A. All patients received a regular diet preoperatively. The administration of the BIO-THREE (6 tables/day) was started three to 15 days prior to the operation, and then was restarted the same day the patient started drinking water. All patients underwent the same intestinal preparation with magnesium citrate (Magcorol P®, Horii Pharmaceutical Co, Ltd, Tokyo, Japan) without peroral administration of antibiotics. Antibiotic prophylaxis was begun with the administration of 1 g Cefmetazole sodium 30 minutes prior to the operation, with additional administrations every three hours during the operation. Intravenous administration of antibiotics continued twice per day until the second postoperative day.

Fecal T-RFLP Assay

Twenty-four patients (n=10: Group A, n=14: Group B) were included in the evaluation. Fecal microbiota was analyzed using terminal-restriction fragment length polymorphism (T-RFLP) of fecal samples. The amplification of the 16SrDNA, digestion of restriction enzymes, size fractionation of T-RFs and analysis of T-RFLP data were performed according to the protocol described by Nagashima (7).
The cluster analyses were performed using the SPSS version 20 software program (IBM SPSS, Tokyo, Japan) based on the BslI T-RFLP patterns. The Pearson similarity coefficient analysis and 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 postoperative days. Twenty patients (n=10: Group A, n=10: Group B) were evaluated by the Cylex Immuknow assay. Sodium heparin anticoagulated whole blood samples were collected at our hospital and tested in our laboratory. The immune response was measured using the FDA-approved Cylex Immuknow (Cylex， Columbia, MD) assay according to the manufacturer's instructions (8). CD4-(Immuknow) T cells were positively selected within the microwells using magnetic particles coated with antihuman CD4 monoclonal antibodies and a strong magnet. The release of ATP was measured using luciferin/luciferase and a luminometer (Berthold， Knoxville， TN or Tuner Biosystems，Sunnyvale，CA). The level of immune response was assessed based on the amount of ATP, expressed in nanograms per milliliter.

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. A SSI was defined as spontaneous or surgically released purulent discharge with positive culture results.

Statistical Analysis

The statistical analysis was performed using the chi-squared test and t-test to compare the two groups, and a logistic regression analysis was used 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 1. 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 timing of the passage of gas and meal intake in Group B were significantly shorter than those in Group A (Table 2) .

A SSI was observed in 27 (17.3%) of the 156 patients. A breakdown of infectious complications showed that 21 (13.4%) patients had a superficial incisional SSI, 16 of whom (19.7%) were in Group A and five of whom (6.7%) were in Group B (p=0.016). All superficial incisional SSI were below grade Ⅱ in Clavien-Dindo(CD) classification. Among the six (3.85%) patients with deep and organ/space SSI, four (4.9%) were in Group A and two (2.7%) were in Group B (Table 3). In Group A, two were grade Ⅱ of and two were gradeⅢb in CD classification. In Group B, two were gradeⅢb. Pneumonie, urinary tract infection, and enteritis were below gradeⅡ.

The rate of SSI by age, sex, BMI, prognostic nutrition index (PNI) (9), history of diabetes mellitus, smoking, chronic renal failure, American Society of Anesthesiologists (ASA) score, state of immunosuppression, open/laparoscopic procedure, length of the operation, volume of blood loss, intraoperative hypotension, hypothermia and oral administration of probiotics was investigated in the univariate analysis. Patients with SSI significantly more often underwent the creation of an ostomy, had more blood loss and required the oral administration of probiotics. In the multivariate analysis, only oral administration of probiotics was identified as independent risk factors for SSI (Table 4).

Immuknow ATP assay

In CRC patients, the Immuknow ATP values peaked on the first postoperative day in both groups. In Group A, the Immuknow value of the first postoperative day (POD) was increased significantly compared to the preoperative value (p= 0.022). In Group B, the value on 1POD did not increase significantly compared to the preoperative value (p=0.28) (Fig. 1). On 4POD and 8POD, the Immuknow ATP values tended to decrease, but there were no significant differences between the two groups.

Changes in fecal microbiota

We investigated the changes in the fecal microbiota resulting from the surgery and subsequent to probiotics administration. Almost no changes in the number of beneficial bacteria, such as *Bifidobacterium*, were observed in Group A. However, the ratio of *Bifidobacterium* increased in Group B from 4.6% to 9.1% in the mean value.

Discussion

The development of various perioperative management techniques has contributed to a decrease in the incidence of SSI. However, the rate of superficial incisional SSI incidence in elective CRC surgery remains 2.5–20.5 % (10). In this study, the rate of SSI was 17.3%.

Previous studies have reported that the incisional SSI were caused by the imbalance of infectious bacteria, surgical technique and the patient's condition. The factors related to infectious bacteria are the use of preoperative non-absorbable oral antibiotics and prophylactic antibiotic use (11). The factors related to the surgical technique are the preoperative skin preparation, the length of the operation, 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 sex, BMI, ASA score, immunosuppression, smoking, wound classification, need for a blood transfusion, the subcutaneous fat thickness and postoperative hyperglycemia (13).

It was recently reported that perioperative probiotic and synbiotic treatment can reduce infectious complications, such as incisional SSI in esophageal cancer, biliary cancer, and colorectal cancer surgery (14-16). However, the evidence in those reports was relatively weak, and both perioperative probiotic treatment and synbiotic treatment were not found to be independently associated with the 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). We selected BIO-THREE as the probiotics in this study, because one BIO-THREE tablet contains 2 mg of *Enterococcus faecalis* T110, 10 mg of *Clostridium butyricum* TO-A and 10 mg of *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 include beneficial, opportunistic and harmful bacteria. Beneficial intestinal microbiota protect the intestinal tract from the invasion of harmful bacteria, while harmful bacteria manifest pathogenicity when the host resistance is decreased (22). In our study, the ratio of beneficial bacteria tended to be increased with perioperative administration of probiotics. Beneficial bacteria increase the concentration of short chain fatty acids (SCFAs) such as acetic, propionic and butyric acids. These SCFAs play various important roles in the colon, including maintenance of the intestinal environment acidity, stimulation of epithelial cell proliferation, stimulation of intestinal motility and enhancement of epithelial mucin secretion, as well as serving as metabolites for energy metabolism in epithelial cells (23). In this study, the intestinal peristalsis was improved earlier in Group B than in Group A.

The clinical validity of the Immune Cell Function Assay (Cylex Immuknow assay) as an objective tool for assessing the immune function has been validated in previous reports when it 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 a patient's immune function. The Food and Drug Administration (FDA) approved the use of the Immune Cell Function Assay to quantify cell-mediated immunity by measuring the concentration of ATP from CD4 T cells in 2002. The Cylex Immuknow assay has since been reported to be clinically useful for assessing 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 magnitude of inflammation (25). During the perioperative treatment of our patients with probiotics, we estimated the inflammation using the Cylex Immuknow assay preoperatively and on 1POD, 4POD, and 8POD. In Group A, the Immuknow value of 1POD increased significantly compared to the preoperative value. In contrast, in Group B, the value of 1POD did not increase significantly. The administration of probiotics may have reduced the inflammation related to the surgical stress after 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). Because it reduced the postoperative inflammatory response, it could lead to a decrease in the hospital stay. Our 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 is helpful for maintaining 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 and an increase in CD4 ATP activity. Therefore, probiotics seem to produce perioperative enhancement of the host immune function. In conclsion, 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 CD4-positive lymphocytes. This reduction in superficial incisional SSI probably 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.

Acknowledgments

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Figures Legends

Figure 1 Immuknow ATP values of both groups

Figure 2 Changes in fecal microbiota (the ratio of *Bifidobacterium*)

Table Legends

Table1 Baseline characteristics of colorectal cancer patients (n=156)

Table2 Postoperative characteristics

Table3 Infectious complications

Table4 SSI (univariate analysis)

Table5 SSI (multivariate analysis)