Preoperative Probiotics Decrease Postoperative Infectious Complications of Colorectal Cancer

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Abstract: *Background:* The objective is to elucidate the effects of oral bifid triple viable probiotics among patients with colorectal cancer. *Methods:* Sixty patients undergoing radical colorectal resection were randomly assigned to 3-day (days −5 to −3) preoperative probiotics (group A, n = 30) or placebo (group B, n = 30) treatment. The alteration of intestinal flora was evaluated by fecal cultures of *Escherichia coli*, *Bifidobacterium longum* and intestinal fungi; the gut barrier function by serum endotoxins and D-lactic acids and the immune and stress responses by peripheral blood immunoglobins, interleukin-6 and C-reactive protein. Postoperative infections were documented physically, radiologically and microbiologically. *Results:* Inverted *Bifidobacterium*/*Escherichia* ratios were preoperatively and postoperatively present in group B (both $P < 0.05$). *Bifidobacterium* counts increased significantly, whereas *Escherichia* counts decreased significantly on postoperative days 3 to 5 ($P < 0.05$), along with reversing the *Bifidobacterium*/*Escherichia* ratio inversion until postoperative days 3 to 5 in group A. Group A also had lower levels of endotoxins, D-lactic acids, serum interleukin-6 and C-reactive protein but higher levels of serum IgG and sIgA (all $P < 0.05$) than group B. The incidences of postoperative infectious complications were 3.3% to 6.7% and 3.3% to 30% in groups A and B (overall, 10.0% versus 33.3%, $P < 0.05$), respectively. *Conclusion:* The preoperative oral bifid triple viable probiotics minimize the postoperative occurrence of infectious complications, with possible mechanisms attributed to the maintenance of the intestinal flora and restriction of bacterial translocation from the intestine. It was representative of the enhancement of systemic/localized immunity and concurrent attenuation of systemic stress response.

Key Indexing Terms: Probiotics; Colorectal cancer; Surgery; Microflora; Infections. **[Am J Med Sci 2012;343(3):199–205.]**

Colorectal cancer (CRC) is the second leading cause of adult cancer deaths in the United States.[1] The previously low incidence of CRC in China has recently increased because of changes in lifestyle and diet; CRC has become the fifth leading cause of cancer mortality.[2] Radical resection is the gold standard treatment in most cases of CRC, although patients with CRC undergoing surgical intervention frequently experience postoperative infection. This risk of infection is particularly high among patients with diabetes mellitus, a history of cerebrovascular accident, hypoalbuminemia, a higher American Society of Anesthesiologists score, anemia, loss of >10% body weight and preoperative use of steroids.[3,4] In addition to increasing the risks of surgical morbidity and mortality, such infectious complications may negatively impact long-term prognoses. As independent risk factors, their concurrent immune defects may predispose patients to CRC recurrence.[5] Conventional preoperative bowel preparation with antibiotics may aggravate the disturbed flora associated with these complications. Furthermore, bacteremia and septicemia may occur after gastrointestinal operations, when viable bacteria may be translocated from the intestine to intestinal mesenteric lymph nodes or distant organs through the intact intestinal mucosal barrier.[6] The gastrointestinal tract is also associated with multiple organ dysfunction syndrome, which amplifies inflammatory mediators and is likely to be fatal, even in the presence of potent broad-spectrum antibiotics.[7] The prevention of postoperative infections must therefore include the restoration of normal intestinal flora, the restriction of bacterial translocation and the protection of the mucosal barrier.

Normal human intestinal floras have been grouped into 30 genera and 500 species, including aerobes, anaerobes, facultative anaerobes and fungi.[8] These floras are present at densities of 10^{11} to 10^{12} bacteria per gram of tissue. Anaerobes usually predominate, and the ratio of *Bifidobacterium* to *Escherichia* (B/E) is used to assess the microbial colonization resistance.[9,10] As intestinal microorganisms that confer healthy benefits to the host, probiotics are involved in the regulation of intestinal flora, immunity and the mucosal barrier.[11] Oral probiotics have been reported to effectively resist gastric acids, pancreatic enzymes and bile acids and are able to enter the colorectum in active forms to colonize the intestinal mucosae and feces.[12] Probiotics have also been successfully used for the treatment of inflammatory bowel diseases and in the chemoprophylaxis of gastrointestinal cancer.[13,14] The preoperative use of probiotics in gastrointestinal procedures has been reported to enhance immune responses, attenuate systemic postoperative inflammatory responses and improve the intestinal microbial environment.[15] Although probiotics have reduced postoperative infectious complications after hepatobiliary resection in patients with biliary tract cancer,[15] their use remains controversial.[16] Reddy et al[17] reported that a combination of mechanical bowel preparation, neomycin and synbiotics reduced the prevalence of fecal Enterobacteriaceae and bacterial translocation but did not reduce inflammatory responses or septic morbidity. Anderson et al[18] observed no measurable effect of synbiotics on gut barrier function in elective surgical patients, likely because of variations in bacterial species, patient characteristics, doses and administration. Recently, Gianotti et al[19] found that preoperative administration of a mixture of *Lactobacillus johnsonii* (La) and *B longum* (BB536) to patients with CRC undergoing colorectal resection affected

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intestinal microbiota by reducing the concentration of pathogens and modulating local immune responses, but they did not study its impacts on clinical parameters, such as gut barrier function, systemic immune responses and postoperative infectious morbidities.

The objective of this study was to elucidate the effects of probiotic supplementation on postoperative intestinal flora profiles, gut barrier function and systemic immune responses among patients with CRC undergoing elective radical surgeries. The patients received oral bifid triple viable probiotics or a placebo as a supplement to preoperative bowel preparation in a randomized double-blind clinical setting. The study aimed to justify the use of preoperative oral probiotics for the minimization of postoperative infections and related complications.

MATERIALS AND METHODS

Patient Enrollment

This study was a single-center prospective randomized control study. The study protocol was approved by the Institutional Review Board at Xin-Hua Hospital, Shanghai Jiaotong University. Eligible patients (n = 82) were consecutively enrolled between August 2006 and June 2007, aged 45 to 90 years. A patient was included if he or she had been diagnosed with pathologically documented colorectal adenocarcinoma and intended to undergo elective radical CRC resection with laparotomy at Xin-Hua Hospital. No patient had received neoadjuvant chemotherapy or radiotherapy before the resection. Patients were excluded from the study if they were unavailable for the collection of fecal samples 6 days before operation (n = 5); if the radical resection failed or the CRC was complicated with malignant ascites (n = 4); if they exhibited moderate to severe cardiac, pulmonary, hepatic or renal dysfunction (n = 4); if they had a preexisting autoimmune disease (n = 2); if they were afflicted with an infectious disease within the 30 days preceding the enrollment (n = 4) or if they had participated in any other clinical trial within the last 6 months (n = 3). All participants volunteered to give informed consent. The eligible patients (n = 60) were randomized and divided into 2 treatment groups: group A (n = 30) received preoperative probiotics and group B (n = 30) received placebos.

Treatment

The probiotic and placebo treatments were identically sealed with aluminum foil and administered in a double-blind manner for 3 days (days −5 to −3) before surgery. The patients in group A received 3 oral bifid triple viable capsules, each of which contained 0.21 g (10^8 cfu/g) of *B longum*, *L acidophilus* and *Enterococcus faecalis* (Shanghai Sine Wangxiang Pharmaceutical Co., Shanghai, China), 3 times a day, whereas the control patients in group B also received 3 placebo capsules containing maltodextrin three times a day. Conventional bowel preparation was performed on preoperative day 2 afterward (days −2 and −1), including the administration of a full liquid diet, oral gentamicin (80,000 U, three times a day; Zhongxi Pharm Co., Shanghai, China), metronidazole (0.4 g, 3 times a day; Sine Wanxiang Pharmaceutical Co.), polyethylene glycol (139.12 g/2,000 mL, every day; Jiangxi Hygecon Pharmaceutical Co., Shangrao, China) and a daily intravenous infusion of 1,000 mL crystal fluid. During surgery, 3 g of cefuroxime sodium (Zhijun Pharmaceutical Co., Shenzhen, China) was administered intravenously under general anesthesia. A postoperative prophylactic regimen of 3 g of cefuroxime sodium and 1 g of metronidazole (Baxter Healthcare Co., Shanghai, China) was intravenously infused twice daily for 3 to 5 days. All the patients received parenteral nutrition from days +1 to +5 and resumed oral intake after the occurrence of the first spontaneous anal passage of gas or feces (days +3–+5).

Fecal Assay

Fresh fecal samples (0.5 g) were collected on preoperative days 6 (day −6) and 3 (day −3) and from the first spontaneous postoperative defecation (postoperative days 3–5, days +3–+5). The samples were dissolved in 4.5 mL of normal saline. The suspensions were homogenized for 3 minutes in a stomacher (PBI, Milan, Italy), followed by continuous 10-fold dilution. The diluted suspensions were inoculated in an aerobic environment onto MacConkey agar plates (Oxoid, Cambridge, United Kingdom) at 37°C for 24 hours for the culture and count of *E coli,* onto modified Sabouraud's agar plates containing 30 μg/mL of imipenem (bioMérieux, Marcy l'Etoile, France) at 20°C to 25°C for 5 to 7 days for the culture and count of fungi and onto Genbox anaer (bioMérieux) at 37°C for 48 hours for the culture in an aerobical environment and count of *B longum*. Available plates were harvested to obtain counts of colony-forming units, which were converted into the number of bacteria per gram.

Peripheral Blood Assay

Peripheral venous blood samples were taken on preoperative days 6 (day −6), 3 (day −3) and 1 (day −1) and postoperative days 1 (day +1), 3 (day +3) and 9 (day +9). Sera were isolated for IgG, IgM, IgA, sIgA, interleukin-6 (IL-6), C-reactive protein (CRP) and D-lactic acid assays using commercially available enzyme-linked immunosorbent assay kits (IgG, IgM, IgA: Zeptometrix, Buffalo, NY; IL-6: Thermo Scientific, Surrey, United Kingdom; D-lactic acid: Roche-Biopharm, Darmstadt, Germany; CRP: RayBiotech, Norcross, GA and sIgA: Shanghai TJFM Co., Shanghai, China). Endotoxins were quantified with a limulus amebocyte lysate assay kit (Yihua Bio, Shanghai, China). Blood cultures were also obtained in cases of pyrexia exceeding 38.5°C at any time postoperatively, regardless of the presence or absence of an infectious source. Whole blood (10 mL) was sterilely drawn from each set of blood cultures and immediately inoculated into separate blood-culture bottles (Organon Teknika, Durham, NC) for aerobic and anaerobic identification, for 7 days or until the detection of bacterial growth. The diagnosis of bacteremia was confirmed with the isolation of any live organism in a single blood culture, unless the isolate was determined to be *Staphylococcus epidermidis* or any other coagulase-negative *Staphylococcus* species. In such cases, blood cultures were repeated to exclude contamination by coagulase-negative *Staphylococci.*

Observation of Postoperative Infectious Complications

Detailed daily records of postoperative courses were maintained for each patient. Postoperative infections included bacteremia and/or septicemia, postprocedural pneumonias, intra-abdominal abscesses, surgical site infections, perineal infections and anastomotic leakage or fistulae. Bacteremia was defined as the presence of viable bacteria in the blood and was diagnosed by blood culture. Postoperative septicemia was recorded prospectively for all patients until their discharge from our hospital. Postprocedural pneumonia referred to the radiographic presence of characteristic pulmonary infiltration complicated with leukocytosis. Intra-abdominal abscess was characterized by purulent discharge from intraoperatively placed peritoneal drains or the peritoneal accumulation of infectious fluids requiring drainage (confirmed by positive cultures). Surgical site infection referred to spontaneous or surgically re-