Synbiotics and gastrointestinal function-related quality of life after elective colorectal cancer resection

George E. Theodoropoulos, Nikolaos A. Memos, Kiriaki Peitsidou, Theodoros Karantanos, Basileios G. Spyropoulos, George Zografos

Annals of Gastroenterology 2016; 29 (1): 56-62

Background Synbiotics (combination of prebiotics and probiotics) may serve as a supportive dietary supplement-based strategy after colectomy for cancer. The potential benefits of early postoperative administration of synbiotics on the gastrointestinal function-related quality of life inpatients were explored.

Methods Patients who underwent elective colectomy were prospectively enrolled and randomized to receive either synbiotics (n=38) or placebo (n=37) on the day they tolerated liquid diet and for 15 days thereafter. Primary endpoints were Gastro-Intestinal Quality of Life Index (GIQLI) questionnaire assessments at 1, 3 and 6 months postoperatively. Secondary endpoints were functional bowel disorders (“diarrhea,” “constipation”) assessed by EORTC QLQ-C30.

Results Patients under synbiotics had a better GIQLI “Global score” compared with those who received placebo [77±1.67 vs. 71.36±1.69, P=0.01 (1 month); 77±1.7 vs. 72.5±1.73, P=0.03 (3 months); 79.23±1.82 vs. 72.75±1.85, P=0.01 (6 months)]. Multivariate linear mixed model analysis showed that synbiotics administration was the only independent significant factor for the “Global score” amelioration (b: 5.42, SE (b)1.8, 95%CI 1.78-9.1, P=0.004). The EORTC QLQ-C30 “diarrhea” domain score differences from baseline were better after synbiotics administration after 3 (P=0.04) and 6 months (P=0.003). No significant effect on “constipation” scores was observed.

Conclusion Synbiotics administration may have a beneficial effect on the postcolectomy gastrointestinal function.

Keywords Synbiotics, colorectal cancer, health-related quality of life, gastrointestinal quality of life index

© 2016 Hellenic Society of Gastroenterology www.annalsgastro.gr

Introduction

The unavoidable anatomical distortion of bowel anatomy due to surgical resections for colorectal cancer (CRC) may lead to intestinal functional disturbances [1]. Preoperative preparation strategies, such as mechanical bowel preparation may disturb the well-established functional balances between enteric flora and normal large bowel function [2]. Bowel disorders may directly or indirectly affect health-related quality of life (HRQoL) and this may be reflected to proportional alterations in the scoring of standardized measurement tools, such as HRQoL questionnaires [3-7]. A validated instrument that assesses gastrointestinal function is the Gastro-Intestinal Quality Life Index (GIQLI) [3-5]. GIQLI may offer valuable insight in rating postoperative gastrointestinal function status at CRC patients. The 30-item questionnaire (QLQ-C30) of the European Organization for Research and Treatment of Cancer (EORTC) has already widely assessed CRC patients’ HRQoL and it includes items on gastrointestinal function, such as the symptom domains “diarrhea” and “constipation” [4,6,7]. HRQoL instruments may offer “functional surveillance” by monitoring, ranking and quantifying the “subjective” patient’s symptoms.

Initial HRQoL postoperative deterioration and postcolectomy gastrointestinal disturbances are expected to be temporary enduring 3-6 months [7]. Interventional strategies and enhancing therapeutic regimens at the immediate postoperative period may have a soothing effect on patients’...
symptoms. Probiotics are cultures of live microorganisms that might beneficially affect the host by improving the composition and the equilibrium of indigenous microflora [8]. Prebiotics are nondigestible ingredients (fibers) that reach the colon and serve as substrate for fermentation by probiotics and indigenous colonic bacteria to nutrients beneficially improving host health [9]. Synbiotics are the combination of probiotics and prebiotics and are believed to be more efficient in terms of gut health and function [10]. Administration of probiotics and/or synbiotics may serve as one of the supportive modulating dietary supplement-based strategies in CRC patients [11-25].

Studies reporting on the effects of post-/synbiotics on functional outcome, gastrointestinal symptoms and HRQoL after CRC surgery are rather scarce [14,22,25]. In an effort to clarify the effects of early postoperative administration of synbiotics on bowel functional outcome, this is measured by gastrointestinal-oriented HRQoL instruments and domains, we prospectively carried out a randomized trial in a selected cohort of patients undergoing colorectal resection for cancer. This study aimed to justify the early postoperative use of oral synbiotics for the minimization of the adverse effect of surgery on the short- and mid-term gastrointestinal symptoms-associated HRQoL.

**Study design**

This was a prospective, double-blind, randomized control trial (RCT) of two groups. The study was carried out in the First Department of Propaedeutic Surgery of Athens Medical School at Hippocrates Hospital, Athens, Greece.

**Participants**

Patients eligible for inclusion were those with histological documentation of cancer of the colon or rectum, operated between July 2008 and April 2012. Patients of both genders, aged between 18 and 80 years, who were candidates for elective colorectal resection for cancer were included in the study. Patients were excluded if they: denied written informed consent; were pregnant; had hereditary cancer; had a history of inflammatory bowel disease; had metastatic disease at presentation; required permanent or temporary stoma; had emergency operation; had major postoperative complications; had disease progression during the study period; or did not tolerate liquid diet by the 5th postoperative day (POD).

**Study intervention**

**Randomization and method of allocation concealment**

The study patients were randomized before surgery to receive either synbiotics (Synbiotics group) or placebo (Control group). Equal randomization was accomplished using a computer-generated random allocation schedule. The method of allocation concealment was sequentially numbered sealed opaque envelopes technique. Both synbiotics and placebo preparations were in foil-sealed sachets stored in identical numbered containers. Both study products were white powders, identical in weight, smell, and taste. Thus, the identity of the specific product was blind to participants, support staff and investigators for the entire duration of the study period.

During the study period, of the 75 patients who initially consented to participate, 38 in the Synbiotics group and 37 patients in the Control group completed the entire trial (Fig. 1).

**Arms assigned interventions and study medications**

A specific multistrain/multifiber synbiotic composition of prebiotics and probiotics (“IONIA” Pharmaceuticals, Athens, Greece) was administered at the active comparator arm of the study. It contained 10 [11] of each of four lactic acid bacteria (LAB): *Pediococcus pentosaceus* 5-33:3, *Leuconostoc mesenteroides* 32-77:1, *Lactobacillus paracasei* spp. *paracasei* 19, and *Lactobacillus plantarum* 2362, and 2.5 g of each of the four fermentable fibers (prebiotics): β-glucan, inulin, pectin and resistant starch. The synbiotics were delivered in sachets and then mixed with water (12 g in 250 mL of water once daily). The treatment started on the day patients tolerated per os liquid intake (2nd-4th POD). The intervention period lasted 15 days. The patients belonging to the placebo comparator arm received only the 4 fibers and no LAB (12 g in 250 mL of water once daily for 15 days). All the subjects were interviewed by a dedicated research fellow (KP) and reactions to the product, and any adverse events occurring in the 15-day period were recorded. During the study period, no parenteral or enteral nutritional supplementation was given. All patients received a regular diet preoperatively, and a low-residue diet 1 day before surgery. For mechanical bowel preparation the afternoon before the operation all patients were given 45 mL of regimen containing monobasic sodium phosphate monohydrate and dibasic sodium phosphate heptahydrate and the dose was repeated 4-6 h later. For chemoprophylaxis, 500 mg of metronidazole and 1 g of cefoxitin were given prior to anesthesia and continued for 24 h after the operation. During the postoperative period, all patients received the regular parenteral hydration infusion.

**Outcome measures and questionnaires**

The primary end point of the study was the assessment of gastrointestinal function-related quality of life at 1, 3 and 6 months postoperatively by the use of the validated questionnaire GIQLI. Secondary endpoint was the assessment of functional bowel disorders (“diarrhea”, “constipation”) at 1, 3 and 6 months postoperatively based on the respective domains of the validated instrument EORTC QLQ-C30.
Patients visited a clinic dedicated to the post-colectomy “functional and oncologic surveillance” and were asked to answer the HRQoL questionnaires at fixed postoperative assessment time-points: 1, 3 and 6 months. Completeness of answers to the questionnaires was regularly checked. Only few patients required a family member’s assistance due to low educational background and vision problems. Baseline HRQoL scores had already been taken at patients’ admission, prior to any treatment.

The GIQLI questionnaire consists of 36 questions referring to a specific symptom, answered by the patient using five-point Likert scales [3-5]. Scoring of individual questions is summarized in five categories (emotional function, physical function, social function, symptoms, and treatment reaction). “Global” GIQLI score is calculated by summarizing the points from all 36 questions and its highest score is 144 points. An elevated GIQLI score means a higher quality of life.

The EORTC QLQ-C30 consists of 30 questions [4,6,7]. All data undergo linear transformation and the scales for severity of symptoms range from 0 (least) to 100 (worst). The symptom scales ‘constipation’ (item No 16) and “diarrhea” (item No 17) were used in the current study.

Ethical considerations

The study was approved by the ethics committee of Hippocratie Hospital, Athens, Greece. The purpose of the study was explained clearly to the patients and their informed written consent was obtained. The trial was registered at ClinicalTrials.gov of the National Institute of Health with the identifier number NCT01479907 and assigned name “SYNBIOTICSCOLON”.

Statistical analysis

Continuous data was presented as mean ± standard error of the mean (SEM) and categorical data as percent (%). SPSS statistical software, version 18 (SPSS, Inc., Chicago, IL) was used for all analyses. For numerical data the t-test while for categorical data the chi-square test with the Fisher exact test when appropriate were used. Modelling was performed with linear mixed models which are a variant of generalized linear models permitting flexibility of modeling not only the means of the data but their variances and covariances as well. The longitudinal nature of the data was also adjusted. Estimators were adjusted for confounding variables as described in each test. For numerical data that did not follow the normal distribution non-parametric tests were used (Mann-Whitney, Kosmolgorov-Smirnov). Tests were considered statistically significant if P<0.05. The goal for the study was set as a change in the “Global score” of the GIQLI questionnaire between the two groups of at least 10%. The calculated sample size was 33 patients per arm giving 80% power. Statistically significant
differences were also evaluated for their magnitude using Cohen’s d classification. If Cohen’s d is <0.2 the difference is considered of small, 0.2<d<0.5 of medium and when d>0.8 of high clinical significance.

Results

No statistically significant differences were observed between the two groups regarding age, gender, site of tumor, type of surgery or stage (Table 1), comorbidities and medications. In general, no bowel motility affecting medications were used by any of patients post-operatively and there was no significant difference between the two groups regarding the use of narcotics after their discharge to home. The same standard peri-operative antibiotic chemoprophylaxis appropriate for colorectal resection (2nd generation cephalosporin and metronidazole first dose pre-operatively and additional doses until the end of the first POD) was delivered to both groups. Inpatient diet was controlled and did not differ between the groups. The percentages of patients subjected to adjuvant treatment were not different between the groups and none of the patients had pre-operative radiotherapy. When the 12 right colectomy specimens of the Synbiotics group patients were compared with the 11 Control group right colectomies, no significant differences were observed in terms of the resected terminal ileum length (mean±SEM: 8.5±1.1 vs. 8.2±1.3 cm, P=0.43) and the amount of large bowel removed (34.8±4.6 vs. 35.7±4.1 cm, P=0.34).

Likewise, no bowel length differences were revealed between the 14 Synbiotics and the 17 Control group sigmoidectomies (24.8±2.6 vs. 27.1±3.1 cm, P=0.18). All low anterior resections for the rectal cancer of the study were restored in a stapled end-to-end fashion at a height of 7.2±1.8 and 6.9±2.1 cm from anal verge at the Synbiotics and Control groups, respectively (P=0.22). No statistically significant difference was observed between the two groups regarding the baseline GIGLI scores and EORTC QLQ-C30 “diarrhea” and “constipation” domains’ scores preoperatively (Table 2).

GIGLI

Measured GIGLI “Global scores” at the postoperative assessment time-points were better at the Synbiotics than in the Control group [77±1.67 vs. 71.36±1.69, P=0.01 (1 month), 77±1.7 vs. 72.5±1.73, P=0.03 (3 months), 79.23±1.82 vs. 72.75±1.85, P=0.01 (6 months)]. Changes in GIGLI scores through time were examined using linear mixed models with dependent variable the scores over time and covariates the group, age, gender, stage, location, chemotherapy, and procedure. Multivariate linear mixed model analysis showed that synbiotics administration was the only independent statistically significant factor for a substantial amelioration of GIGLI “Global score” (b 5.42, SE (b)1.8, 95% CI 1.78-9.1, P=0.004, Fig. 2A). The assessment time-point was not a significant factor for GIGLI score in the model except for the 6 month- score, which was significantly better than the preoperative one (b 3.69, SE (b)1.58, 95% CI:6.58-0.58, P=0.02). The effect size of the difference expressing the magnitude of this association, measured by Cohen’s d, was 0.69 and that was considered as medium to high. Interaction between group and time was also tested in the group but was not found statistically significant.

No statistically significant change occurred between the two groups regarding GIGLI “symptoms” domain over time. The change in each group is shown in Fig. 2B. Linear mixed

Table 1 Demographic data of patients receiving synbiotics or placebo

|              | Synbiotics group | Control group | p   |
|--------------|------------------|---------------|-----|
| Age (years)  | 66.8±2.17        | 69±1.37       | 0.42|
| Gender (%)   |                  |               |     |
| Male         | 20 (52.6)        | 23 (62.16)    | 0.54|
| Female       | 18 (47.4)        | 14 (37.8)     |     |
| Location (%) |                  |               |     |
| Colon        | 26 (68.4)        | 28 (75.6)     | 0.65|
| Rectum       | 12 (31.6)        | 9 (24.3)      |     |
| Stage (%)    |                  |               |     |
| 0            | 5 (13.2)         | 8 (21.6)      | 0.43|
| 1            | 9 (23.7)         | 9 (24.32)     |     |
| 2            | 15 (39.5)        | 10 (27)       |     |
| 3            | 9 (23.7)         | 10 (27)       |     |
| 4            | 0 (0)            | 1 (2.7)       |     |
| Chemotherapy (%) |          |               |     |
| Yes          | 26 (68.1)        | 24 (64.8)     | 0.80|
| No           | 12 (31.6)        | 13 (35.2)     |     |
| Laparoscopic (%) |         |               |     |
| Yes          | 27 (71.1)        | 24 (64.8)     | 0.80|
| No           | 11 (28.9)        | 11 (29.7)     |     |

Table 2 Baseline scores for GIGLI and EORTC QLQ-C30 “diarrhea” and “constipation” domain

|               | Group (mean±SEM) | p   |
|---------------|------------------|-----|
| GIGLI         |                  |     |
| Global        | 74.27±1.78       | 70.94±1.55 | 0.17|
| Symptoms      | 42.54±0.97       | 42.02±0.82 | 0.69|
| Emotional     | 8.69±0.54        | 7.61±0.59 | 0.18|
| Physical      | 13.98±0.67       | 12.75±0.62 | 0.19|
| Social        | 6.56±0.28        | 6.06±0.27 | 0.13|
| EORTC QLQ-C30 |                  |     |
| “Constipation”| 26.11±5.65       | 27.44±6.67 | 0.83|
| “Diarrhea”    | 25.21±5.54       | 17.63±4.92 | 0.28|

SEM, standard error of mean
modeling for the emotional subdomain showed a substantial amelioration during followup in Synbiotics group patients (b1.83, SE(b)0.68, 95% CI0.48-3.2, P=0.009, Fig. 2C). Emotional subdomain scores amongst assessment time-points did not differ significantly. Females had significantly lower physical function subdomain scores compared to males (b-1.75, SE(b)0.69, 95% CI-3.14--0.37, P=0.013). Finally, the social domain showed a substantial increase in Synbiotics group patients (mean steady contrast between the two groups: 0.72±0.30, P=0.03, Fig. 2D).

EORTC QLQ-C30

Domains referring to “diarrhea” and “constipation” were isolated. The greater the score of the EORTC QLQ-C30 symptoms domains was, the worse the outcome. The “diarrhea” score was analyzed by subtracting the score at each time point from the baseline. Each difference was then compared between the two groups. The equation was ScC30ti- ScC30t0. The lower the difference was the better the outcome. As shown at Fig. 3, the mean difference in the Synbiotics group remained negative at all time-points. Therefore, at all time-points the score was always lower than that at baseline, highlighting a postoperative improvement. The values reached statistical significance between the two groups when compared at 3 months and 6 months assessment time-points (-13.33±5.45 vs. 4.04±5.6, P=0.04 and -19.1±6.3 vs. 2.01±5.45, P=0.003, respectively). The same analysis was used for the “constipation” domain. However, no statistically significant change was observed (data not shown).
Discussion

In the current randomized study early postoperative synbiotics administration appeared to have a beneficial effect on postcolectomy gastrointestinal function, as that was indicated by the better GIQLI scores at all postoperative assessment time-points, as well as the significant amelioration of the symptom “diarrhea”, as assessed by the respective EORTC QLQ-C30 domain. The beneficial action of probiotics supplementation in gastrointestinal disorders has been recognized. Restoration of gut balance, inhibition of the epithelial and mucosal adherence of pathogens, introduction of lower colonic pH favoring the growth of nonpathogenic species, and stimulation of immunity during or after antibiotic treatment through receptor competition may be related to the positive gastrointestinal traits and properties of pro-/synbiotics [26]. In keeping with our results, the use of pro-/synbiotics to postoperative CRC patients has been reported to be beneficial in alleviating gastrointestinal symptoms, including diarrhea, the first defecation time, abdominal pain and flatulence [14,24,25]. Probiotics have been associated with a reduction and prevention of non-colectomy-related diarrheas [27,28].

In general, early postoperative oral nutrition is regarded as an essential part of fast track recovery after colorectal surgery. Food intake can stimulate gastrointestinal peristalsis in the early postoperative period, attenuates catabolism and potentially decreases infectious complications. Synbiotics may play an additional beneficial role on the context of fast track and enhanced recovery programs after colorectal surgery but definite suggestions are not currently plausible since no fast track protocols integrating the administration of synbiotics have been tried yet in the clinical practice.

The strength of our study lies at the repetitive assessment of patients at fixed assessment time-points and the use of validated questionnaires to objectify the patients’ subjective symptomatology. Only one other group of investigators has utilized a general, non-specific HRQoL questionnaire in symptomatology. Only one other group of investigators has validated questionnaires to objectify the patients’ subjective of patients at fixed assessment time-points and the use of pro-/synbiotics [22]. In keeping with our results, the use of pro-/synbiotics to postoperative CRC patients has been reported to be beneficial in alleviating gastrointestinal symptoms, including diarrhea, the first defecation time, abdominal pain and flatulence [14,24,25]. Probiotics have been associated with a reduction and prevention of non-colectomy-related diarrheas [27,28]. Improvements in the functional outcome and/or HRQoL were observed in all groups after administration of probiotics, with the right colectomy and proctectomy groups mostly benefited [22].

Different types of colorectal resections were included in our study and this constitutes a potential limitation on deriving definitive conclusions on the accurate beneficial role of synbiotics on specific subsets of patients. Another limitation of our study is its complete clinical orientation in regards to outcome measures and the lack of any theoretical explanation that could be provided by associated laboratory or microbial assays. Albeit, it would be more compelling if supporting data regarding actual changes in the bowel microbiota had been included. It has been, though, sufficiently demonstrated that the use of probiotics after surgery has markedly improved intestinal microbial populations [14-16]. Although local immune function restoration by synbiotics is easily conceivable, improvement infunctional state may not be fully explainable. An explanation could involve immune-mediated neural local and systemic mechanisms [29]. Dendritic cells in the gastrointestinal tract are able to interact with commensal bacteria and the nerves embedded in the gut wall [29]. The vagus nerve has an important role in signaling from the gut to the brain and can be stimulated by bacterial products or inflammatory cytokines [30]. Synbiotics-induced modulation of vagal responses indirectly affecting the brain function itself may be speculated. Indeed, in our study, non-gastrointestinal GIQLI domains, such as emotional and social function were improved after synbiotics supplementation. Administration of a probiotic formulation has significantly attenuated psychological distress in human volunteers and reduced anxiety-induced behavior in a rat model [31].

Probiotics may alleviate irritable bowel syndrome (IBS) symptoms [32]. Bowel resections may induce an IBS-like transient gut dysfunction, both due to sensitive and motor disruptions of the visceral nerves, as well as changes in the bacterial ecosystem of the intestine. Implicating pathophysiological mechanisms may be similar. It has been suggested that the generation of nitric oxide (NO), a gas with immuno-modulating but also neuron-modulating properties, by lactobacilli could play an important role [33]. Synbiotics positive effects on neuro-motor/sensitive locoregional transmission may be more complex and needs further evaluation. The sustained positive effects of synbiotics over time in our study may be related to the early “conditioning” of bowel to a better state of function via its repopulation by the “non-pathogenic” microbiota and the restoration of a “better”

---

**Summary Box**

**What is already known:**

- Synbiotics are used as a supportive dietary supplement-based strategy in patients after colectomies for cancer
- Synbiotics administration has been shown to alleviate gastrointestinal symptoms such as diarrhea, abdominal pain and flatulence in patients after colectomies for colorectal cancer
- Improvement in the functional outcome of colectomized patients has been shown only by one group using a non-specific HRQoL questionnaire

**What the new findings are:**

- Early postoperative synbiotics administration improved post-colectomy gastrointestinal function based on better GIQLI scores at all postoperative assessment time-points
- The early administration of synbiotics in patients after colectomy for colorectal cancer led to significant amelioration of “diarrhea” based on the EORTC QLQ-C30 questionnaire
mucosa. Nevertheless, these tempting speculations need further confirmation by additional studies.

Despite the tantalizing multitude of trials on the potentially positive role of pre-/pro-/synbiotics, a straightforward translation into a clinical evidence-based strategy remains still unlikely. Incorporation of pre-/pro-/synbiotics formulations in the perioperative management of the CRC patient could be fully supported if further evidence from RCTs with all clinical relevant end-points will be accumulated in the future.

References

1. Schoetz DJ Jr. Postcolectomy syndromes. World J Surg 2005;15:605-608.
2. Prakash S, Rodes L, Coussa-Charley M, Tomaro-Duchesneau C. Gut microbiota: next frontier in understanding human health and development of biotherapeutics. Biologics 2011;5:71-86.
3. Eypasch E, Williams JI, Wood-Dauphinee S, et al. Gastrointestinal functional outcome after laparoscopic sigmoid colectomy. J Am Coll Surg 2007;194:49-56.
4. Schwenk W, Neudecker J, Haase O, Raue W, Strohm T, Müller JM. Comparison of EORTC quality of life core questionnaire (EORTC-QLQ-C30) and gastrointestinal quality of life index (GIQLI) in patients undergoing colorectal cancer resection. Int J Colorectal Dis 2004;19:554-560.
5. Forgione A, Leroy J, Cahill RA, et al. Probiotics, prebiotics, and functional outcome after laparoscopic sigmoid colectomy. Ann Surg 2009;249:218-224.
6. Schwarz R, Hinz A. Reference data for the quality of life of colorectal cancer patients. Nord Med 2002;5:1028-1034.
7. Theodoropoulos GE, Karantanos T, Stamopoulos P, Zagoras G. Prospective evaluation of health-related quality of life after radiation-induced diarrhea: results from multicenter, randomized, placebo-controlled nutritional trial. Int J Radiat Oncol Biol Phys 2008;71:1213-1219.
8. Delia P, Sansotta G, Donato V, et al. Use of probiotics for prevention of radiation-induced diarrhea. World J Gastroenterol 2001;13:912-915.
9. Ohigashi S, Hoshino Y, Ohde S, Onodera H. Functional outcome, quality of life, and efficacy of probiotics in postoperative patients with colorectal cancer. Surg Today 2011;41:1200-1206.
10. Peitsidou K, Karantanos T, Theodoropoulos GE. Probiotics, prebiotics, symbiotics: is there enough evidence to support their use in colorectal cancer surgery? Dig Surg 2012;29:426-438.
11. He D, Wang HY, Peng JY, Zhang MM, Zhou Y, Wu XT. Use of probiotics as prophylaxis in patients undergoing colorectal resection for cancer: A meta-analysis of randomized controlled trials. Clin Res Hepatol Gastroenterol 2013;37:406-415.
12. Li Y, Wang D, Kong X. A study of enteral eco-nutrition in patients received surgery for colorectal cancer [in Chinese]. Med J Qilu 2008;4:303-305.
13. Rolfe RD. The role of probiotic cultures in the control of gastrointestinal health. J Nutr 2000;130:396S-402S.
14. Hempel S, Newberry SJ, Maher AR, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA 2012;307:1959-1969.
15. Mcfarland LV. Systematic review and meta-analysis of saccharomyces boulardii in adult patients. World J Gastroenterol 2010;16:2202-2222.
16. Goehler LE, Gaykema RP, Nguyen KT, et al. Interleukin-1β in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? J Neurosci 1999;19:2799-2806.
17. Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 2000;405:458-462.
18. Messaoudi M, Lalande R, Violle N, et al. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br J Nutr 2000;105:755-764.
19. Lombardo L. New insights into Lactobacillus and functional intestinal disorders. Minerva Gastroenterol Dietol 2008;54:287-293.
20. Sobko T, Huang L, Midvedt T, et al. Generation of NO by probiotic bacteria in the gastrointestinal tract. Free Radic Biol Med 2006;41:985-991.