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Prebiotic and Synbiotic Treatment before Colorectal Surgery – Randomised Double Blind Trial

Bojan Krebs

UCC Maribor, Department for Abdominal Surgery, Maribor, Slovenia

ABSTRACT

The aim of our study was to demonstrate higher concentrations of lactic acid bacteria (LAB) on the colonic mucosa in operated colorectal cancer patients treated with oral intake of synbiotics or prebiotics preoperatively. We also tried to prove that the systemic inflammatory response after surgery is not so severe in patients who took synbiotics or prebiotics, furthermore these patients have less postoperative complications and a favorable postoperative course. 73 patients with preceding colorectal operations were recruited. They were randomized into three groups. One group received preoperatively prebiotics, the second synbiotics in and third was preoperatively cleansed. We have defined the number of four different probiotic bacteria on colonic mucosa with polymerase chain reaction (PCR). Serum levels of interleukin-6, CRP, fibrinogen, white cell count and differential blood count were measured pre- and postoperatively to determine systemic inflammatory response. We succeed in confirming that in the synbiotic group there were considerably more LAB presented on the mucosa. They did pass the upper gastrointestinal tract and were isolated in colonic mucosa. On the other hand, we did not find any statistical differences in systemic inflammatory response measured by upper factors and no differences in postoperative course and complications rate between all three groups.

Key words: *probiotics, prebiotics, synbiotics, systemic inflammatory response syndrome, polymerase chain reaction, colorectal surgery*

Introduction

Despite advances in medicine and especially oncology and surgery in last decades there is no significant rise of survival rate following colorectal surgery for cancer and also the complication rate remains almost the same[1].

One of the important postoperative complications is without doubt postoperative infection or even sepsis. Despite the broad use of antibiotic prophylaxis these infections are still a very important reason for postoperative morbidity leading to prolonged hospital stay, increased costs or even higher mortality[2].

The exact pathophysiological mechanism that predisposes patients undergoing major abdominal surgery to infection is not known. However, of major importance for the pathogenesis of postoperative infections is undoubtedly bacterial translocation from the gastrointestinal tract to the systemic circulation[3–6]. The main causes of bacterial translocation are generally an injury of the colonic mucosa leading to disruption of the gut barrier and increased intestinal permeability as well as microbial imbalance and decreased immunodeficiency of the patient after major surgery[7–9].

The use of mechanical bowel cleansing before colorectal surgery is commonly practiced from almost the beginning of this type of surgery[10–11]. This has been a standard procedure for surgeons for more than 100 years. The rationale is based on hypothesis that feces and bacteria in the operation field could facilitate infection[12]. The idea is that the removal of GIT content preoperatively by mechanical cleansing will reduce the bacterial content and thus reduce the risk of wound infection, abdominal abscesses, and even anastomotic dehiscence. However, recently, several significant trials and three meta-analyses suggest that there is no significant benefit of preoperative cleansing[13–16].

Taking into account that preoperative cleansing can obviously be harmful and that hazard is likely related to the change in the quantity and composition of bacteria in the colon, it would be necessary to find a procedure which would preserve the composition of bacteria and their positive effects.

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Prophylactic treatment with beneficial bacteria might be one of the ways to achieve this. The presence of such bacteria in the gut results in prevention of overgrowth and predominance of potentially pathogenic microbes, which is also called colonization resistance[17].

There is possibility that by adding so called »good bacteria« to severely ill and surgical patients we could prevent the overgrowth of pathogenic bacteria and all consequences of such an event. Such »good bacteria« is called probiotic. The Greek term probiotic means »for life« and is defined as a preparation of living microorganisms that change the micro flora and has positive effects on human health.

Probiotics are as a matter of fact live microorganisms thought to be beneficial to the host organism and there have been many similar definitions in history[18–20]. According to the definition by FAO/WHO, probiotics are live microorganisms which, when administered in adequate amounts have a health benefit on the host[21]. The most common types of microbes used as probiotics are lactic acid bacteria (LAB) and bifid bacteria. In fact, probiotics are bacteria from normal gut flora, which are administered orally.

It is very important that the probiotics utilized remain viable during GIT passage and that at least 107 cfu/ml of viable bacteria reach the intestine[22]. Only in this way we can aspect the clinical effect of ingested probiotics. Most probiotics currently advertised and sold do not meet this condition. As a matter of fact, the ability to survive the acidity of the stomach and bile acid content of the small intestine seems to be limited to very few microorganisms.

Another important fact to remember in choosing the right probiotic for clinical use is that only a small minority of LAB can ferment semi-resistant fibers such as oligofructans: inulin and phleins. When the ability of 712 different LAB to ferment oligofructans was studied only 16 of 712 were able to ferment the phleins and as little as 8 of 712 the inulin type fibre. Only four LAB species fermented these fibers: Lactobacillus plantarum (several strains), Lactobacillus paracasei subspecies paracasei, Lactobacillus brevis and Pediococcus pentosaceus[23,24].

LAB also have the ability to control various pathogens, found in gut. This property is strain-specific and often limited to a few strains. When the ability of fifty different LAB to control 23 different pathogenic Clostridium difficile strains was tested, only 5 proved effective against all, 8 were antagonistic to some, but 27 were totally ineffective[25]. The five most effective strains were Lactobacillus paracasei subspecies paracasei (2 strains) and Lactobacillus plantarum (3 strains). Clearly information like this is important for choice of probiotics for clinical use.

For bacterial fermentation in lower GIT the presence of some plant dietary fibres are of great necessity. Such fibres also had a strong self-bioactivity. A recent definition by the American Association of Cereal Chemists suggests that dietary fibre is: „the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine[26]. Such dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances and are called prebiotics. Those are non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the digestive system in ways claimed to be beneficial to health.

The combination of specific bioactive microorganisms – probiotics and specific plant fibers – prebiotics which can stimulate growth of certain microbes and which together have a synergistic effect is called synbiotic.

There are several possible mechanisms by which synbiotics have a beneficial effect on the organism:

– antagonistic activity against pathogenic bacteria either by inhibition of adherence and translocation or by production of antibacterial substances,
– modulation of barrier function,
– modulation of intestinal cytokine production,
– anti-inflammatory properties, and
– improvement of gut permeability[27].

On the whole our idea was to administer »good bacteria« (probiotics) and fibers that stimulate their growth (prebiotics) to the patients with preceding colorectal surgery and totally evade preoperative bowel cleansing. Our first aim was to demonstrate the difference in the number of LAB in the colon mucosa between patients who received them and patients who did not. However, as we already mentioned above, the problem is that most of the LAB do not even reach the large bowel. Secondly, the degree of systemic inflammatory response after surgery and possible positive clinical effect resulting in a smaller number of postoperative complications was also of crucial importance when conducting this study.

The results of our study may alter the algorithm and guidelines of the preoperative preparation not only in patients with colorectal cancer but also in patients undergoing any other major abdominal surgery and thus diminish the patient's morbidity and mortality as well as the hospital stay.

Materials and Methods

This was a prospective randomized, controlled, double blind trial of three groups. The study was performed at University Hospital Maribor, Slovenia and microbiology tests with polymerase chain reaction (PCR) were made at the Institute of Public Health Maribor, Slovenia.

A total number of 73 patients with preceding large bowel operation for colorectal cancer were recruited of whom 19 were excluded for various reasons.

Exclusion criteria were: Any chronic disease or health condition which in opinion of investigators may interfere with the patient's ability to comply with protocol, place the subject at unnecessary risk, or interfere with the evaluation of the study drugs, patients with signs or symptoms of bowel obstruction, patients who could not take the mechanic preoperative bowel preparation, and patients with chronic inflammatory bowel disease. Some patients were removed from the study if one or more of the following had

occurred: significant protocol violation or non-compliance on the part of the patient, refusal of the patient to continue treatment or decision of the investigator that termination is in the patient's best medical interest. In the end 54 patients were eligible for statistical analysis.

Procedures and treatment

Patients were randomized in three groups with sealed opaque envelopes. Once a patient was consented to enter a trial an envelope was chosen and opened. According to this, the patient entered either group A, B or C.

Both synbiotics and prebiotics were stored in identical sachets with different labels, AA and AB. They were both, white powders, identical in weight, smell and taste. Thus the identity of the product was not known to participants or researchers until the end of the study.

Patients in all three groups started with a liquid diet two days prior to the operation. Patients in group A got one sachet marked AA twice a day three days before the operation. Patients in group B got one sachet marked AB twice a day three days before operation. Finally patients in group C received standard mechanic preoperative bowel preparation. On the day before surgery those patients were allowed a light breakfast. Afterwards they drank 75 ml of ColoclensR. After few hours, afternoon patients had to consume 4 liters of hyperosmolar liquid Golitely, provided by the hospital pharmacy.

We estimated systemic inflammatory response by repetitive measuring of C reactive protein (CRP) levels, proinflammatory cytokine interleukin-6 (IL-6) levels, fibrinogen levels, white blood count and differential blood count. Samples of 10 ml blood were taken on the operation day, two hours prior to surgery, on day one and day three following the operation.

ASA scores (American Society of Anesthesiologists) were calculated at the time of surgery for each patient.

All patients received preoperatively one dose of antibiotic cefuroksim and metronidasole.

Several clinical data, first oral solid food intake, first peristalsis, passing of gasses and stool and complications were observed and recorded.

Patients were operated on in accordance with standard oncological principles.

There were only elective operations and no emergencies.

Two mucosal samples were taken immediately after the surgical specimen removal. They were store on ice and immediately transported to the laboratory for PCR examination. Oligonucleotide sequences specific for bacteria Lactobacillus paracasei, Lactobacillus mesenteroides, Lactobacillus plantarum, and Pediococcus pentosaceus were determined with real time PCR.

Isolation and purification of DNA was performed with Qiagen DNA MiniKit under the strict manufacturer's protocol.

Primers and probes were designed for detection of specific oligonucleotide sequences in target genes listed in

TABLE 1
TARGET GENES FOR THE PRIMERS AND PROBES

Bacteria	Target gene
Lactobacillus paracasei	putative pheromone PcrA
Leuconostoc mesenteroides	acetat kinase
Lactobacillus plantarum	luxS
Pediococcus pentosaceus	collagen adhesion gene 1158

Table 1. Detection of specific products was carried out by using fluorescently labeled hydrolising probes (Taqman probes). Amplification and detection was performed in ABI Prism 7000 Sequence Detection System.

In all samples, in which we confirmed the oligonucleotide sequence characterized by at least one of the four bacteria, we performed relative quantification of bacterial species with real-time PCR. As reference gene we used the gen for beta-globin, which is part of the human genome.

All the procedures were in accordance with the Helsinki Declaration. The study protocol received a full local research and ethics committee approval. Written informed consent was obtained from all patients.

Patient data were collected in special designed protocols until discharge from hospital and data were analyzed on an intention to treat basis.

Medications used

The sachets marked AA contained Synbiotic 2000 FORTE. It consists 10^{11} of each of four LAB: Pediacoccus pentosaceus 5–33:3, Leuconostoc mesenteroides 32–77:1, Lactobacillus paracasei subsp paracasei 19, and Lactobacillus plantarum 2362. This makes 400 billion LAB per dose or if supplemented twice daily 800 billion LAB per day. Also included in the sachet is 2.5 g of each of the four fermentable fibres – probiotics: betaglucan, inulin, pectin and resistant starch. Synbiotic 2000 FORTE was produced and marketed by Medipharm, Kageröd Sweden and Des Moines, Iowa, USA.

The sachets AB contained only prebiotics in the same amounts: 2.5 g of each of the four fermentable fibres (prebiotics): betaglucan, inulin, pectin and resistant starch.

Coloclens (Senna glykosides) is an antraquinon laxative specially prepared for thorough preparation prior to abdominal surgery.

Golitely (Polyethylene glycol) is an osmotic agent, which is effective in cleansing the colon prior to elective colonic surgery.

Statistics

Qualitative data were presented with absolute frequencies and numerical data with median and range values

TABLE 2
DEMOGRAPHIC ANALYSIS

Group	A	B	C
No.	18	20	16
Sex; male/female	11/7	13/7	9/7
Age; median (range)	62 (43–87) y	64 (46–81) y	67 (52–78) y
ASA score; (median)	1.72 (1–3)	2.15 (2–3)	2.06 (1–3)

due to distribution not following normal. Data analysis was performed using repeated measurements analysis of variance, and presented with distribution values F and P-values for within subject factor, between subject factor and the model as a whole. Only P-values lower than 0.05 were considered significant. Statistics was done using MedCalc (MedCalc software, ver. 11, Mariakerke, Belgium).

Results

54 patients were eligible for the study. 20 in group A, 18 in group B and 16 in group C. There were 21 women and 33 men. Median age was 65, for years between 43 and 87. According to the ASA score we divided the patients into three groups: ASA 1, ASA 2 and ASA 3 and 4 together. Patients in all three groups were well matched for age, sex distribution, and ASA score and there were no significant statistical differences between them (Table 2).

We performed 26 sigma resections, 16 right hemicolectomies, 8 anterior rectal resection, 3 left hemicolectomies and one resection of transversal colon.

With PCR we determined a relative concentration of all four ingested LAB on colonic mucosa of all patients. The Kruskal-Wallis test was used to prove higher concentrations of lactobacilli in patients who received synbiotics preoperatively. The difference was statistically significant for all four LAB ($p < 0{,}0001$). There was always a difference between group A and B or C, however there was no difference between groups B and C (Table 3).

On the other hand we found that there was no statistical significant difference in systemic inflammatory response which was valued with three consecutive measurements of CRP, IL-6(Table 4), fibrinogen, leukocyte count and differential blood count prior to operation, on the first and third postoperative day.

There was the slight difference within groups but this was not statistically significant.

TABLE 3
RELATIVE NUMBER (MEDIAN) OF ISOLATED BACTERIA ON BOWEL MUCOSA CONSIDERING GROUPS A, B AND C

	PAR	MES	PENT	PLANT
	median (min–max)	median (min–max)	median (min–max)	median (min–max)
A	0.47 (0.1–33.2)	3.71 (0–2534) x 10E-3	3.36 (0.1–57.5)	0.27 (0–14.6)
B	2.18 (0–000) x 10E-2	0 (0–0.01)	0 (0–2.7)	0 (0–3.3)
C	8.73 (0–224) x 10E-3	0 (0–0.03)	0 (0–4.1)	0 (0–0.4)
Chi-Square	24.976	25.126	37.110	29.455
P	< 0.01	< 0.01	< 0.01	< 0.01

PAR – Lactobacillus paracasei, MES – Lactobacillus mesenteroides, PENT – Pediococcus pentosaceus, PLANT – Lactobacillus plantarum

TABLE 4
THE VALUES OF THREE MEASUREMENTS OF IL-6 IN THE GROUPS AND THEIR COMPARISON ANALYSIS OF VARIANCE FOR REPEATED MEASUREMENTS (F, F-VALUE ANALYSIS OF VARIANCE, P, LEVEL OF SIGNIFICANCE).

IL-6	Groups				F
repeated measur.	A	B	C	Together	p
first	2.6 (2–16)	5 (2–25)	4.05 (2–49)	3.95 (2–49)	96.70*
second	103.5 (6–235)	67.9 (9.9–323)	63.7 (21.5–224)	67.6 (6–323)	<0.001
third	13.4 (4.2–96.4)	15.3 (3.2–56.7)	12.6 (6.7–132)	14.3 (3.2–132)	
F	1.36**				0.67***
p	0.701				0.611

* Comparison of repeated measurements (post-hoc test with $p<0.05$ differs first from the second and first from the third measurement, while the second and third measurements did not differ).

** Comparison of values between groups A, B and C.

*** Comparison of combined effect, ie the impact of belonging to groups A, B and C on repeated measurements.

TABLE 5
COMPARISON OF FIRST PERISTALSIS, FIRST SOLID FOOD INTAKE, FIRST PASSING OF GASSES AND FIRST PASSING OF STOOL BETWEEN THREE GROUPS (P, LEVEL OF SIGNIFICANCE)

Group	A	B	C	p
	median (min–max)	median (min–max)	median (min–max)	
FP	1.9 (1–3)	1.5 (1–3)	2.2 (1–4)	0.580
FSOFI median(min–max)	5.2 (2–10)	4.95 (3–10)	5.4 (3–9)	0.621
POG median(min–max)	2.7 (2–4)	2.5 (1–4)	2.8 (2–5)	0.873
POS median(min–max)	4.2 (2–8)	3.8 (2–6)	3.8 (2–6)	0.752

*First peristalsis (FP), first solid oral food intake (FSOFI), passing of gasses (POG) and passing of stool (POS).

We also could not confirm any statistically significant differences for observed clinical data: first peristalsis (FP), first solid oral food intake (FSOFI), passing of gasses (POG) and passing of stool (POS).We detected a slight advantage in pre and synbiotic group, but there were no significant differences (Table 5).

Medial hospitalization time after operation was 10.68 days with minimum 7 and maximum 25 days. In group A median hospitalization time was 10.16, in group B 11.30 and in group C 10.5 days. The hospitalization was longer in the control group but the difference was not statistically significant (p=0.512).

There were no major complications. 9 patients had postoperative bowel paresis, four patients had wound infection and two pneumonia. All complications were treated conservatively. The complication rate was low and we found no statistical differences between groups.

Discussion

The results of this study confirmed that our choice of synbiotic was correct. We succeeded to detect the ingested LAB in much higher concentrations than in other groups. We decided to choose a mixture of four probiotics which had already been tested in several other studies and demonstrated safe, and four different prebiotics[28–32]. The composition was constructed after large studies of > 350 human and >180 plant strains by microbiologists Asa Ljungh and Torkel Wadström from Lund university, Sweden (33, 34). They have chosen the four LAB to be used in the composition based on the ability of the various LAB to produce bioactive proteins, transcribe nuclear factor kappa-light-chain-enhancer of activated B cells (NF-.B), produce pro- and anti-inflammatory cytokines ant antioxidants, and to functionally complement each other. The four LAB individually operate differently, but show synergistic effects when supplemented together.

It is well known that LAB in commercial probiotics which are currently generally available in many cases do not pass the stomach, duodenum, and small bowel. They are destroyed and fermented by the strong gastric and duodenal juices and they do not even reach the large bowel. And the large bowel is the only part of digestive tract, where probiotics could be extremely useful.

This was the reason for us to try to prove and did prove that LAB in Synbiotic 2000 Forte were able to reach the final part of digestive tract, the large bowel. PCR tests showed that all four LAB were present on the colonic mucosa in significantly higher ratio than in patients, who received only prebiotics and in patients who were preoperative cleansed.

We also hoped that synbiotics would have had a beneficial effect on our patients in spite of lower postoperative inflammation response, lower complication rate and of course shorter hospitalization which are very important goals for surgeons. Unfortunately we were not able to prove those benefits although we still believe in them.

In recent years several randomized controlled studies regarding pro and prebiotics in surgical patients have been published. Santvoort and al reviewed 14 randomized control studies on probiotics in surgical patients[35]. Nine showed a significant decrease of total infectious complications in the patients treated with probiotics, but 5 studies could not demonstrate such an effect.

Rayes and al performed a review of 15 randomized control studies published up to 2008 (36). 12 studies showed at least a slight benefit of probiotics, while three studies performed by the same group show no significant positive effect of such treatment. Rayes explains those results by the relative short period of probiotic administration, oral route of administration and inhomogeneous distribution of operations with a high percentage of simple operations with low overall rates of infection.

In 2010 Gianotti et all published a randomized double blind trial regarding the preoperative application of probiotics in patients with colorectal cancer (37). The probiotics given were Bifidobacterium longum and Lactobacillus johnsonii. The conclusion was that only La1 adheres to colonic mucosa and affects intestinal microbiota by reducing the concentration of pathogens and modulates local immunity.

In conclusion, the results of this prospective randomized double blind study suggest that after oral intake of Synbiotic 2000 Forte, which contains four LAB; those are found on colonic mucosa. The study also suggests that the application of synbiotic and prebiotic has no effect on systemic inflammatory response, measured by repetitive measurement of IL-6, CRP, differential blood count and

fibrinogen and there is no effect considering postoperative course. However we feel that we should not generalize these results and that further studies with perhaps larger samples are required.

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B. Krebs

UCC Maribor, Department for abdominal surgery, Ljubljanska ulica 5, SI-2000 Maribor, Slovenia
e-mail: bojan@guest.arnes.si

PREBIOTSKO I SINBIOTSKO LIJEČENJE PRIJE KOLOREKTALNOG OPERACIJE – SLUČAJNI, DVOSTRUKO SLIJEPI TEST

SAŽETAK

Cilj našeg istraživanja bio je pokazati veće koncentracije bakterija mliječne kiseline (LAB) na sluznici debelog crijeva kod operiranih pacijenata oboljelih od raka debelog crijeva liječenih preoperativnim oralnim unosom sinbiotika ili prebiotika. Također smo pokušali dokazati da je sustavni upalni odgovor nakon operacije nije tako težak kod bolesnika koji su uzimali sinbiotika ili prebiotike, nadalje, ovi pacijenti imaju manje postoperativnih komplikacija i povoljan postoperativni tijek. 73 bolesnika s prethodnim kolorektalnim operacijama su regrutirani. Podijeljeni su u tri skupine. Jedna skupina je preoperativno dobila prebiotike, druga sinbiotike, a kod treće je preoperativni postupak čist. Definirali smo broj četiri različite probiotičkih bakterija na sluznici debelog crijeva s lančanom reakcijom polimeraze (PCR). Serumske razine interleukina-6, CRP, fibrinogen, brojem bijelih krvnih stanica i diferencijalne pretrage krvi su vršene prije i nakon operacije radi određivanja sustavnih upalnih odgovora. Mi smo uspjeli potvrditi da je u sinobiotičkoj skupini bilo je znatno više LAB-a prisutno na sluznici. Oni su prošli gornji dio gastrointestinalnog sustava te su izolirani na sluznici debelog crijeva. S druge strane, nismo pronašli nikakve statističke razlike u sustavnim upalnim reakcijama mjerenih gornjim faktorima i nije bilo razlike u postoperativnom tijeku i broju komplikacija između sve tri skupine.