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Supply of Pre- and Probiotics Reduces Bacterial Infection Rates After Liver Transplantation—A Randomized, Double-Blind Trial

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Bacterial infections frequently occur early after liver transplantation. We recently reported significant progress with a synbiotic composition, consisting of one lactic acid bacteria (LAB) and one fiber, which reduced the infection rate from 48% (with selective bowel decontamination) to 13%. Now, our aim is to study if a combination of different LAB and fibers would further improve outcome.

A prospective randomized double-blind trial was undertaken in 66 liver transplant recipients. All patients received enteral nutrition immediately post-operatively. Comparison was made between one group (A) receiving a composition of four LAB and four fibers and another group (B) receiving the fibers only. The treatment started the day before surgery and continued for 14 days. Thirty-day infection rate, length of hospital stay, duration of antibiotic therapy, non-infectious complications and side effects of enteral nutrition were recorded.

The incidence of post-operative bacterial infections was significantly reduced; being 48% with only fibers and 3% with LAB and fibers. In addition, the duration of antibiotic therapy was significantly shorter in the latter group. In both groups, mainly mild or moderate infections occurred. Fibers and LAB were well tolerated.

Early enteral nutrition supplemented with a mixture of LAB and fibers reduces bacterial infection rates following liver transplantation. Treatment with only fibers led to a low incidence of severe infections.

Key words: Liver transplantation, bacterial infections, probiotics, prebiotics, synbiotics

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Introduction

Liver transplant recipients are particularly susceptible to infection. Recent studies report infection rates between 30% and 86% (1–3). Hollenbaek et al. found out that surgical site infections after liver transplantation incur approximately 24 additional hospital days per patient, 159 967 U.S. dollar in excess charges and 10% increased mortality (4). Furthermore, post-operative infections and antibacterial and antiviral treatments are associated with a significantly higher incidence of graft loss (5).

Many of the observed infections are gut derived, and bacterial translocation has shown to be an important pathogenic factor for their development (6). A new concept to decrease bacterial translocation is the administration of fibers (prebiotics) and living lactic acid bacteria (LAB; probiotics) in order to restore the physiological gut flora (7). In addition to their impact on translocation, probiotics have several other beneficial functions that might prevent bacterial infections. Of special interest is their ability to enhance immune function, presumably via activation of macrophages and stimulation of secretory IgA and neutrophils without further release of inflammatory cytokines (8). We previously reported, from a randomized trial in liver transplant patients, a significant reduction in infections with supply of a synbiotic composition consisting of one LAB (*Lactobacillus plantarum* 299) and one fiber (oat fiber), compared to selective bowel decontamination (9).

Recent experimental and clinical studies analyzed the outcome of combination therapy using different strains of probiotic bacteria. Results of these studies implicate a synergistic effect. As example, eight highly concentrated probiotic strains could prevent the onset of acute pouchitis in patients with ileal pouch-anal anastomosis (10).

In an experimental study, different bacterial strains were tested for their potential usefulness in clinical trials. Only eight tested strains could survive the transport through the gastrointestinal (GI) tract and produce antimicrobial substances. Four LAB of the extensively studied strains were chosen, two *lactobacilli*, one *Pediococcus* and one *Leuconostoc*, together with four fibers, known for their strong bioactivities: betaglucan, inulin, pectin and resistant starch, to form a synbiotic composition (11,12).

The present study was undertaken to investigate if the infection rate in human liver transplantation could be further reduced when this novel composition is supplemented.

Patients and Methods

Patients

Sixty-six adult patients scheduled for liver transplantation were included in the double-blind study.

Exclusion criteria were decompensated renal insufficiencies (creatinine clearance <50 mL/min) and cerebral disorders with danger of aspiration. Patients with roux and Y-anastomosis were also excluded, because of assumed danger of anastomotic leak due to early enteral nutrition. The study was approved by the local ethics committee, and all patients gave written informed consent before study entry.

Patients were evaluated with complete medical history and clinical examination, analysis of laboratory parameters and disease-specific further examinations.

Serum prealbumin was measured to evaluate the nutritional status. The patients with liver cirrhosis were classified according to the Child's-Pugh classification. All patients were stratified using the classification of the American Society of Anaesthesiologists (ASA).

Then patients were randomized by sealed envelope into one of the two study groups. In all patients a nasojejunal tube, with the tip behind the ligament of Treitz, was placed intra-operatively.

Enteral nutrition and study groups

Enteral nutrition with a low-fiber formula (Stresson®, Pfrimmer Nutricia, Erlangen, Germany) was started within the first hour after operation. The initial infusion rate was 25 mL an hour. If well tolerated, the enteral infusion rate was increased to 1 mL/kg body weight/h from post-operative day 1, and continued for at least 8 days. If the patient did not have sufficient oral intake on POD 8, enteral nutrition was further continued. The formula contains per liter; 1250 kcal, 75 g protein, 145 g carbohydrates and 42 g lipids. Crystalloids were infused when judged clinically necessary and oral intake started on the second post-operative day.

Group A: A novel specific synbiotic composition of pre- and probiotics (Synbiotic 2000® Medipharm, Kågeröd, Sweden and Des Moines, IA, USA) was administered twice daily via the feeding tube or orally. All the strains used are deposed at the Belgian Coordinated Collection of Microorganisms (BCCM) deposition number provided below within parenthesis. Each dose of the combination contains four different LAB: 10^{10} *Pediacoccus pentosaceus* 5-33:3 (dep. no. LMG P-20608), *Leuconostoc mesenteroides* 77:1 (dep. no. LMG P-20607), *Lactobacillus paracasei* ssp. paracasei F19 (dep. no. LMG P-17806) and *L. plantarum* 2362 (dep. no. LMG P-20606) plus four bioactive fibers: 2.5 g of each betaglucan, inulin, pectin and resistant starch, totally 10 g/dose, or 20 g/day. The treatment started on the day of the operation and continued during the first 14 days after the operation.

Group B: Identical treatment as group A, with the only difference being that the patients received only the four fibers and no LAB. The sachets and its content looked identical in both groups. The only person who knew the type of treatment received, was a study nurse who was not involved in the trial and did not treat the patients.

Regimen of antibiotics and catheters

All patients received intravenous prophylaxis with cefuroxime (1.5 g) and metronidazole (500 mg) twice daily for 36 h starting 30 min before operation. After that, antibiotics were only given in case of bacterial infection.

If infections occurred, patients were initially treated empirically and then following resistance testing of the isolated bacteria.

H2-blockers (150 mg ranitidine) were routinely supplied once daily during the whole study period.

During operation, all patients received a central line, an intra-abdominal drainage and an urinary catheter. These catheters were removed as soon as possible, normally on the third post-operative day except in case of serious complications. In addition, a T-tube, which remained in place for 6 weeks, was placed in the bile duct intra-operatively.

Analyzed parameters

Primary study end point was the occurrence of post-operative bacterial infection during the first 30 post-operative days. Therefore, incidence, type of infections, and type of isolated bacteria were specifically recorded. In addition, total hospital stay and days on intensive care unit, side effects of enteral nutrition and duration of antibiotic therapy were evaluated. The duration of antibiotic therapy was determined by counting the number of days on which the patients received antibiotic therapy. The period of antibiotic prophylaxis without signs of infection was excluded. Total length of hospital stay was defined as the period between day of operation and discharge.

In order to rule out differences in intra- and post-operative risk factors for infections, antibiotic therapy 1 month prior to operation, operating time, number of transfused units of blood, plasma and albumin intra- and post-operatively were analyzed. The following well-known non-infectious complications were specifically looked at: biliary fistulas, anastomotic leaks, intra-abdominal hemorrhage, vascular complications, rejections and impaired kidney function requiring hemodialysis. In addition, relaparotomies were specifically registered.

Laboratory values were measured pre-operatively and on post-operative days 1, 4 and 8, including hematology, clinical chemistry with bilirubin, bun, CRP, IgA, transferrin and prealbumin. In addition, the cellular immune status was measured using FACScan (FACSCalibur, Becton-Dickinson, Heidelberg, Germany): CD4+, CD8+, CD19+, NK-cells and CD4/CD8 ratio.

Surveillance and definition of infection

Body temperature was measured twice daily. Surveillance cultures from urine, blood, bile (t-tube), wound and intra-abdominal drainages were done routinely in each patient three times a week and in case of suspected infection. The respective specimens were cultivated on agar plates for aerobic and anaerobic bacteria (blood agar, McConkey agar and gentamicin agar plates). Lactobacilli were also specifically looked for. Differentiation of bacteria was performed by using routine clinical methods. Results of the surveillance cultures were reported to the clinicians, but only patients with clinical signs of infection plus positive cultures were treated.

The diagnosis of bacterial infection was based on fever (>38°C), elevation of C-reactive protein, specific clinical symptoms of infection as shown below and a positive bacterial culture:

- *Low urinary tract infection*: dysuria, leucocyturia and a positive urine culture with >10^5 colony forming units/mL.
- *Upper urinary tract infection*: symptoms as listed above plus fever.
- *Wound infections*: detection of pus in the wound and a positive bacterial culture.
- *Pneumonia*: fever, cough, dyspnea, reduced arterial oxygen, typical pulmonary infiltrate on chest x-ray, positive culture from sputum or bronchoalveolar lavage.
- *Cholangitis*: fever, elevation of cholestatic enzymes, positive culture from T-tube.

Statistical analysis and sample size calculation

Statistical analysis was performed using SPSS 10.0 (Chicago, IL, USA). To compare discrete variables, the χ^2 test was used. For non-parametric analysis of continuous variables, the Mann-Whitney U test and the Kruskal-Wallis test were done. A p-value < 0.05 was regarded as statistically significant. The sample size was calculated by the Institute of Medical Biometry at the Humboldt University, Berlin. From the results of our previous study (9), we assumed that the study substance would be able to reduce infection rates from 50% (placebo) to 15%. Therefore, the calculated sample size was 33 patients for each group with an α-error of 2.5%, a power of 80% and a drop-out rate of 10%.

Results

Demographic and operative data

In total, 66 patients completed the study, 33 in each group. Age, gender, ASA-classification and Child's-Pugh classification of cirrhosis were equally distributed between the two groups (Table 1). Four patients in group A compared to two patients in group B received antibiotic therapy during the month before operation. The operating time, amount of intra- and post-operatively transfused units of blood, fresh frozen plasma and albumin did not differ significantly between the groups (Table 2). There were no relevant differences regarding routine immunosuppression, which consisted of a triple regimen including prednisolone, either tacrolimus or cyclosporine and an induction therapy with an IL2 antibody.

Table 1: Demographic data and pre-operative state of health

Group	A	B
Age (years)	53 ± 2	50 ± 2
Male/female	22/11	16/17
Child		
A	7	8
B	16	19
C	8	6
ALF	2	0
ASA		
1	0	0
2	8	11
3	22	22
4	3	0

Mean and standard error of mean, ALF = acute liver failure.

Table 2: Operative data, length of hospital stay, days on intensive care unit and length of antibiotic therapy

Group	A	B
Operating time (min)	283 ± 10	299 ± 10
EC (n) intra-post-operative	3.4 ± 0.6	2.4 ± 0.5
	4.2 ± 0.7	7.5 ± 3.7
FFP (n) intra-post-operative	8.8 ± 0.9	7.0 ± 0.7
	5.3 ± 1.5	6.5 ± 2.9
HA 20% (ml)	1953 ± 412	1956 ± 384
Hospital stay (day)	27.8 ± 2.4	27.9 ± 2.1
ICU (day)	8.8 ± 0.9	10.2 ± 1.8
Antibiotics (day)	0.1* ± 0.1	3.8* ± 0.9

Mean and standard error of mean.
*Difference between the groups statistically significant.
EC, erythrocyte concentrates; FFP, fresh frozen plasma, HA, human albumin, ICU, intensive care unit.

Length of hospital stay and antibiotic therapy

The mean total length of hospital stay did not differ between the groups. The stay on intensive care unit was slightly shorter in group A than in group B, but the difference was not statistically significant (Table 2). The duration of antibiotic therapy (without prophylaxis) was significantly shorter in the patients receiving the synbiotic combination compared to those receiving only fibers (Table 2).

Side effects of enteral nutrition

Enteral nutrition, containing the synbiotic combination, was well tolerated in all patients. In group A, 3 out of 33 patients developed diarrhea and 5 out of 33 patients abdominal cramps; and in group B, 4 out of 33 patients had signs of diarrhea and 6 out of 33 patients abdominal distension and cramps. All side effects disappeared under temporary reduction in the amount of enteral nutrition.

Post-operative bacterial infections, other complications and mortality

Peri-operative mortality was 0% in both groups. A total of 16 (48%) out of 33 patients receiving only the four fibers, developed some sort of infection (Table 3). Predominantly, mild-to-moderate infections of the upper (n = 5) or low urinary tract (n = 7) occurred. Only one severe infection (pneumonia) was observed. The number of days with

Table 3: Post-operative bacterial infections and isolated bacteria

Group	A	B
Patients with infection	1/33* (3%)	16/33* (48%)
Urinary tract	1	12
Wound	0	1
Pneumonia	0	1
Cholangitis	0	2
Bacteria		
E. faecalis/faecium	1	11
E. coli	0	3
Enterobacter cloacae	0	2
Pseudomonas aeruginosa	0	2
Staphylococcus aureus	0	1

*Difference between groups significant.

temperature elevations > 38.5°C was one in group A compared to 22 in group B. All infections were treated with antibiotics. Most of the isolated bacteria were gut-derived with a predominance of *Enterococcus faecalis* and *Enterococcus faecium*. In contrast, only one bacterial infection was observed in the group receiving the synbiotic combination (3%). The duration of urinary or central line catheterization was the same in both groups. In 10 of the 13 patients with urinary tract infections, urine catheters were removed on the third post-operative day. In the remaining three patients the catheters remained for 5–6 days (one patient from group 1, two patients from group 2). The urinary tract infections were diagnosed at a mean of 8 days (5–14 days) following transplantation. The catheter had been removed before occurrence of infection in all 13 patients.

The incidence of acute rejections was 6 (18%) out of 33 in group A and 7 (21%) out of 33 in group B. Twelve patients (36%) in group A had non-infectious complications; biliary tract stenosis or fistulas treated endoscopically with stents (n = 4), lienalis-steal syndrome requiring intervention with angiography (n = 4), abdominal hemorrhage requiring relaparotomy (n = 2) and acute renal failure (n = 2). Four patients in group B (12%) developed non-infectious complications; abdominal hemorrhage requiring relaparotomy (n = 2), acute renal failure (n = 1) and initial non-function of the liver followed by re-transplantation (n = 1).

Surveillance cultures

Results of the surveillance cultures are listed in Table 4. Eight patients in group 1 compared to 16 patients in group 2 had positive bacterial cultures. Enterococci were the most frequent bacteria. LABs were not isolated in the group with live probiotics.

Table 4: Results of the surveillance cultures in the two study groups: number of patients with positive surveillance cultures, number of positive/total cultures per site, time between transplantation and positive culture

Specimen	A	B
Urine		
No. of pos pts	n = 5/33 (4 EF, 1 PA)	n = 12/33 (10 EF, 1 EC, 1 PA)
No. of pos /total cultures	8/18	20/40
Time after OLT (days)	5 (2–8)	8 (5–14)
Ascites		
No. of pos pts	n = 2/33 (EF, SA)	n = 4/33 (EF)
No. of pos/total cultures	2/2	4/4
Time after OLT (days)	2	3 (2–4)
Bile		
No. of pos pts	n = 1/33 (EC)	n = 1/33 (EF)
No. of pos/total cultures	1/4	2/3
Time after OLT (days)	4	4
Wound		
No. of pos pts	n = 0	n = 1/33 (SA)
No. of pos/total cultures		2/3
Time after OLT (days)		6

Mean and range of days. OLT, orthotopic liver transplantation; EF, *E. faecalis* or *faecium*; PA, *Pseudomonas aeruginosa*; EC, *E. coli*; SA, *Staphylococcus aureus*.

Laboratory parameters and cellular immune status

The mean laboratory values including nutritional parameters did not differ significantly throughout the groups. There was a great variety in the cellular immune parameters between the patients, but the differences between the groups did not reach statistical significance.

Discussion

Prevention of nosocomial bacterial infections is a major issue in surgical patients. Despite advanced surgical techniques and broad-spectrum antibiotic prophylaxis and treatment, bacterial infection is the most common cause of morbidity within the first 3 post-operative months in patients following liver transplantation (13). Reported risk factors for the development of infections are advanced age, accompanying liver or renal disease, malnutrition, a high number of intra- and post-operatively transfused blood products and unsuccessful operation (1,6). None of these parameters were statistically different between the two groups presented in this analysis so that the pre- and intra-operative risk profile was comparable.

In the present prospective, randomized, double-blind trial, early enteral nutrition supplemented with a mixture of four LAB and fibers significantly reduced the incidence of bacterial nosocomial infections following liver transplantation compared to only fibers. The difference is even more astonishing as more non-infectious complications, known as risk factors for infections, occurred in the group with LAB. Because the present infection rates following liver transplantation are even lower than in our previous study with only one strain of probiotics, *L. plantarum* 299 (9), and one fiber, the combination of different pro- and prebiotics could in fact act synergistically although the studies are not directly comparable. The infection rate in the group with only fibers was similar to those observed in other studies with parenteral or standard enteral nutrition (1).

In our previous study, mentioned above, 15 out of 32 liver transplant recipients receiving selective bowel decontamination and a fiber-free enteral diet developed bacterial infections, the majority being cholangitis, pneumonia and sepsis caused by gram-negative, gut-derived bacteria. In contrast, only 4 out of 31 patients treated with one living lactic acid bacterium plus oat fiber developed infections. Therefore, we assumed that prevention of bacterial translocation by pre- and probiotics was the major mechanism of action (9), an assumption supported by other similar studies (14,15).

Fibers alone have been suggested to reduce translocation by stimulation of the growth of the commensal microflora and subsequent increase in production of short chain fatty acids, which are known to stabilize the intestinal barrier and the local immune system (16,17). These findings could explain why, in the patients of the present study who received only the four bioactive fibers, mainly mild and only few so called gut-derived infections were observed in comparison to the fiber-free group in the previous study.

The majority of infections in this study occurred in the urinary tract. There was no difference between the two study groups with regard to the length of duration of urinary catheters or number of patients with pre-existing diseases of the urinary tract. Therefore, probiotics are likely to be responsible for the reduction of these infections. In addition to their impact on bacterial translocation, probiotics act via several other mechanisms: they can reduce and eliminate potentially pathogenic microorganisms (18), reduce and eliminate various toxins and mutagens from urine and faeces (19), modulate innate and adaptive immune defense mechanisms, promote apoptosis and release numerous nutrients, antioxidants and growth factors from consumed fibers (20)—functions which might all be of importance for reduction of infections in surgical patients. In clinical studies, certain *Lactobacillus* strains were able to reduce the risk of urinary tract infections, bacterial vaginosis and yeast infections. It is interesting that these effects were achieved not only by intra-vaginal administration, but also through oral supply of LAB (21). Therefore, it seems that probiotics not only act locally in the gut, but also by enhancing especially the innate immune system. This hypothesis is supported by experimental studies in mice, showing that oral administration of probiotics results in an increase in activity of natural killer cells, peripheral blood mononuclear cells and peritoneal macrophages, and also in the proliferative response of spleen cells to mitogens (22).

A recently published randomized, double-blind study of 55 cirrhotic patients with minimal hepatic encephalopathy with a similar study design and the same synbiotic combination as the present study, compared the effects of oral supplementation of the synbiotic combination during 30 days, to those of non-fermentable fiber (23). A significant decrease of venous ammonia and serum endotoxin levels, and prevention of cecal overgrowth with *Escherichia coli* and *Staphylococcus* spp. were observed. Furthermore, supply of the synbiotic composition led to reversal of minimal hepatic encephalopathy and improvement of liver function in approximately half of the patients. Interestingly, fermentable fibers alone were also effective in a substantial proportion of patients.

So far, clinical experience with pre- and probiotics in surgical patients is limited. One trial was performed in 45 patients with acute pancreatitis. In the group who received *L. plantarum* 299 plus oat fiber, infected pancreas necrosis or abscess occurred in 1 out of 22 patients compared to 7 out of 23 patients in the control group (24).

Although the prevention of mainly mild or moderate infections does not seem to be an important advantage, several additional positive effects were noted. As a consequence of the lower infection rates, the mean duration of antibiotic therapy was significantly shorter in group A. In addition, the length of stay on intensive care unit was not significantly different despite the higher rate of non-infectious complications in group A. Especially in high-risk patients who develop post-operative complications, this kind of prophylaxis could have the greatest benefit. Total length of hospital stay was similar in both groups. This is well explained by the present policy of our Medical center, as well as of other German Medical centers, to routinely keep liver transplant recipients in the hospital for 25 days.

Mean laboratory values including nutritional parameters such as prealbumin, transferrin and immunoglobulin A, did not significantly differ between the groups, implicating that the lower infection rates in the living probiotic group were not caused by a more effective nutrition. Cellular immune parameters also showed no significant difference, but they are difficult to interpret due to immunosuppression.

In conclusion, early enteral nutrition with a mixture of different pre- and probiotics is an effective means to prevent post-operative bacterial infections in high risk surgical patients, and since it causes no resistant strains and has no serious side effects, it could be widely used. Although no placebo group was included in the present study, data from our previous study and from other clinical studies implicate that prebiotics alone seem to have a beneficial effect. In the present study, they probably prevented more serious infections. Further clinical studies with larger patient numbers that also include measurement of special immune parameters are needed to confirm these preliminary results and to clarify the exact mode of action of pre- and probiotics.

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