INTESTINAL INFECTION

A prospective randomised study of the probiotic *Lactobacillus plantarum* 299V on indices of gut barrier function in elective surgical patients

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Background: Bacterial translocation occurs in surgical patients and may predispose to postoperative septic morbidity. Many factors are thought to influence the prevalence of bacterial translocation, one of which is the composition of the gut microflora. The aim of this prospective and randomised study was to assess the effect of the probiotic *Lactobacillus plantarum* 299v on the incidence of bacterial translocation, gastric colonisation, and septic complications in elective surgical patients.

Methods: Patients undergoing elective major abdominal surgery were randomised to either a treatment or control group. The treatment group received an oral preparation containing *Lactobacillus plantarum* 299v (*Proviva*) for at least one week preoperatively and also in the postoperative period. Bacterial translocation was determined by culture of a mesenteric lymph node and serosal scraping obtained at laparotomy. Gastric colonisation was assessed by microbiological culture of nasogastric aspirates. All postoperative septic complications were recorded.

Results: A total of 129 patients completed the study (probiotic group n=64). There was no significant difference between the two groups in terms of bacterial translocation (12% *v* 12%; p=0.82), gastric colonisation with enteric organisms (11% *v* 17%; p=0.42), or septic morbidity (13% *v* 15%; p=0.74).

Conclusions: Administration of *Lactobacillus plantarum* 299v in elective surgical patients does not influence the rate of bacterial translocation, gastric colonisation, or incidence of postoperative septic morbidity.

Septic complications remain a major cause of morbidity in patients undergoing elective abdominal surgery despite the routine use of antibiotic prophylaxis. The majority of nosocomial infections are caused by organisms derived from the gastrointestinal tract. There is increasing evidence to suggest that the passage of these organisms across the intestinal barrier to distant sites is the cause of this septic morbidity.[1] The gut origin of sepsis hypothesis is based on the phenomenon of bacterial translocation (BT), which is the term given to the process by which bacteria cross the intestinal epithelium. Many factors are thought to promote the occurrence of BT, including increased intestinal permeability, host immunodeficiency, and alterations in the composition of the gut microflora.[2]

In recent years, scientific knowledge in the field of microbiology has expanded and it has been suggested that the gastrointestinal microflora has a role to play in maintaining human health.[3] This has led to attempts to manipulate the composition of the gut microflora in a beneficial way, in the hope of achieving health benefits in the host. The gastrointestinal ecology in normal subjects may be altered by administration of live microorganisms, termed *probiotics*. These organisms have been shown to inhibit the growth and adherence of potentially pathogenic bacteria to enterocytes, an important step in the process of BT.[4 5] There is therefore a sound theoretical basis for proposing that probiotic induced alteration of the gastrointestinal microflora may influence the rate of BT and subsequent septic morbidity in surgical patients. This hypothesis has been investigated in this prospective and randomised clinical trial using the probiotic *Lactobacillus plantarum* 299v.

PATIENTS AND METHODS

Approval for the study was obtained from the Scarborough Local Research Ethics Committee. All patients listed for major elective abdominal surgery were eligible for entry into the study. Patients were excluded if they had received antibiotics in the week prior to surgery. Randomisation to the treatment or control group was achieved by opening of a sealed envelope with numbers generated by random number sequence. Patients in the treatment group were asked to consume 500 ml per day of *Proviva* (Skanemejerier, Malmo, Sweden). *Proviva* is a commercially available oatmeal based drink containing 5×10^7 colony forming units per ml of *Lactobacillus plantarum* 299v. Duration of preoperative consumption of the drink was from the time of study entry to the day prior to surgery. Patients were asked to avoid other probiotic products for the duration of the study. Patients were supplied with a diary sheet and asked to record their intake of the drink. All patients otherwise continued their normal diet until the day of surgery. In the postoperative period *Proviva* was gradually reintroduced in the treatment group according to tolerance. Patients were not blinded as to which group they were in, and no placebo product was used.

Gastric colonisation and bacterial translocation

Gastric colonisation and BT were assessed using techniques described previously.[6 7] A 5 ml nasogastic aspirate was obtained at the time of induction of anaesthesia and transported to the laboratory in a sealed sterile container for culture. This technique has been shown to yield reliable and reproducible results, comparable with those obtained by direct sampling of gastric contents at laparotomy.[8] Immediately after

Abbreviations: BT, bacterial translocation; CRP, C reactive protein; POSSUM, Physiological and Operative Severity Score for enUmeration of Morbidity; IQR, interquartile range; MLN, mesenteric lymph node; GALT, gut associated lymphoid tissue.

Table 1 Details of the patients in the initial treatment group who did not receive any prophylactic antibiotics

Patient No	Age (y)	Sex	Diagnosis	Operation	Septic complication	Causative organisms
1	42	M	Enterocutaneous fistula	Small bowel resection	Wound	*E faecalis*, coliforms, mixed anaerobes
2	78	M	Colorectal Ca	Hartmann's	Wound/UTI	Coliforms/*E coli*
3	34	M	Colorectal Ca	AP resection	Wound/line	Mixed anaerobes/ *S epidemidis*
4	67	M	Colorectal Ca	Right hemicolectomy	Wound	Mixed anaerobes/*S aureus*
5	66	M	Colorectal Ca	Anterior resection	Chest	*H influenzae*
6	62	F	Pancreatic Ca	Triple bypass	None	
7	72	M	Pancreatic Ca	Whipples	None	
8	74	M	Leiomyosarcoma	Laparotomy	None	
9	77	F	Colorectal Ca	AP resection	None	
10	68	M	Colorectal Ca	AP resection	Wound	Mixed anaerobes, *E faecalis*, coliforms
11	78	F	Colorectal Ca	Right hemicolectomy	Wound	*S aureus*

Ca, cancer; UTI, urinary tract infection; AP, abdominoperineal.

opening the peritoneum, a lymph node was excised from the ileocolic mesentery, and a serosal scraping taken from the antimesenteric border of the terminal ileum using a fresh surgical blade. Both samples were transported immediately in sterile saline to the laboratory for culture.

The lymph nodes and serosal samples were separately homogenised in sterile saline using a stomacher (Seward Medical, London, UK). These homogenates and the nasogastric aspirates were inoculated onto blood agar and cystine-lactose-electrolyte deficient medium for aerobic incubation, and blood and neomycin blood containing media for anaerobic incubation. All cultures were maintained at 37°C for 48 hours. Isolates grown from lymph nodes, serosal scrapings, and nasogastric aspirates were identified using standard microbiological techniques.[9 10]

Antibiotic prophylaxis

The original intention of this study was to evaluate the use of probiotics instead of prophylactic antibiotics in surgical patients. All of the control patients received standard antibiotic prophylaxis, but the first 11 patients enrolled in the treatment group were not given any antibiotics. Seven of these patients developed one or more postoperative septic complications, with a high incidence of anaerobic wound infection. Details of these patients are summarised in table 1. As a consequence of this significant increase in septic morbidity, the study protocol was amended so that all subsequent patients received a single dose of intravenous cefuroxime and metronidazole on induction. The ethics committee was informed of this amendment.

Systemic inflammatory response, POSSUM, and septic morbidity

Serum C reactive protein (CRP) levels were measured preoperatively and on postoperative days 1 and 7 as a measure of the systemic inflammatory response. POSSUM scores (Physiological and Operative Severity Score for enUmeration of Morbidity) were calculated at the time of surgery for each patient.[11] Septic complications were defined as the presence of recognised pathogens in body tissues that are normally sterile, confirmed by the results of culture and supported by clinical, haematological, or radiological evidence.

Statistical analysis

Patient data were collected until discharge from hospital, and data were analysed on an intention to treat basis. Statistical analysis was performed using the XLStatistics Excel Workbooks for Data Analysis software package. Qualitative data were compared using the χ^2 test or Fisher's exact test for small samples. Quantitative data were expressed as medians (interquartile range (IQR)), and were compared using the Mann-Whitney test for non-parametric data. A p value of 0.05 or less was taken to signify statistical significance. A sample size calculation based on the published prevalence of BT demonstrated that approximately 44 patients would be required in each group to show a significant decrease in the prevalence of BT from 15% to 0% at the 5% significance level with a power of 80%.[6]

RESULTS

Demographic details

A total of 129 patients were entered into the study, with 64 randomised to the probiotic group and 65 to the control group.

Table 2 Demographic details

	Probiotic group	Control group
No of patients	64	65
Median age (y) (IQR)	68 (58–74)	69 (58–77)
Sex (M:F)	39:25	36:29
Median POSSUM scores (IQR)		
Physiological	18 (15–20)	18 (14.5–23)
Operative	16.5 (13–25)	15 (12.5–20)
Diagnosis		
Colorectal Ca	30	36
Gastro-oesophageal Ca	4	7
Pancreatic Ca	3	1
Crohn's disease	3	3
Ulcerative colitis	2	2
Diverticular disease	3	3
AAA/aorto-iliac disease	9	6
Miscellaneous	10	7
Malignant disease	41 (64%)	45 (69%)

Ca, cancer; IQR, interquartile range; AAA, abdominal aortic aneurysm.

Table 3 Bacterial translocation to mesenteric lymph node (MLN) and small bowel serosa

	Probiotic group	Control group
MLN obtained	59	60
MLN positive	7 (12%)	7 (12%)
Organisms cultured		
*Escherichia coli**	4	3
*Citrobacter freundii**	1	0
*Proteus mirabilis**	0	1
Staphylococcus epidermidis	1	3
Staphylococcus aureus	1	1
Streptococcus spp.	2	0
Bacillus spp.	0	1
Serosa obtained	54	54
Serosa positive	3 (6%)	3 (6%)
Organisms cultured		
*E coli**	2	1
*Klebsiella oxytoca**	1	0
S epidermidis	1	2

*Potentially pathogenic enteric species.

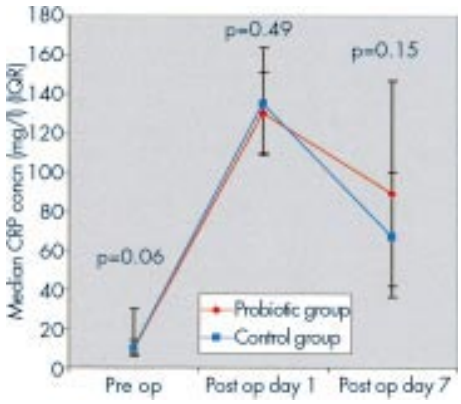

Figure 1 Serial C reactive protein (CRP) measurements in the probiotic and control groups.

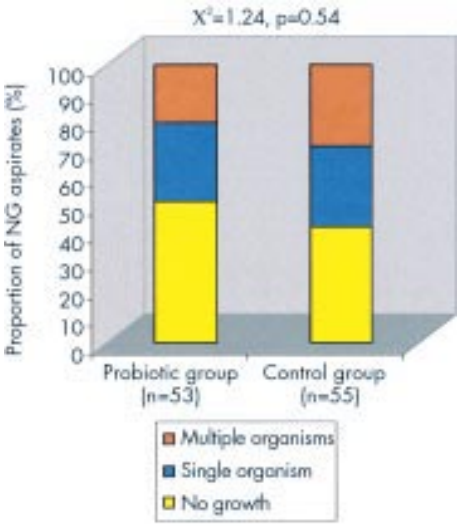

Figure 2 Gastric colonisation in the probiotic and control groups; proportion of nasogastric (NG) aspirates.

The two groups were similar in terms of age, sex, POSSUM physiological and operative scores, and indications for surgery (table 2). Exactly two thirds of patients were operated on for malignant disease.

Probiotic intakes

Median volume of *Proviva* consumed during the preoperative period was 4000 ml (IQR 3100–5500). Median duration of consumption was nine days (IQR 7–12 days). Only five patients (8%) received *Proviva* for less than five days, three of whom received none. The reasons for shortfalls in intake were: *Proviva* temporarily unavailable (n=6), operation postponed (n=4), patient did not like the taste (n=2), nausea (n=1), and operation earlier than anticipated (n=1).

Median volume consumed postoperatively was 800 ml (IQR 380–2045) for a median duration of five days (IQR 4–9 days). Four patients (6%) received none. Median volume consumed per day was 150 ml (IQR 103–227). Only 11 patients (17%) had no problems with intake in the postoperative period, the most common problems of which were: drink not to patient's taste (n=19), nausea (n=16), and paralytic ileus (n=12).

Other than *Proviva* there was no difference between the two groups in terms of additional oral intake.

Bacterial translocation

A mesenteric lymph node (MLN) was harvested in 119 patients. Fourteen MLNs yielded bacterial growth, an overall prevalence of BT of 12%. There was no difference between the two groups, with seven positive MLNs in each. Nine (50%) of the 18 organisms isolated were enteric species (table 3). Serosal scrapings were obtained in 108 patients, six of which yielded growth (table 3). In five of these six patients the MLN was also positive for the same organism (*Escherichia coli* n=3 and *Staphylococcus epidermidis* n=2). There was no morbidity from tissue sampling.

Systemic inflammatory response

Serial serum CRP measurements preoperatively and on postoperative days 1 and 7 are shown in fig 1. CRP measurements on day 7 in patients who did not receive prophylactic antibiotics were excluded from this analysis. There were no significant differences between the two groups at any time point.

Gastric colonisation

Nasogastric aspirates were obtained from 108 patients, 58 (54%) of which yielded growth. A total of 107 isolates were obtained, with *Candida* the most frequently cultured organism. Sixteen (15%) of the isolates were potentially pathogenic enteric species (*E coli* n=7, *Klebsiella* spp n=4, *Serratia* spp n=2,

Proteus mirabilis n=1, *Hafnia alvei* n=1, and *Enterococcus faecalis* n=1). Six of the isolates were *Lactobacillus* species although none were *Lactobacillus plantarum*.

There was no difference between the probiotic group (n=53) and the control group (n=55) in terms of extent of gastric colonisation (fig 2). The prevalence of colonisation with potentially pathogenic enteric species was lower in the probiotic group although this was not statistically significant (6/53 (11%) *v* 9/54 (17%); χ^2=0.57, p=0.45).

Septic morbidity and mortality

Excluding patients who did not receive prophylactic antibiotics, a total of 28 septic complications occurred in 17 patients, an overall incidence of 14%. Potentially pathogenic enteric bacteria were implicated in 18 (64%) of the complications. There was no significant difference in the incidence of septic morbidity between the probiotic and control groups (7/53 (13%) *v* 10/65 (15%); χ^2=0.11, p=0.74). Nine patients died within 30 days of surgery, a mortality rate of 7%. Mortality was slightly higher in the probiotic group although this was not statistically significant (7/64 (11%) *v* 2/65 (3%); p=0.16, Fisher's exact test mid p).

DISCUSSION

There is no evidence from this study that administration of *Lactobacillus plantarum* 299v to preoperative patients conferred any advantage with respect to the prevention of BT, colonisation of the upper gastrointestinal tract, or subsequent septic morbidity and mortality. In addition, no attenuation of the systemic inflammatory response was detected. The limited data from the pilot study suggest that *Lactobacillus plantarum* 299v does not confer the same protection against wound infection than is achieved by prophylactic antibiotic administration. In this study, gastric colonisation with enteric organisms predisposed to postoperative sepsis, confirming earlier work from our unit.[6] BT was not associated with increased septic morbidity in this series of patients.

Despite developments in antisepsis and antibiotic prophylaxis, septic complications are common in surgical patients. Realisation that the majority of postoperative infections are caused by gut derived organisms has focused attention on the functioning of the gut mucosal barrier and the role of the indigenous gut microflora. There is good evidence from animal and in vitro studies that alteration of the gastrointestinal microflora with probiotic organisms can reduce the rate of BT to MLNs.[4 12 13] Although the significance of BT in humans remains to be determined, its postulated links with sepsis and multiple organ failure make it an obvious target for therapeutic intervention.[14] This is the first study to assess the effect of probiotic administration on the incidence of BT and septic morbidity in elective surgical patients.

Lactobacillus plantarum 299v was chosen for this study as it fulfils the criteria necessary to achieve probiotic status.[3] The organism has been shown to survive in the gastrointestinal tract during oral administration and can be isolated from rectal mucosal biopsies of healthy volunteers up to eight days after cessation of the strain.[15 16] *Lactobacillus plantarum* 299v, in common with other lactobacilli, interacts with the host in a number ways to exert a beneficial effect. *Lactobacillus plantarum* 299v attaches strongly to human intestinal cells via a mannose specific adhesin and can inhibit adherence of pathogenic organisms, such as *E coli*.[13 17] There is also increasing evidence that probiotic organisms can interact with the gut associated lymphoid tissue (GALT) and influence the mucosal and systemic immune systems.[18 19] In addition, lactobacilli can secrete antimicrobial compounds called bacteriocins which inhibit a broad spectrum of enteric organisms.[20]

Bacterial translocation

The primary objective of this study was to assess the effect of probiotic administration on gut barrier function. Confirmation of BT by microbiological identification of organisms in normally sterile extraintestinal tissues remains the "gold standard" for assessing the integrity of the gut barrier.[21] Microbiologically confirmed BT occurred in 12% of patients entered into the study. This is similar to previous research from our unit which documented translocation in 25 (10.3%) of 267 general surgical patients.[6] Administration of *Lactobacillus plantarum* 299v for one week or more prior to surgery had no effect on the occurrence of translocation. This finding is somewhat surprising given the consistent antitranslocation effect of probiotics in animal and in vitro models. It is possible that these models do not reflect the pathological processes occurring at the gut mucosal barrier in humans. There is however increasing evidence from human clinical trials to suggest that probiotics can alter the progression of disease states which affect the intestinal mucosa. For example, probiotics have been used successfully to treat diarrhoea in children[22 23] and prevent the relapse of ulcerative colitis and chronic pouchitis in adults.[24–26] Although these studies have not attempted to elucidate the mechanism of probiotic action in humans, they suggest that probiotics have the ability to interact with their human host at its epithelial interface.

The systemic inflammatory response

In an attempt to evaluate the systemic inflammatory response that occurs following surgery, which may well be influenced by alterations in gut barrier function, we measured serial serum CRP levels. Theoretically, if the probiotic *Lactobacillus plantarum* 299v were to prevent BT and endotoxin exposure to the GALT, the resulting cytokine and systemic inflammatory response would be diminished. Serial serum CRP measurements were unaffected by preoperative administration of *Lactobacillus plantarum* 299v. In both groups, a significant rise in CRP was observed postoperatively, in keeping with the surgical stress response. CRP levels subsequently fell towards normal in the week after surgery. A recent study in our unit of *Lactobacillus plantarum* 299v administration in critically ill intensive care unit patients also did not demonstrate any change in serum CRP.[27] This study did demonstrate however that the proinflammatory cytokine interleukin 6 was significantly reduced in the probiotic group after two weeks. We concluded that *Lactobacillus plantarum* 299v can modify the acute inflammatory response. Measurement of serial cytokines may have been a more sensitive marker than serum CRP in this study.

Gastric colonisation and septic morbidity

In agreement with previous work from our unit, nasogastric colonisation with enteric organisms at the time of surgery predisposed to subsequent septic morbidity.[6] Patients who received the probiotic were more likely to have a sterile nasogastric aspirate and less likely to be colonised with enteric organisms than controls. Although these results were not significant, they suggest that preoperative probiotics may modulate the gastrointestinal microbial environment. On a theoretical basis, this may confer some advantage with respect to nosocomial aspiration pneumonia. Bacterial overgrowth is known to promote BT in animal models but this study failed to demonstrate a similar phenomenon in humans. There is no doubt that BT occurs in humans but its clinical significance remains elusive.

Lactobacillus plantarum 299v and gut barrier function

There are a number of possible reasons that might account for the failure of *Lactobacillus plantarum* 299v to confer any apparent benefit in this study. It might be argued that the probiotic was given for an insufficient period of time prior to surgery. We accept this and recognise that further studies using much longer preoperative periods of administration would be worthwhile. However, there is a major ethical restraint in

delaying elective surgery for patients with malignant disease. Another criticism of this study might be that we had no microbiological confirmation of patient compliance. Culture of *Lactobacillus* ingested in a probiotic preparation is difficult and necessitates either colonic biopsy or faecal sampling. The first poses ethical problems and the second major logistical problems for microbiology departments. On any account we consider poor compliance unlikely. Patients readily accepted the need to take the *Proviva*, returns of the product were low, written diaries were adequately completed, and only a minority of patients recorded poor tolerance. Finally, another possible explanation is that the prevention of translocation in humans is dependant on the species of *Lactobacillus* or *Bifidobacteria* administered. The ability of probiotic organisms to adhere, colonise, and modulate the human gastrointestinal tract varies from species to species. It is quite possible that another probiotic may have superior qualities in relation to preventing translocation.

One of the most interesting findings of this study is that *Lactobacillus plantarum* 299v did not confer any protection against wound infection. With the emergence of antibiotic resistance, the World Health Organization has promoted the use of probiotics as a form of microbial interference therapy.[28] Unfortunately, our results do not support the contention that probiotics, or at least *Lactobacillus plantarum* 299v, be used independently of routine antibiotic prophylaxis. This finding is at variance with the reports from Bengmark who have demonstrated no increase in postoperative infections in liver resection patients who received *Lactobacillus plantarum* 299v instead of antibiotics.[29]

In conclusion, the results of this prospective randomised trial suggest that preoperative administration of the probiotic *Lactobacillus plantarum* 299v for two weeks has no effect on the human gut mucosal barrier, as measured by BT, gastric colonisation, and the systemic inflammatory response.

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