Effects of probiotic therapy in critically ill patients: a randomized, double-blind, placebo-controlled trial

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ABSTRACT

Background: Multiple organ dysfunction syndrome (MODS) is a major cause of mortality in intensive care units. A breakdown in gut barrier function and immune dysfunction are associated with the onset of MODS. Probiotic bacteria have been shown to modulate intestinal barrier and immune function.

Objective: This study assessed the efficacy of a probiotic compound in a viable and nonviable formulation in modulating intestinal permeability and immune function and preventing the onset of MODS in patients in the intensive care unit.

Design: A double-blind, randomized controlled trial was conducted in the intensive care unit of a tertiary care teaching hospital. Twenty-eight critically ill patients admitted to the intensive care unit were randomly assigned to receive 1 of 3 treatments daily for 7 d: 1) placebo, 2) viable probiotics, or 3) equivalent probiotic sonicates. MODS scores and systemic concentrations of immunoglobulin (Ig) A and IgG were measured on days −1, 4, and 7, and intestinal permeability measurements were taken daily.

Results: The patients responded to viable probiotics with a significantly larger increase in systemic IgA and IgG concentrations than in the patients who received placebo or sonicates (P < 0.05). MODS scores were not significantly affected by probiotic treatment. Over the study period, intestinal permeability decreased in most patients.

Conclusion: Patients receiving viable probiotics show a greater enhancement in immune activity than do patients receiving either placebo or probiotic bacterial sonicates.

KEY WORDS Intestine, multiple organ dysfunction syndrome, Lactobacillus sp., Bifidobacterium, sepsis

INTRODUCTION

Multiple organ dysfunction syndrome (MODS) is a hyperinflammatory state that is a major cause of death in adult intensive care unit (ICU) patients (1–3). The gastrointestinal system appears to play a key role in the pathogenesis of MODS due to a breakdown of intestinal barrier function and increased translocation of bacteria and bacterial components into the systemic circulation (4). This leads to a vicious downward spiral, culminating in immune system dysfunction and multiple organ failure (4).

Intestinal microbes are a major source of systemic infection in postsurgical and trauma patients (5–7). In contrast, endogenous probiotic bacteria of the gut, such as Bifidobacterium and Lactobacillus, play a vital role in maintaining the intestinal mucosal barrier and enhancing immune responses. Feeding probiotics to experimental animals can improve gut barrier function and reduce populations of gram-negative bacteria (8). Because gram-negative organisms account for a significant proportion of infections that arise from the abdominal system, this effect of probiotic bacteria may contribute to their observed benefits (9).

Probiotics have shown efficacy in a wide range of applications, including prophylactic and maintenance treatment of pou- chitis (10–14), treatment of radiation-induced diarrhea (15), and adjuvant treatment of ulcerative colitis (16). In mouse models of colitis, live probiotics fed daily enhanced colonic permeability and reduced gut inflammation (8). In vitro studies have shown certain Bifidobacterium strains to release a proteinaceous factor that directly influences epithelial permeability and prevents invasion by potential pathogens (8). Although live probiotics clearly modulate gut immune and barrier function, other studies have shown immunomodulatory effects of probiotic DNA (17). Indeed, some studies have suggested that isolated probiotic bacteria DNA is equally as efficacious in attenuating intestinal inflammation as is treatment with live bacteria (18, 19). The use of isolated bacterial DNA instead of live bacteria in the treatment of sepsis would alleviate the possibility of Lactobacillus sepsis occurring in patients, which, although rare, has been documented (20). Thus, the purpose of this double blind, placebo-controlled, randomized clinical trial was to determine whether the administration of probiotics would maintain gut barrier function and prevent the development of MODS in critically ill, enterally fed patients in the critical care unit, and secondly, to determine whether the effects of bacterial sonicates containing bacterial DNA would be comparable to those effects seen with viable bacteria.

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SUBJECTS AND METHODS

Study participants

Patients admitted between January and December of 2004 to the ICU of the Royal Alexandra Hospital (Edmonton, Alberta, Canada) were eligible for the study. The following inclusion criteria were applied to patients: 1) >18 y of age, 2) could be fed enterally within 48 h of ICU admission, and 3) anticipated to require enteral nutrition for >48 h. Exclusion criteria included the following: 1) inability to be fed via the gastrointestinal tract; 2) presence of renal failure, pancreatitis, short gut syndrome or pre-existing sacral ulcers; 3) HIV positive; 4) previous bone marrow, lung, or liver transplant; 5) patient had received mannitol, lactulose or anticipated initiation of aforementioned drugs over the upcoming week; or 6) not expected to survive 7 d given their current uncorrectable medical condition. Twenty-eight patients were enrolled in the present study. Consent was provided by the patient or, if the patient was unable to, by immediate family members.

The Ethics Committee for Medical Research at the University of Alberta approved the study protocol. Before their admission into the study, written and oral informed consent were provided from all study participants or their relatives. The study was performed in accordance with International Conference on Harmonisation Good Clinical Practice guidelines based on the Declaration of Helsinki.

Treatment

Patients who met the inclusion criteria and provided consent were randomly assigned to one of 3 treatment groups: 1) placebo; 2) viable probiotics—2 sachets daily; and 3) bacterial sonicates (not viable). Each sachet of probiotics (VSL#3; VSL Pharmaceuticals, Ft Lauderdale, FL); contained 900 billion viable lyophilized bacteria consisting of 4 strains of Lactobacillus (L. casei, L. plantarum, L. acidophilus, and L. delbrueckii subsp. Bulgaricus), 3 strains of Bifidobacterium (B. longum, B. breve, and B. infantis) and Streptococcus salivarius subsp. Thermophilus. Bacterial sonicates were prepared by rehydrating probiotic sachets in sterile water and sonicating twice for 30 s. Homogenates were centrifuged at 100 000 × g for 10 min at 4°C and filtered through a 0.22 μm filter to ensure removal of all live bacteria. Bacterial DNA was present in the sonicate. A sample was taken for culture to ensure no live bacteria remained in the supernatant, and the supernatant immediately frozen at −70°C until use. All treatment groups also received a polymeric enteral formula containing 22 g fiber/1000 mL, which includes 10 g fructooligosaccharides/1000 mL and 12 g of a patented soluble and insoluble fiber blend (Jevity Plus; Abbott Laboratories, Columbus, OH). The treatment solutions were identical in appearance.

Study design

This was a single center double-blind, placebo controlled trial. Enteral nutrition was provided to study patients within 48 h from the time of ICU admission. Enteral feeds were initiated and progressed according to Capital Health Region ICU protocol. By protocol, nasoenteric feeds were started at 25 mL/h and increased by 25 mL/h every 4 h until the target rate was achieved. When gastric residual volumes exceeded 150 mL, prokinetic agents were initiated and feeds were resumed and advanced until the target rate was achieved. All study treatments were administered twice daily at 0900 and 2100. Within 60 min of reconstitution, the study treatment and placebo preparations were dispensed in identical packaging and administered to the patient via a feeding tube. The probiotics group received 2 sachets of probiotics twice daily providing a total of 9 × 10^11 bacteria. Bacterial sonicate treatments were prepared from an equivalent dosage; on preparation, they were immediately frozen, transported, and infused to the patients within 1 h of thawing. Patients received the study treatment for 7 consecutive days. If the patient was to discontinue enteral nutrition or was ready to be transferred out of the ICU before the study completion, the study was discontinued prematurely. All patients in the study received concomitant therapy, including antibiotics, as considered appropriate by the attending physician.

Apache II scores

Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were calculated utilizing data obtained during the 24 h before initiation of enteral nutrition (21).

Nutritional assessment

Energy requirements were calculated as 25–30 kcal/kg and protein requirements as 1.2–1.5 g/kg protein. Daily energy and protein intake were recorded. Body mass index was calculated by the formula weight (in kg)/height^2 (in m) (2), and subjective global assessment was assessed at the initiation of enteral nutrition.

Indirect calorimetry

On achieving the target rate of enteral feeding, an indirect calorimetric measurement was performed to confirm the adequacy of enteral nutrition. Patients were assessed by using a Sensormedics Deltrac II indirect calorimeter (Sensormedics, Yorba Linda, CA) for ≥20 min. The patients did not receive any analgesia, stimulation, or undergo any ventilatory changes for 30 min either before the test or during the measurement. Acceptable variations in VO2 (volume of oxygen utilized, in mL/min) and VCO2 (volume of carbon dioxide produced, in mL/min) were defined as <15%, and acceptable variation in respiratory quotient was defined as <10%. Measurements that exceeded these limits were not interpreted. Energy requirements were reassessed based on the indirect calorimetric results, and enteral feeding rates were adjusted to meet resting energy expenditures.

Outcome measures

Multiple Organ Dysfunction Score

A MODS score was calculated on day −1, 4, and 7 of the study. Briefly, the parameters used to calculate MODS for each individual system were as follows: 1) respiratory (partial pressure of oxygen/fraction of inspired oxygen), 2) renal (serum creatinine), 3) hepatic (bilirubin), 4) cardiovascular (pressure adjusted heart rate), 5) hematologic (platelets), and 6) neurologic (Glasgow Coma Scale) (22).

Biochemical analysis

C-Reactive protein (CRP), immunoglobulin (Ig) A, and IgG baseline measurements were made on day −1, before initiation of
the study treatment. Measurements were repeated at the completion of the study (day 7) after a 6 h urine collection for intestinal permeability. In those cases where a subject completed the study before Day 7, blood was drawn at the conclusion of the last intestinal permeability collection.

Diarrheal episodes

Diarrheal episodes were measured daily by the Hart & Dobb diarrheal scale. Diarrhea was defined as a score of ≥12 in a 24 h period (23). Diarrhea incidence was calculated by the number of days with Hart and Dobb Score of ≥12 divided by the number of days patients received treatment and enteral nutrition.

Intestinal permeability

Intestinal permeability was measured daily for 7 d by using a standardized protocol (24). The first measurement was performed on Day −1 before the patient received treatment. A syringe containing 7.5 mL lactulose was sent daily to the bedside with 2 g mannitol. The mannitol was reconstituted with 20 mL distilled water and administered enterally daily during the ICU stay. Twenty mL water was given to rinse the feeding tube after administration of the sugar solution. Feeding with enteral preparations was temporarily interrupted during administration of the sugar solution but was immediately resumed after the rinse solution. The excreted portion of each sugar marker was collected for 6 h in urine via a standard urinary catheter collecting system to which gentamicin was added. Collected urine was placed in a bottle containing 5 mL 10% thymol. The collected urine was drained from the catheter bag every quarter hour. The collection jug was kept on ice at the bedside for the 6 h collection time. The collection jug was refrigerated at 4 °C and then stirred, before taking two 15-mL aliquots of urine. All samples were frozen to −70 °C within 24 h. Measurement of the urinary concentration of sugars was made by using standardized HPLC methodology (24).

Statistical analysis

The data were analyzed by using the statistical software program SPSS 12.0, Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL). Independent t tests were performed on all baseline data between groups. Differences in variables at baseline and after treatment were assessed with a repeated-measures analysis of variance that included a time × treatment interaction. Differences between means were evaluated by using analysis of variance or paired t tests where appropriate. Data were further analyzed with a Bonferroni adjusted t test for multiple comparisons. The Mann-Whitney U test was used to compare nonparametric data. Intestinal permeability measures were reported as the lactulose mannitol ratio (LMR). LMR results were converted to their natural log (ln) values to normalize the distribution for analysis. Cohorts were compared for daily changes in permeability through the use of linear mixed-effects model, a technique that allows for comparisons between the means of cohorts, unit changes in permeability per unit change per day, and inclusion of effects of daily changes of physiologic dysfunction and accounts for individual variability between patients. Reported P values are two-tailed; P values <0.05 were considered significant for all statistical tests. A total sample size of 45 subjects was calculated based on an α of 0.05 and power of 90% by using independent t test calculations for differences in intestinal permeability. Interim analysis was required as numerous subjects stopped enteral nutrition before study completion.

RESULTS

Participant characteristics

Twenty-eight patients were enrolled into the trial (n = 9 for placebo, 10 for viable probiotics, and 9 for probiotic sonicates). No significant differences in age, sex, APACHE II scores, MODS, body mass index, or use of antibiotics were observed between the groups. (Table 1)

Nutritional variables

Patients in the treatment groups were not significantly different in terms of nutritional status (Table 2). Mean daily energy intake was compared with energy requirements derived from indirect calorimetric measurements as described in the methodology section. Mean protein intakes were compared with protein requirements calculated by formulaic methods. No significant differences existed between treatment groups for mean energy and protein intake. The 2 most common reasons for interrupting enteral nutrition included temporary cessation for medical procedures or increased gastric residuals >150 mL as per ICU enteral feeding protocol.

Development of Multiple Organ Dysfunction Syndrome

MODS analysis was completed on days −1, 4, and 7. No statistically significant differences in MODS scores were observed either within groups from days −1 to 7 or between the groups on days −1, 4, or 7. In the group receiving viable probiotics, 50% (5 of 10) of the patients transferred out of the ICU on day 4 of the study (Table 3). In the placebo group, 33% of patients (3 of 9) transferred out of the ICU on day 4 of the study, and in the group receiving probiotic sonicates, 22% (2 of 9) transferred out of the ICU on day 4. No significant difference in the proportion discharged from the ICU was observed between the groups.

Immune variables

The patients who received viable probiotics showed significantly greater increases in IgG and IgA than did the patients who received placebo or probiotic sonicate (P < 0.05) (Figure 1). The increase in IgG and IgA concentrations was not significantly different in the patients who received sonicates compared with the patients who received placebo (P = 0.64). Overall, there was a significant increase in IgG and IgA concentrations over the treatment period for all patients (P < 0.05). A significant decline in CRP concentrations occurred in all treatment groups (P < 0.05). No significant differences in the change in CRP values were observed between the placebo (73 ± 28), probiotic (9 ± 35), and probiotic sonicates (63 ± 25) groups.

Incidence of diarrhea

Diarrhea incidence was calculated by number of days with Hart and Dobb Score of ≥12 divided by the number of days patients received treatment and enteral nutrition. Patients who received placebo had a 23% incidence of diarrhea compared with
Nutritional variables of the study participants by treatment group

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<td>Energy intake (kcal/d)</td>
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<tr>
<td>Energy requirements met (%) (^3)</td>
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<td>Protein intake (g/d)</td>
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<td>Protein requirements met (%) (^3)</td>
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\(^1\) Independent \(t\) tests were performed on all baseline data between the groups, and variables were assessed with ANOVA. No significant differences were observed between the groups.

\(^2\) SGA, subjective global assessment. A, well-nourished; B, moderately malnourished; and C, severely malnourished.

\(^3\) \(\bar{x} \pm SD\) (all such values). Determined by energy intake from enteral nutrition/energy requirements assessed through indirect calorimetry.

\(^4\) \(\bar{x} \pm SD\) (all such values). Determined by grams protein consumed via enteral nutrition/grams protein required from formulaic assessment of 1.2–1.5 g protein \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1}\) (mean).
patient in each of the 3 treatment groups died during their ICU admission. The causes of death for the patients who died in ICU included respiratory failure (one patient on sonicates), congestive heart failure (one patient on viable probiotics), and myocardial infarction (one patient on placebo). The deaths in the treatment groups occurred after the probiotic therapy had been discontinued. The patient randomly assigned to viable probiotics died on day 9 of ICU admission, and the patient randomly assigned to sonicates died on day 128 of ICU admission.

**DISCUSSION**

The present pilot study used a double-blind, placebo-controlled, randomized design to determine the effects of viable probiotics and probiotic sonicates on the development of MODS in critically ill, enterally fed patients. Overall, the patients who received viable probiotics showed a greater enhancement in immune activity and reduction in intestinal permeability than did the patients who received either placebo or sonicates.

MODS scores calculated over the first 24 h of ICU admission correlate strongly with ICU mortality rates (25). In validation studies, ICU mortality rates have been shown to be 100% for MODS scores > 20, 50% for those patients with scores between 13 and 16, and <5% for those patients with scores between 1 and 4. Patients entered into the current study had pretreatment MODS scores ranging from 2 to 14. Logistic regression analysis has shown that an increase in MODS scores of one point increases the odds of death by 1.59. Patients who received viable probiotics had an average reduction of 1.2 in their MODS scores after 3 days of treatment.

Probiotics modulate the innate and adaptive immune system in a dose- and strain-dependent manner (26, 27). In particular, some Lactobacilli and Bifidobacteria strains have been shown to induce the production of secretory IgA and IgG (26, 28, 29). High concentrations of IgA activity in the gut are crucial to maintaining a barrier against pathogenic bacterial translocation, especially of gram-negative organisms (30). In our study, administration of viable probiotics significantly increased IgA and IgG production. A similar finding in a mouse model was reported by Galdeano and Perdigon (27), who showed that viable L. casei and L. acidophilus increased the number of IgA cells in the intestine of mice to a much greater extent than did nonviable bacterial cells. However, in contrast to our results, 2 studies showed no increases in IgA in surgical patients who consumed ProViva, an oatmeal-based drink containing Lactobacillus plantarum 299v or a probiotic compound containing Lactobacillus acidophilus

**TABLE 3**

<table>
<thead>
<tr>
<th>Day</th>
<th>Placebo group</th>
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<th>Viable probiotics group</th>
<th></th>
<th></th>
<th>Probiotic sonicates group</th>
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<tbody>
<tr>
<td>−1</td>
<td>MODS$^2$</td>
<td>n</td>
<td>MODS$^2$</td>
<td>n</td>
<td>MODS$^2$</td>
<td>n</td>
<td>MODS$^2$</td>
<td>n</td>
</tr>
<tr>
<td>4</td>
<td>3.8 ± 1.6$^7$</td>
<td>9</td>
<td>4.6 ± 3.8</td>
<td>10</td>
<td>4.0 ± 1.8</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4.2 ± 1.5</td>
<td>9</td>
<td>3.4 ± 2.7</td>
<td>10</td>
<td>4.6 ± 1.3</td>
<td>9</td>
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$^7$ Data were analyzed by using a linear mixed-effects model for periods between days $−1$ and 4 and between days 4 and 7. No significant differences or time × treatment interactions were observed.

$^2$ Variables used to calculate MODS included partial pressure of oxygen/fraction of inspired oxygen, serum creatinine and bilirubin, pressure-adjusted heart rate, platelet count, and the Glasgow Coma Scale.

$^3$ $x$ ± SD (all such values).
Intestinal permeability was assessed daily by measuring the excretion of mannitol and lactulose in urine by HPLC. The first measurement was performed on day −1 before the patients received treatment. Results are reported as a ratio between excreted lactulose and mannitol (lactulose-to-mannitol ratio, LMR) in individual patients who received placebo (A; n = 7), viable probiotics (B; n = 7), and probiotic sonicates (C; n = 8). Independent t tests were performed on all baseline data between the groups. Differences in permeability at baseline and after treatment were assessed with a repeated-measures ANOVA that included a time × treatment interaction. Tukey’s post hoc tests were used to assess differences between the treatment groups. LMRs were converted to their natural log values to normalize the distribution for analysis. Cohorts were compared for daily changes in permeability by a linear mixed-effects model that compared means of cohorts, unit changes in permeability per unit change per day, inclusion of effects of daily changes of physiologic dysfunction, and accounted for individual variability between patients. A significant decrease in small intestinal permeability over time was observed in all treatment groups (P < 0.003).
La5, Lactobacillus bulgaricus, Bifidobacterium lactis Bb-12, and Streptococcus thermophilus (31). Whether these negative results are due to the selected strain or dose of bacteria, the duration of treatment, the type of patients studied (undergoing major surgery), or the possibility of variability in consumption of the study compound before surgery by patients (and corresponding lack of control over possible consumption of over-the-counter probiotic-containing compounds by patients taking placebo) remains to be determined. However, several factors are known to be important in probiotic therapy, with strain selection, time point when therapy is initiated, and dose being critical (32).

CRP, commonly used as a marker of systemic inflammation (33), is an acute-phase protein produced by the liver and by endothelial cells (34). CRP inhibits the production of proinflammatory cytokines and chemokines, including tumor necrosis factor α and interferon γ (35) and also demonstrates significant antimicrobial activity (36). Although most of the patients in the present study showed a reduction in CRP concentrations over the treatment period, those patients who received viable probiotics had a lesser decline in CRP concentrations than did those patients who received either placebo or bacterial sonicates. Interestingly, a recent study by Viljanen et al (37) showed that Lactobacillus GG treatment of infants with atopic eczema-dermatitis and cow milk allergy resulted in higher concentrations of CRP than did placebo treatment. CRP has been shown to have a protective effect in preventing the onset of disease in lupus-prone transgenic mice (38), which suggests that the role of CRP in sepsis is likely more complex than simply being a nonspecific marker of inflammation. Further, a probiotic-induced maintenance of CRP concentrations may actually be beneficial in treating systemic infection through its antimicrobial actions (36). Additional studies are necessary to clearly define the role of CRP in sepsis and also the effect of probiotic bacteria in modulating CRP production.

A decrease in intestinal permeability was observed over time in most patients in all 3 groups. Probiotics have been shown to have positive effects in reducing small intestinal permeability (39, 40) but also to be ineffective (41). It is interesting that colonic permeability has not routinely been assessed in critically ill patients, because it is possible that the primary effects of probiotic therapy may be seen in the maintenance of colonic, rather than small intestinal, permeability. However, a recent systematic review suggests that the addition of probiotics to enteral nutrition can enhance the beneficial effects of enteral nutrition on patient outcomes, including the modulation of inflammation and systemic immunity, as was seen in the present study (39). It is clear that the therapeutic potential of probiotics to prevent increases in intestinal permeability and its complications requires further investigation.

Administration of both viable probiotics and probiotic sonicates decreased the incidence of diarrhea. However, absolute power was low at 0.176. The effect size of 0.065 may also account for the statistical insignificance of the finding. Previously, 64 patients per treatment group were randomly assigned to receive placebo or Saccharomyces boulardii with their tube feedings (40). The investigators concluded that S. boulardii decreased the incidence of diarrhea by 4.7% in critically ill tube-fed patients (40).

The percentage of energy requirements achieved with enteral nutrition was not significantly different between the groups. Difficulties establishing enteral nutrition are greater in patients with higher severities of illness because of gut dysmotility and hypoperfusion to the gastrointestinal tract. In the current study, the most frequent reasons for stopping or interrupting enteral feeds were for medical procedures and for high gastric residuals. Two of the 28 patients received <50% of the prescribed energy and protein provisions. In light of the findings on intestinal permeability and immune variables, the most advantageous route seems to be enteral nutrition administered with probiotic therapy. However, the absolute power and effect size were low in all outcome measures, indicating that the study needs to be replicated in a larger sample size to be conclusive. The second limitation of the study was the heterogeneity of the ICU patients enrolled, because the study subjects included medical, surgical, and trauma patients. Future studies may consider attempting to enroll patients with a higher severity of illness but with a similar disease profile. In addition, larger studies are required to confirm the efficacy and safety of probiotics in preventing the onset of MODS in ICU patients.

KM, CA, LG, and RF designed the study. CA, JM, CF, LM, and KM analyzed the data and prepared the manuscript, which was reviewed by all authors. JM performed the HPLC for intestinal permeability data. CA enrolled all patients and collected samples. DK performed the statistical analysis. None of the authors had a financial or personal conflict of interest.

REFERENCES