Benefits of early enteral nutrition with glutamine and probiotics in brain injury patients

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ABSTRACT

Brain injury patients have higher energy and protein expenditures and are prone to infections. The aim of the present study was to evaluate the results of early enteral feeding with glutamine and probiotics in brain injury patients. Twenty-three brain injury patients (Glasgow score between 5–12 and therapeutic intervention scoring system > 20) were studied. Three patients were excluded to leave 20 remaining patients. Patients were randomized to receive either an early enteral diet (control group, \( n = 10 \)) or the same formula with glutamine and probiotics added (study group, \( n = 10 \)) for a minimum of 5 days (range, 5–14 days). The diets were isocaloric and isonitrogenous [35 kcal \( \cdot \) kg\(^{-1} \) \cdot day\(^{-1} \) (where 1 kcal \( \approx \) 4.184 kJ) and 1.5 g of protein \( \cdot \) kg\(^{-1} \) \cdot day\(^{-1} \)]. Main outcome measures were the incidence of infection, the length of stay in the intensive care unit and the number of days requiring mechanical ventilation. The two groups were homogeneous in gender, age, nutritional status and severity of trauma. There was no mortality during the study period. The infection rate was higher in controls (100 %) when compared with the study group (50 %; \( P = 0.03 \)) and the median (range) number of infections per patient was significantly greater (\( P < 0.01 \)) in the control group [3 (1–5)] compared with the study group [1 (0–3)]. Both the critical care unit stay [22 (7–57) compared with 10 (5–20) days; \( P < 0.01 \); median (range)] and days of mechanical ventilation [14 (3–53) compared with 7 (1–15) days; \( P = 0.04 \); median (range)] were higher in the patients in the control group than in the study group. We conclude that the enteral formula containing glutamine and probiotics decreased the infection rate and shortened the stay in the intensive care unit of brain injury patients.

INTRODUCTION

Patients with head trauma present important metabolic alterations that trigger increased energy and protein expenditure compared with patients without brain injury. Protein catabolism reaches values ranging from 14–25 g of nitrogen/day in individuals with brain injury, which is much higher than a normal fasting individual (3–5 g of nitrogen/day) [1]. The neurological clinical condition associated with the use of sedatives, steroids, barbiturates and muscle-relaxing drugs postpones the use of nutrients in these patients and, thus, complications, including infection and longer hospitalization, may occur [2]. Weight loss, negative nitrogen balance and immune dysfunction constitute a characteristic response in both surgical and trauma patients. This condition facilitates the onset of acute malnutrition and infectious complications and, consequently, there is an increased incidence of morbidity and mortality [3]. Thus the prevention or minimization of acute malnutrition is one of the objectives of enteral nutritional therapy and may guarantee the survival of these patients.

In addition to digesting and absorbing nutrients, the gastrointestinal tract is thought to be important in the immune response. Translocation of indigenous bacteria or their products from the gut into the circulation, due

Key words: glutamine, probiotics, enteral nutrition, trauma, brain injury.
Abbreviations: BMI, body mass index; ICU, intensive care unit; TISS, therapeutic intervention scoring system.
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to increased permeability of the intestinal mucosa, is a well-known feature of the metabolic response in trauma patients. The persistence of these events may promote a continuous systemic inflammatory response syndrome that, in some cases, may result in multiple organ and system dysfunction and eventually death [4].

Enteral nutrition lessens the catabolic response to trauma and decreases the incidence of abdominal infection, possibly due to enhancing mucosa barrier function and reducing bacterial translocation [5]. An ideal enteral nutrition has not yet been developed, but it is believed that formulas containing trophic effects for the intestinal mucosa, immune-enhancing nutrients or ingredients to decrease the inflammatory acute phase reaction would be valuable [6]. Early enteral nutrition has also been reported [6–10] as an infection-reducing factor even when administered below the daily patient requirements. Enteral nutrition with specific nutrients, such as glutamine, arginine, omega-3 fatty acids, glycine, probiotics, symbiotics and nucleotides, has been reported recently with promising results [6–10].

Hypercatabolism is a common finding in trauma patients. In this context, increased muscle proteolysis and concomitant translocation of peripheral amino acids to visceral organs is a common feature. In these circumstances, glutamine is the amino acid released most by the muscle [11]. Although abundant in the plasma, glutamine consumption is accelerated and is greater than its production under stress, resulting in a negative balance. The plasma glutamine concentration decreases by up to 50 % during the acute postoperative period [12].

On the other hand, the intestinal microflora is responsible for a variety of functions, many of which are only now beginning to be understood. The large intestine is the most densely colonized portion, with levels ranging from $10^{10}$ to $10^{12}$ colony forming units/ml of faeces. About 400 different species of bacteria may live in the colon [13]. In this context, probiotics are currently considered to be organisms that provide some benefit to the host, promoting the intestinal microbial balance. Nevertheless, when this ecosystem is altered, either by antibiotic use or associated diseases, these bacteria can displace and colonize other organs and, thus, serious infection may occur [14]. The effect of probiotics in reducing infections has been investigated in recent randomized studies [15,16]. In acute pancreatitis, nutritional support with supplementary *Lactobacillus plantarum* was effective in reducing sepsis and the number of surgical interventions [15]. In another randomized trial, patients undergoing major abdominal operations had lower incidences of bacterial infections with early enteral nutrition enriched with both fibre and probiotics in comparison with conventional nutrition [16]. However, to date, in the trauma setting no reports have investigated the effect of enteral nutrition enriched with probiotics on the incidence of infectious morbidity.

Glutamine alone or associated with other immune-enhancing nutrients may diminish infection rates and hospital stay in stressed patients [5,7,10]. Enteral nutrition with immune-enhancing nutrients may decrease the occurrence of sepsis and effectively reduce the inflammatory response, the necessity of mechanical ventilation and hospitalization [17–19].

No previous studies have investigated the effect of probiotics in brain-injured patients under controlled conditions. Hypothetically, the association of glutamine with probiotics may promote a combined and favourable action in the brain trauma patient. Although glutamine would improve the nutrition of both the gut mucosa and immune cells, the probiotic bacteria would favourably alter the intraluminal environment, competing for nutrients and adhesion sites with pathogenic bacteria. These co-operative actions may reduce the rate of bacterial translocation and, thus, decrease both the incidence of infection and the length of hospitalization. Thus the aim of the present study was to investigate the effects of early administration of an enteral diet enriched with glutamine and probiotics in patients with brain injury in an intensive care unit (ICU).

**MATERIAL AND METHODS**

The present study was approved by the Committee of Ethics in Research of the Júlio Muller University Hospital. The patients were included after their family had been informed about the study and had given consent.

Twenty-three consecutive patients admitted to the ICU were randomized to receive a standard diet (control group, $n = 12$) or a glutamine- and probiotics-enriched diet (study group, $n = 11$). The main end points in the study were the frequency of infectious complications, the length of time in the ICU and the period of mechanical ventilation required. The ICU team, who manage both medical and surgical therapies for all the patients, had no information about either the above end points or the study design.

Eligibility for the study included victims of brain trauma alone (patients of either sex and nutritionally eu trophic or overweight, with age ranging from 16–50 years), requiring ICU treatment and enteral feeding, with a score of between five and 12 on the Glasgow scale and a therapeutic intervention scoring system (TISS) score $>20$. The exclusion criteria were: (i) multiple organ and system dysfunction; (ii) immune-suppressive conditions (AIDS, chronic corticoid use and immune-suppressive drugs); (iii) severe brain injury (Glasgow scale score $<5$); (iv) other associated trauma such as pulmonary contusions, extremity fractures and chest or intra-abdominal injuries; (v) chemotherapy or radiotherapy in the last 6 months; (vi) cancer; (vii) insulin-dependent diabetes mellitus; (viii) morbid obesity [body
mass index (BMI) > 40] or malnutrition (BMI < 17); (ix) chronic obstructive pulmonary disease (partial pressure of CO₂ > 45 mmHg on admission); (x) renal failure requiring peritoneal or haemodialysis or creatinine > 2.5 mg/dl; (xi) hepatic dysfunction, cirrhosis or bilirubin > 3 mg/dl on admission; (xii) previous organ transplantation; (xiii) pregnancy; and (xiv) indication of total parenteral nutrition. Patients were excluded if transferred to another institution before the fifth day after the onset of the diet, if the diet was interrupted for 2 or more consecutive days or for more than 5 days during the whole hospitalization period.

Definitions
Respiratory infection was defined as a compatible X-ray seen by the senior radiologist (all patients had normal X-rays during admission), fever and yellow secretion collected from the tracheal tube with positive culture. Urinary infection was defined as a positive urine culture (more than 100,000 colony forming units/ml). Wound infection was defined as abscess and purulent secretion drainage with positive culture. Sepsis was defined as systemic inflammatory response syndrome with bacteriological evidence of infection [4].

Diet and anthropometry
Both groups received a diet containing 35 kcal·kg⁻¹ of mean ideal weight·day⁻¹ (where 1 kcal = 4.184 KJ). A polyurethane tube in the nasoenteral position was used to administer the enteral nutrition, with the patient raised at a 30° angle. The mean ideal weight was calculated by the adapted BMI [20]. Height was measured by the same examiner with the bed in a horizontal position and the patient in dorsal decubitus, with the feet together and parallel. A metric measuring tape was stretched along the body, and the length was considered as the height of the patient.

The patients received a pure polymeric diet (control group) or the same diet supplemented with 30 g of glutamine and 240 ml of fermented milk with the probiotic strain *Lactobacillus johnsonii* (La 1) (LC1®; Nestlé, São Paulo, Brazil) as shown in Table 1. The two diets were isocaloric and isonitrogenous.

Formula administration
Diet administration was started when the patient was haemodynamically stable. The diet was administered via an enteral route with a polyurethane catheter for 6 days or at most for 14 days, beginning no later than 48 h after admission and continuing for a minimum of 5 days. All the diets were prepared in a single 24-h shift and kept in a refrigerator at 4°C, according to the composition specified in Table 1 by the Nutrition and Diet Service at the Julio Muller University Hospital.

The diet was administered by an infusion pump at a speed of 30 ml/h, reaching 60 ml/h at 96 h after the start of enteral nutrition to meet the nutritional needs of the patients. The calorie and protein needs were estimated from the mean ideal weight multiplied by 35 and 1.5 to give the calories and proteins required respectively, of the individual. Glutamine was administered as a bolus, as the first component of the diet of each day, followed by the fermented milk (probiotic) that was dispensed by the infusion pump.

Patient monitoring
Blood samples were collected over three periods (days 0, 4 and 7) and, if the patient was still hospitalized, on days 14 and 21. Samples were collected in the morning and routine examinations were performed, including a haemogram and determination of total proteins, globulins, albumin and urinary nitrogen. Nitrogen balance was determined in all the patients on days 4, 7 and 14 of study if they remained hospitalized. The following formula was used for the calculation of nitrogen balance: nitrogen balance = nitrogen in the diet – (urinary nitrogen + 2 g for each 50 kg of ideal weight) [21]. Urinary nitrogen was calculated from the amount of urea plus ammonia present in 24-h urine samples. Nitrogen balance was a monitored variable and was not a treatment parameter.

The site and the number of infections and sepsis occurrence were also recorded in each patient, according to the previous definitions. The ICU physician reported the diagnosis of any infectious complications during the daily morning rounds. The length of stay in the ICU was defined as the number of days from the start of the diet to the discharge of the patient from the hospital. The number of days that the patient required mechanical ventilation was also registered.

Statistical analysis
Statistical analyses were performed using the SPSS 8.0 program for Windows. Student’s t test or Mann–Whitney test was used to compare the continuous variables

<table>
<thead>
<tr>
<th>Table 1 Composition of the two diets</th>
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<tbody>
<tr>
<td><strong>Composition</strong></td>
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<tr>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Milk without lactose* (g)</td>
</tr>
<tr>
<td>Calcium caseinate (g)</td>
</tr>
<tr>
<td>Dextrin-maltose† (g)</td>
</tr>
<tr>
<td>Glutamine‡ (g)</td>
</tr>
<tr>
<td>Fermented milk§ (probiotics) (ml)</td>
</tr>
<tr>
<td>Calories (kcal)</td>
</tr>
<tr>
<td>Protein (g)</td>
</tr>
<tr>
<td>Lipid (g)</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
</tr>
</tbody>
</table>
between the groups whether they were homogeneous (assessed by the Levene's test) or not. The Fisher's exact test was used to compare the category variables. The data are expressed as means ± S.D. or medians (range) according to the homogeneity of the samples, and \( P < 0.05 \) was established as the significance level (type I error). Two-tailed power analysis was done on all end-point variables (infection rate, number of infections/patient, length of stay in the ICU and days of mechanical ventilation) aiming to give a minimum power of 80 % (type II error).

### RESULTS

There were no deaths recorded during the present study. Three patients were excluded from the study, one because he was released before the fifth day of the enteral diet, another because he was unable to receive the diet for more than 48 h, and the third because it was indicated that total parenteral nutrition was required. The remaining patients \((n = 20)\) were assigned to the two groups (control group, \( n = 10 \); study group, \( n = 10 \)), which were homogeneous for age, sex, nutritional state, Glasgow scale score, TISS score and calorie and protein ingestion \((P > 0.05)\), as shown in Table 2. Only one patient in the study group had a penetrating brain injury, due to a gunshot, whereas all others had closed brain trauma. None of the patients had a stroke associated with the trauma injury.

There was only one female patient; most patients were young men and all were in a good nutritional state. There were no complications during the infusion of the diet and there was no interruption for more than 6 h in any of the cases. Nitrogen balance did not differ statistically between the two groups either on the fourth or the seventh day. The protein and calorie intake was similar between the two groups. The haemogram, total proteins, globulins and albumin were similar between the groups during the experimental period (results not shown).

All the patients (100 %) in the control group during the experimental period presented at least one infectious event, in contrast with only five cases (50 %) in the study group \((P = 0.03\); Table 3). The most frequent site of infection was the lungs either alone or in combination with other infections (13 cases; 86.7 %). The median number of infections/patient in the control group was significantly greater \((P < 0.01)\) when compared with the study group (Table 4). Three patients (33.3 %) in the control group had sepsis, whereas no cases were detected in the study group \((P > 0.05)\). The length of stay in the ICU was significantly longer \((P < 0.01)\) in the control group than in the group of patients treated with the diet under study (Table 4). The number of days of mechanical ventilation for the patients in the control group was approx. 2-fold greater than the

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**Table 2** Demographics and clinical characteristics of the two groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group ( (n = 10) )</th>
<th>Study group ( (n = 10) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>26 (19–49)</td>
<td>27 (19–46)</td>
<td>0.86</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>9/1</td>
<td>10/0</td>
<td>1.00</td>
</tr>
<tr>
<td>Glasgow score*</td>
<td>7 (5–9)</td>
<td>7 (6–10)</td>
<td>0.98</td>
</tr>
<tr>
<td>TISS score†</td>
<td>32 ± 5</td>
<td>34 ± 8</td>
<td>0.56</td>
</tr>
<tr>
<td>BMI (kg · m(^{-2}))*</td>
<td>22 (21.5–24.5)</td>
<td>22 (21.5–24.5)</td>
<td>0.62</td>
</tr>
<tr>
<td>Protein received/day (g)†</td>
<td>103 ± 10</td>
<td>103 ± 9</td>
<td>0.99</td>
</tr>
<tr>
<td>Calories received/day (kcal)†</td>
<td>2400 ± 232</td>
<td>2390 ± 206</td>
<td>0.96</td>
</tr>
<tr>
<td>Days of enteral nutrition†</td>
<td>13 ± 5</td>
<td>9 ± 4</td>
<td>0.55</td>
</tr>
<tr>
<td>Total of infused volume (ml)†</td>
<td>18 494 ± 6 826</td>
<td>12 259 ± 6 907</td>
<td>0.72</td>
</tr>
<tr>
<td>Nitrogen balance (g/day)*</td>
<td>−1.47 (−28.9 to +6.5)</td>
<td>−1.64 (−28 to +7.4)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

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**Table 3** Distribution of infection cases in the two groups

<table>
<thead>
<tr>
<th>Infection</th>
<th>Control group ( (n = 10) )</th>
<th>Study group ( (n = 10) )</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>10 (100 %)*</td>
<td>5 (50 %)</td>
<td>15 (75 %)</td>
</tr>
<tr>
<td>No</td>
<td>0 (0.0 %)</td>
<td>5 (50 %)</td>
<td>5 (25 %)</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

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**Table 4** Clinical characteristics in the two groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group ( (n = 10) )</th>
<th>Study group ( (n = 10) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infections/patient</td>
<td>3 (1–5)</td>
<td>1 (0–3)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ICU stay (days)</td>
<td>22 (7–57)</td>
<td>10 (5–20)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Mechanical ventilation (days)</td>
<td>14 (3–53)</td>
<td>7 (1–15)</td>
<td>0.04</td>
</tr>
</tbody>
</table>
study group ($P = 0.04$; Table 4). Power analysis was 85% for infection rate, 95% for number of infections/patient, 81% for length of stay in the ICU and 51% for the time mechanical ventilation was required.

**DISCUSSION**

Although the incidence of infection in the brain injury patients detected in the present study was high, this is in line with other studies [2,22]. In the literature, the incidence of infection in these patients varies from 6 to 65%, with the lung being the most common site of infection. In patients with any deficit in the level of awareness and nutrition provided by naso- or oro-gastric tubes, aspiration of the gastric contents at some point during treatment is one of the main causes of this high incidence of pneumonia [2]. Associated thoracic trauma and lung aspiration, due to vomit prior to the nutritional treatment, are also causes of lung infection [23]. In this context, the positioning of the feeding tube at the jejunum and lung aspiration, due to vomit prior to the nutritional treatment, are also causes of lung infection [23]. In this context, the positioning of the feeding tube at the jejunum or duodenum is associated with less retrograde reflex and, consequently, less possibility of lung infection than when situated in a gastric position [24,25].

No difference in nitrogen balance was detected in the two groups, and this finding probably reflects the similar quantity of calories and nitrogen in the two diets. Thus the addition of glutamine and probiotics to enrich the enteral nutrition did not influence nitrogen gain. Some criticisms may arise due to the use of urinary urea nitrogen plus ammonia for the calculation of nitrogen balance instead of total urinary nitrogen. In fact, in post-injured patients, protein metabolism is altered and total urinary nitrogen is more reliable than urinary urea nitrogen; however, urinary urea nitrogen plus ammonia does provide a greater level of reliability as an estimate of total urinary nitrogen [26]. In addition, nitrogen balance in the present study was used only for monitoring purposes and not as an outcome measure.

Reduced mortality in trauma patients with the use of immune-enhancing nutrients has been reported previously [18]. However, we have not found any prospective studies in the literature focusing on the effect of the use of probiotics associated with glutamine in patients with trauma. Trauma patients characteristically present with marked depression of the immune system and constitute a risk group for nosocomial infections. This immune suppression seems to have a multifactorial origin and its mechanism has not been well characterized yet [18,19,21]. The use of some specific nutrients, such as glutamine, arginine, omega-3 fatty acids and nucleotides, have been related with an improvement in the immune function in critical patients, especially in a group of patients with trauma [21].

The possible beneficial effects of a diet with immune-enhancing nutrients are various and are described in the literature [5–9,19,21]. In particular, glutamine is used as a source of energy for cells of the intestinal epithelium and immune system. Furthermore, glutamine supplies nitrogen for purine and pyrimidine synthesis, which are essential for cells in mitosis. Consequently, the use of glutamine seems to be able to decrease the occurrence of bacterial translocation and inflammatory response, reducing the possibility of events such as systemic inflammatory response syndrome and sepsis. Moreover, diets enriched with immune-enhancing nutrients appear to improve healing and reduce the number of days of mechanical ventilation and hospitalization [7,17].

The central hypothesis of the present study was that the association of glutamine with probiotics might promote a synergistic and favourable action in trauma patients. Glutamine would improve both the enterocyte and immune cell nutrition, whereas the presence of probiotic bacteria would beneficially alter the intraluminal environment. Probiotics may improve the intestinal barrier by decreasing its permeability and hindering pathogen adhesion [27] and, thus, may prevent bacterial passage and decrease the occurrence of infection [28]. Theoretically, the combination of these nutrients might synergistically contribute to diminish bacterial translocation rates.

In the present study, enhancing enteral nutrition with glutamine and probiotics significantly reduced the incidence of infection in head trauma patients. There was not only a reduction in the number of patients with infections, but also a smaller number of infections per patient in the group that received the combination of immune-enhancing nutrients. Both groups received early enteral nutrition as recommended for critical surgical patients [17]. These findings suggest a potential benefit of early enteral nutrition enhanced with glutamine and probiotics to prevent the possibility of infections in patients with brain injury in the ICU. However, the small number of patients in the present study is certainly a limitation of this finding and, thus, further investigations are necessary.

Brain injury patients frequently have longer stays in the ICU, and causes of this beyond the severity of the brain injury include the presence of infectious complications, sepsis and acute malnutrition [19,24]. Weight loss, negative nitrogen balance and impaired immune response in these injured patients increase their susceptibility to infectious diseases and longer hospitalization [29]. Another potential benefit of early enteral nutrition enriched with probiotics and glutamine for patients with brain injury in the present study was the reduction in the period of time in the ICU and the number of days requiring mechanical ventilation. However, the significant decrease in the number of days patients in the study group required mechanical ventilation was restricted by the non-optimal statistical power found, i.e. below 80%.
Overall, the results obtained in the present study suggest that patients with brain injury may benefit from the use of early enteral nutrition enriched with glutamine and probiotics. Thus it can be concluded that early enteral nutrition containing glutamine and probiotics in brain trauma patients decreases infectious morbidity and shortens the period of time in the ICU. However, the reduction in the length of mechanical ventilation in these patients needs to be confirmed in further investigations. These conclusions are also limited by the small number of patients included in the present study.

REFERENCES