Role of glutamine administration on cellular immunity after total parenteral nutrition enriched with glutamine in patients with systemic inflammatory response syndrome

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Keywords: SIRS; Immunity; Glutamine

Abstract Glutamine is an important substrate for enterocyte and other rapidly proliferating cells. Low plasma and tissue levels present in glutamine in critically ill patients suggest that demand may exceed endogenous supply. Because commercially available amino acid solutions do not contain glutamine because of its instability in aqueous solution, conventional total parenteral nutrition (TPN) does not prevent stress-induced glutamine depletion. In this study, we administered intravenous glutamine-supplemented TPN to patients with systemic inflammatory response syndrome (SIRS) to investigate the effect of glutamine supplementation on immune states. This study is a prospective, randomized clinical trial. All patients received TPN given continuously for 6 days. Thirty patients with SIRS were allocated to either a glutamine group (L-glutamine 0.4g/[kg d]) (n = 15) or a control group (n = 15). Blood samples were collected on day 1 and day 6 after admission for C-reactive protein, immunoglobulin (Ig) M, IgG, IgA, C\textsubscript{3}, C\textsubscript{4}, and lymphocyte analysis. The Acute Physiologic and Chronic Health Evaluation II score and the Simplified Acute Physiologic II (SAPS II) score were used to evaluate the patients after admission. Although there was a tendency for decreased T cytotoxic cells and natural killer cells in the control group, no significant difference was observed between the 2 groups. However, an increase in lymphocyte and lymphocyte subgroups in the glutamine group was observed; but there was no difference between the groups. A low SAPS II score was observed on the sixth day in the glutamine group, whereas no difference in SAPS II and Acute Physiologic and Chronic Health Evaluation II scores was observed between the 2 groups. There was no difference in IgM, IgG, IgA, C\textsubscript{3}, and C\textsubscript{4} levels and numbers of B-lymphocytes between the groups. Glutamine-added TPN significantly decreases leukocyte and natural killer cell count and therefore suppresses inflammation. Furthermore, total lymphocyte count, B- and T-lymphocytes, and their subgroups (helper T-lymphocytes, cytotoxic T-lymphocytes) are increased; although not statistically significant, these increases might be playing a role in improving the immune system.

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1. Introduction

Systemic inflammatory response syndrome (SIRS) is one of several conditions related to systemic inflammation, organ
dysfunction, and organ failure. It is a subset of cytokine storm, in which there is abnormal regulation of various cytokines. Systemic inflammatory response syndrome is also closely related to sepsis, in which patients satisfy criteria for SIRS and have a suspected or proven infection [1-3]. Fever and leukocytosis are features of the acute-phase reaction, whereas tachycardia is often the initial sign of hemodynamic compromise. Tachypnea may be related to the increased metabolic stress due to infection and inflammation, but may also be an ominous sign of inadequate perfusion resulting in the onset of anaerobic cellular metabolism [2-4].

Protein synthesis and degradation increase in critical patients. Human cells use mostly branched-chain amino acids. In critical patients, glutamine levels normally decrease [5,6]. Glutamine is abundantly found in plasma and tissue. Glutamine is synthesized in the skeletal system and secreted into the bloodstream so that it may reach tissues. Kidneys, liver, small intestine, and immune system cells are the most important consumers of glutamine [7]. Glutamine provides optimum immune defense; it is the essential component in the optimum function of neutrophils and macrophages and also in lymphocyte proliferation [8].

In our study, we aim to investigate the effects of glutamine dipeptide−added total parenteral nutrition (TPN) therapy on intensive care unit (ICU) patients who meet at least 2 of the SIRS criteria.

2. Materials and method

Eskişehir Osmangazi University’s Faculty of Medicine’s hospital ethics committee approval and patients’ informed consent (or relitives on behalf of patients) were provided. We conducted this prospective clinical study on 30 adult patients aged 20 to 82 years who met at least 2 SIRS criteria. Patients who had sepsis, multiple organ dysfunction, and immune deficiency or were enterally fed were excluded from the study.

Criteria for SIRS were established in 1992 as part of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference [1]. The conference concluded that the manifestations of SIRS include, but are not limited to, the following: body temperature less than 36°C or greater than 38°C, heart rate greater than 90 beats per minute, tachypnea (high respiratory rate) with greater than 20 breaths per minute, an arterial partial pressure of carbon dioxide less than 32 mm Hg, white blood cell count less than 4000 cells per cubic millimeter or greater than 12 000 cells per cubic millimeter, or the presence of greater than 10% immature neutrophils (band forms). Systemic inflammatory response syndrome can be diagnosed when 2 or more of these criteria are present.

After being accepted to the ICU, medical treatment directed at primary disease, ventilatory support for respiratory failure, and antibiotherapy according to cultures were initiated. Total parenteral nutrition was randomly introduced in both groups: 15 patients with and 15 patients without glutamine dipeptide. The daily energy provided by isonitrogenous TPN solution is standardized as 30 kcal/(kg d) for all patients. This TPN solution is composed of 70% carbohydrates and 30% lipids; proteins (Traumamine 6.9%; Eczacibasi, Baxter; Eskisehir, Turkey) are added as 1.5 g/(kg d). Group I received standard TPN without glutamine dipeptide, whereas group II received TPN with 0.4 g/(kg d) glutamine dipeptide (l-alanyl-l-glutamine dipeptide, Dipeptiven, Fresenius Kabi; Eskisehir, Turkey). Both groups received TPN for 6 days.

Blood samples were taken from every patient at admission and on the sixth day. Sedimentation; C-reactive protein (CRP); white blood cell count (WBC); and, with the flow cytometer method, lymphocytes, lymphocyte subgroups, absolute percentage of T-lymphocytes, absolute percentage of B-lymphocytes, absolute percentage of helper T-lymphocytes (Th), absolute percentage of cytotoxic T-lymphocytes (Ts), and ratio of Th to Ts were measured. The levels of products of immune system cells—immunoglobulin (Ig) A, IgG, and IgM—and the mediators of immune system—C3 and C4—were measured from the blood samples taken at admission and on the sixth day. Based on the WBC taken at simultaneous blood samples and the absolute percentage of lymphocytes, we calculated the total lymphocyte count (WBC × absolute percentage of total lymphocytes = total lymphocyte count per cubic millimeter). Based on the total lymphocyte count, lymphocyte subgroup count (Th count, Ts count, B-lymphocyte, natural killer [NK] cell count) was calculated (eg, total lymphocyte count per cubic millimeter × absolute percentage of B-lymphocytes = B-lymphocytes count per cubic millimeter).

Simplified Acute Physiologic Score (SAPS II) and Acute Physiologic and Chronic Health Evaluation (APACHE II) scores of all critical patients in ICU are recorded for the sake of objectivity. To evaluate clinical recovery, progress of disease, and mortality risk, we calculated daily SAPS II and APACHE II scores using the parameters.

In our statistical analysis, we used SPSS (Chicago, IL) 10.0 program and the free t test and Mann-Whitney U test for nonparametric data. We accepted P < .05 as significant, and data are presented as mean ± standard deviation. Statistical analyses were conducted both within and intergroup.

3. Results

There was no statistical difference according to age, sex, and body weight in both groups (P < .05) (Table 1).

Both groups had an increased erythrocyte sedimentation rate. The increase in group I was found to be statistically significant (P < .01). The difference between groups was not statistically significant (P > .05). Both groups had increased CRP, but it was not statistically significant (P > .05) (Fig. 1).
The mean leukocyte count of group I increased at the sixth day, and the average leukocyte count of group II decreased. This difference was not statistically significant ($P > .05$). Both groups showed an increased average absolute percentage of total lymphocyte count at the end of the sixth day, but this difference was not statistically significant ($P > .05$) (Fig. 2A). Both groups had increased average total lymphocyte count at the end of the sixth day, but this difference was not statistically significant ($P > .05$). Group I showed a decreased average absolute percentage of total lymphocyte count, and group II had increased average absolute percentage of total lymphocyte count; but this difference was not statistically significant ($P > .05$). Group I had decreased average T-lymphocyte count, and group II had increased average T-lymphocyte count; but this difference was not statistically significant ($P > .05$).

The mean absolute percentage of $T_h$ count of group I decreased on the sixth day, but average absolute percentage of $T_h$ count of group II increased. This difference was not statistically significant ($P > .05$). The average $T_h$ count of group I decreased on the sixth day, but average $T_h$ count of group II increased. This difference was not statistically significant ($P > .05$) (Fig. 2B).

Mean absolute percentage of the $T_s$ count in group I decreased at the sixth day. This difference was statistically significant ($P < .05$). Average absolute percentage of the $T_s$ count in group II decreased, but this difference was not statistically significant ($P > .05$). The average $T_s$ count in group I was decreased at the sixth day, but the average $T_s$ count in group II increased. This difference was not statistically significant ($P > .05$) (Fig. 2C).

The mean $T_h/T_s$ ratio of group I decreased at the sixth day, but the mean $T_h/T_s$ ratio of group II increased. This difference was not statistically significant ($P > .05$) (Fig. 3). Both groups had decreased mean in absolute percentage of NK cell count. The decrease in group I was found to be statistically significant ($P < .01$). The difference between the groups was not statistically significant ($P > .05$). Both groups had decreased average NK cell count. The decrease in group I was found to be statistically significant.

### Table 1: Clinical outcomes in patients receiving control TPN (group I) or glutamine-supplemented TPN (group II)

<table>
<thead>
<tr>
<th>Glutamine group (group I, n = 15)</th>
<th>Control group (group II, n = 15)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>54.46 ± 5.32</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.40 ± 9.37</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>3/12</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

Fig. 1  Median serum concentration of CRP in patients receiving control TPN (group I) or glutamine-supplemented TPN (group II).
The mean absolute percentage of B-leukocyte count of group II was increased at the sixth day, but this difference was not statistically significant ($P > .05$). Both groups had increased average B-leukocyte cell count. The difference between the groups was not statistically significant ($P > .05$).

Both groups had increased IgM and IgG cell count, but this difference was not statistically significant ($P > .05$). Group I had a decreased average IgA cell count, and group II had an increased mean IgA cell count; but these differences were not statistically significant ($P > .05$).

Group I had an increased mean $C_4$ count, and group II had a decreased mean $C_3$ count; but these differences were not statistically significant ($P > .05$). Group I had a decreased average $C_4$ count, and group II had an increased average $C_4$ count; but these differences were not statistically significant ($P > .05$).

Mean APACHE II scores were increased in group I, and mean APACHE II scores were decreased in group II; but these differences were not statistically significant ($P > .05$) (Fig. 5A). The decrease of SAPS II scores in group I at the sixth day was found to be statistically significant ($P < .05$).

4. Discussion

Many cells in the immune system proliferate very rapidly. Glutamine facilitates this process, functioning as a biosynthetic precursor and as an energy source [9,10]. Glutamine heals the defense system, probably by increasing antioxidant protection and also by increasing the effectiveness of the bowel-barrier function. Glutamine is very important in optimizing the immune system, by way of promoting lymphocyte proliferation and optimum functioning of neutrophils and macrophages [11].

Glutamine has essential properties in lymphocyte proliferation and optimum function of neutrophils and macrophages. In conditions of decreased plasma glutamine levels, T-lymphocytes are suppressed, phagocytic properties of macrophages decrease, and IL-1 production also decreases [4]. These very intense uses of glutamine by immune system cells show that glutamine is very important for protection of immune system [3].

In our study, we investigated the effect of glutamine (0.4 g/[kg d]) -added TPN on the immune system in SIRS patients for 6 days. At admission and at the sixth day, we investigated immune cells, specifically lymphocytes and lymphocyte subgroups (T-lymphocytes, $T_h$, $T_s$, $T_h/T_s$ ratio, B-lymphocytes, NK cells), immune system products (IgM, IgA, IgG),...
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serum and membrane proteins required for protective function of immune system (C₃, C₄), serum CRP, and sedimentation parameters for evaluation of inflammation.

Wischmeyer et al [12] investigated infection morbidity and nitrogen control by administering glutamine-added TPN to severe burn patients for 14 days. They used CRP as a parameter of all inflammations. Their results show that glutamine-added TPN statistically decreases total inflammation measured by CRP. Our study shows that a glutamine-added TPN group has lower CRP levels than the control group, but this difference is not statistically significant. We believe that the differences between these studies may be associated with different patient groups and the duration of glutamine administration.

In trauma patients, the effect of administering glutamine, arginine, and omega-3 fatty acid solutions added to the enteral nutrition on the duration of hospital stay and healing rate of immune system was investigated [13]. C-reactive protein is used as a parameter for inflammation. Total lymphocytes, lymphocyte subgroups (T-lymphocytes, Th, Tₘ, B-lymphocytes), immune system products (IgM, IgA, IgG), and serum and membrane proteins required for protective function of immune system (C₃, C₄) are measured as parameters of the immune system. They showed a decrease in respect to the control group, but this decrease was not statistically significant. In our study, we showed that both groups had increased total lymphocyte count and that group II had higher levels of T-lymphocytes, Tₘ, Tₘ/Tₜ, and B-lymphocytes but that this difference was not statistically significant. Immunoglobulin M levels are increased in both groups, but IgA levels are increased only in group II. Average C₃ levels are increased in group I but decreased in group II, but none of the parameters were significant. Although, in the Wischmeyer et al study, they also added omega-3 and used different means of nutrition, the results are similar to our study.

The effect of glutamine-added vs glutamine-free multifiber nutrition solutions on the immune system of 16 acute pancreatitis patients [14] was investigated. They evaluated IgG, IgM, C₃, and C₄ parameters for immune system and CRP as acute phase reactant. There was no significant difference in CRP, but IgG levels were clinically improved in the glutamine-added group; also, IgM levels were markedly improved. There was no significant difference in C₃ and C₄ parameters. Similar to our study, CRP, IgG, C₃, and C₄ levels were correlated in their study.

T-lymphocyte response according to glutamine level was investigated in 20 postoperative colorectal resection patients receiving glutamine-added TPN for 6 days [15]. They found that T-lymphocyte count elevation was statistically significant. Similar to our study, the T-lymphocyte levels of group II increased; but this increase was not statistically significant. We think that the difference in these studies might be associated with different patient groups and age intervals.

Cellular immunity changes due to perioperative glutamine infusion after cardiopulmonary bypass were investigated [6]. They found that T-cell-derived inflammatory response, indicating a sufficient supply of glutamine, shows no significant change. We think that the difference between these studies might be associated with different durations of glutamine administration and patient numbers. Crawford and Cohen [16] investigated glutamine effect on lymphocyte count and immunoglobulin production. They showed that immunoglobulin production improved, but that there was no change in B-lymphocytes, T-lymphocytes, Tₘ, and Tₚ. Therefore, it is suggested that glutamine support might improve lymphoblastic transformation and differentiation of plasma cells. Boelens et al [17] investigated glutamine-added enteral nutrition on lymphocyte subgroup count and immunoglobulin production in 38 trauma patients. They found out that both groups had increased levels of serum IgM, IgA, and IgG, but that these increases were not statistically significant. These results are similar to our study.

L-Glutamine and L-alanine effects on immune system cells were investigated in vitro [18]. It was shown that L-glutamine and L-alanine--added nutrition leads to increased T-lymphocyte count. This increase in T-lymphocyte count depends on glutamine dose. In the same study, they could not find any change in NK cells and Tₘ cells. In our study, we found a statistically significant decrease in both groups; group I showed a more marked significance.

Jones et al [19] investigated glutamine-added enteral nutrition effect on survival. They used APACHE II scores for this purpose and found no difference between groups. In our study, we observed decreased APACHE II scores in group II, but found no significant difference between groups. Nevertheless, we found a statistically significant decrease in SAPS II scores.

In conclusion, glutamine dipeptide--added TPN significantly decreases SAPS II scores, leukocytes, and NK cell count, which might be associated with suppressing inflammation and improving clinical recovery. Furthermore, total lymphocyte count, B- and T-lymphocytes, and their subgroups (Tₘ, Tₚ) are increased. Although not statistically significant, these increases may play a role in improving the immune system. Larger and controlled clinical trials are needed to determine the potential efficacy of glutamine-supplemented nutrition as an adjunctive therapy in critically illness.

References


