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The effect of parenteral glutamine supplementation on the immune system and nitrogen balance in critically ill patients receiving glutamine enriched enteral nutrition

Glutaminden zengin enteral nütrisyon desteği alan yoğun bakım hastalarında parenteral glutamin desteğinin immün sistem ve azot dengesi üzerindeki etkileri

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Summary

Aim: Dietary glutamine is mostly utilized by enterocytes and gut associated lymphoid tissue. Glutamine is also an essential amino acid for the rest of the lymphoid cells. We aimed to investigate the effects of the addition of parenteral glutamine to enteral glutamine on lymphocyte subpopulations and nitrogen balance.

Materials and Methods: 33 patients (24 M, 9 F; aged 19-76) formed the two groups. Daily energy requirements were measured by indirect calorimetry for 4 days and the amount of glutamine enriched enteral nutrition was adjusted accordingly. In IV-GLN group (n=18) 0.3 g/kg/day parenteral L-alanyl-L-glutamine solution was infused within 6 hours. In the IV-STD group (n=15) isonitrogenous standard amino acid solution was administered. 24 hour urine samples were collected and the nitrogen balance was calculated daily. Blood samples were obtained for lymphocyte subpopulations (CD3, CD4, CD8, CD19, NK, active T cell, IL-4 and IFN-gamma) at the beginning and after 96 hours of supplementation.

Results: Demographic variables and APACHE II scores were similar between the groups. There were no significant differences between the groups in terms of lymphocyte subpopulations and cumulative nitrogen balance.

Conclusion: In our study, the addition of L-alanyl-L-glutamine or standard amino acid solution parenterally to glutamine enriched enteral nutrition had similar effects in terms of lymphocyte subpopulations and nitrogen balance. This is one of the limited number of studies investigating the effects of the addition of parenteral glutamine to enteral glutamine. Optimal dose and route of glutamine supplemantation for ICU patients still needs to be clarified.

Key Words: Glutamine, enteral nutrition, parenteral nutrition, critical care, immune system.

Özet

Amaç: *Diyetle alınan glutaminin çoğu enterositler ve barsakla ilişkili lenfoid doku tarafından kullanılır. Glutamin vücuttaki diğer lenfoid hücreler için de gerekli bir amino asittir. Bu çalışmada enteral glutamine parenteral glutamin eklenmesinin lenfosit alt grupları ve azot dengesi üzerindeki etkilerinin araştırılması amaçlanmıştır.* ***Gereç ve Yöntem:*** *33 hasta (24 erkek, 9 kadın; 19-76 yaş) iki grubu oluşturdu. Günlük enerji gereksinimleri 4 gün boyunca indirekt kalorimetre ile ölçüldü ve glutaminden zengin enteral nütrisyon solüsyonu miktarı belirlendi. IV-GLN grubunda (n=18) 0.3 g/kg/gün parenteral L-alanyl-L-glutamin solüsyonu 6 saatte infüze edildi. IV-STD grubunda (n=15) izonitrojen standart amino asit solüsyonu verildi. 24 saatlik idrar örnekleri toplanarak günlük azot dengeleri hesaplandı. Lenfosit alt gruplarının (CD3, CD4, CD8, CD19, NK, active T cell, IL-4 and IFN-gamma) analizi için çalışma başlangıcında ve 96 saat sonra kan örnekleri alındı.*

Bulgular: *Gruplar arasında demografik veriler ve APACHE II skorları açısından fark belirlenmedi. İki grup arasında lenfosit alt grupları ve kümülatif azot dengesi açısından anlamlı fark saptanmadı.*

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Sonuç: *Çalışmamızda glutaminden zengin enteral nütrisyon uygulamasına parenteral olarak L-alanyl-L-glutamin veya standart amino asit solüsyonu eklenmesi lenfosit alt grupları ve azot dengesi üzerinde benzer etki göstermiştir. Bu çalışma, enteral glutamine parenteral glutamin desteğinin eklendiği az sayıda çalışmalardan bir tanesidir. Yoğun bakım hastalarında glutaminin optimal dozu ve ideal uygulama yolu halen netlik kazanmamıştır.*

Anahtar Sözcükler: *Glutamin, enteral nütrisyon, parenteral nütrisyon, yoğun bakım, immün sistem.*

Introduction

Glutamine is a non-essential amino acid, however it becomes conditionally essential under conditions like extreme stress, particularly of prolonged duration (1). Glutamine is a necessary substrate for nucleotide synthesis in rapidly proliferating cells like lymphocytes. During inflammatory states, glutamine consumption may exceed endogenous production and a relative glutamine deficiency state may exist. Several animal and clinical studies suggest that outcome may be improved by providing glutamine in the appropriate dose and by the appropriate route to obtain adequate tissue concentrations. It was also stated that glutamine plays an important role in protein turnover and it may be important in preventing muscle loss and negative nitrogen balance (2). But there are also studies indicating negative effects of glutamine on intestinal permeability, nitrogen balance and nosocomial sepsis (3,4). Because dietary glutamine is utilised primarily by the enterocytes and gut-associated lymphoid tissue, high glutamine requirement of immune cells outside the gastrointestinal tract should be met by the systemic circulation and influenced by circulating levels of glutamine (5). It may be hypothesized that the addition of parenteral glutamine to enteral glutamine might improve immunological functions and nitrogen balance. Therefore, we aimed to investigate the effects of the addition of parenteral glutamine to glutamine enriched enteral nutrition on the immune system and nitrogen balance.

Materials and Methods

This is a randomized and controlled clinical trial. Critically ill patients (APACHE II score>10) who expected to stay more than 5 days in the ICU were included after Ege University Hospital Ethics Committee approval and obtaining informed consent. A total of 38 patients were enrolled but 3 patients who died and 2 patients who developed intolerance to enteral feeding were excluded from the study. The remaining 33 patients (24 male, 9 female; aged 19-76 years) were analyzed. Exclusion criteria were existing contraindication for enteral nutrition, renal and hepatic failure, congestive heart failure, pregnancy and age below 18 years. After hemodynamic, respiratory and metabolic stabilization of the patients, venous blood was drawn for basal measurements. Daily energy requirements were determined by indirect calorimetry at every consecutive day for 4 days and the amount of glutamine (15.97 g/L) enriched enteral nutrition (Alitraq, Abbott) was adjusted according to measured energy expenditure in all patients. The enteral solution was infused continuously during the study period. The patients were randomized into two groups. In the IV-GLN group, (n=18) 0.3 g/kg/day parenteral L-alanyl-L-glutamine solution (Dipeptiven, Fresenius Kabi) was infused for 6 hours each study day. In the IV-STD group (n=15) isonitrogenous protein was administered as standard amino acid solution (FreAmine, Eczacibasi-Baxter). In order to calculate the daily and cumulative nitrogen balance, 24 hour urine samples were collected each day during the study period and urinary urea nitrogen was measured. In order to avoid the possible detrimental effects of this high protein diet, ammonia levels were measured at 72 hrs and at the end of the study. Blood samples were obtained for lymphocyte subpopulations (CD3, CD4, CD8, CD19, NK, active T cell, IL-4 and IFN-gamma) at the beginning of the study and after 96 hours of supplementation. Lymphocyte subpopulations were analyzed by flow cytometry.

Data was analysed by the Statistics Department of Ege University Hospital by using SPSS statistics program. Data was analyzed by the t-test for parametric and the Mann-Whitney U test for nonparametric data. $p < 0.05$ was accepted as statistically significant and data was presented as mean ± standard deviation.

Flow cytometry

A fresh anticoagulated peripheral blood sample was collected by venipuncture and stained within 6 hours with each of the antibodies from the Simultest IMK Plus kit (Becton Dickinson, San Jose, California). A white blood cell count (WBC) was obtained from the same sample of whole blood before staining. Samples with counts greater than 9.8×10^3 WBC/dL were diluted with phosphate-buffered saline (PBS) containing 0.1% sodium azide. The kit contained six pairs of Simultest murine monoclonal antibody reagents conjugated with fluorescein isothiocyanate (FITC) and phycoerythrin (PE) that includes LeucoGATE^{TM} (CD45/14) for establishing a lymphocyte acquisition gate and Simultest Control IgG_1 FITC/IgG_{2a} PE for setting fluorescence markers around the negative population and detecting nonantigen-specific antibody binding. Other pairs were Anti-Leu-

4/Anti-Leu-12 (CD3/CD19), Anti-Leu-3a/Anti-Leu-2a (CD4/CD8), Anti-Leu-4/HLA-Dr (CD3/HLA-DR), Anti-Leu-4/11c+19 (CD3/CD16+56). Labelled cells were washed three times and all sample tubes were run through the flow cytometry Facscalibur (Becton Dickinson), and acquired data was analyzed by the CELLquest 3.0 software program.

Results

Demographic variables and APACHE II scores were similar between the groups (Table-1). There was no significant difference between the two groups in terms of lymphocyte subpopulations (Table 2). In none of the patients was the IL-4 level within the detectable limits. Cumulative nitrogen balance at the end of the study period was – 4.2 ± 17.6 g in IV-STD group and 3.6 ± 23.7 g in IV-GLN group. There was no statistically significant difference between groups in terms of nitrogen balance (p = 0.29).

Table-1. Demographic variables and APACHE II scores.

	IV-GLN Group (n = 18)	IV-STD Group (n = 15)
Sex (M/F)	12 / 6	12 / 3
Age (years)	52.5 ± 18.9	49.7 ± 17.8
Height (cm)	167.2 ± 7.1	169.8 ± 7.8
Weight (kg)	70.6 ±6.9	71.1 ± 8.5
APACHE II score	15.7 ± 6.9	16.4 ± 5.4

Table-2. Lymphocyte subpopulations in the study groups.

(%)	IV-GLN 1 / IV-GLN 2	IV-STD 1 / IV-STD 2
CD3	60.7±14.4 / 60.8±14.3	66.0±11.4 / 67.3±13.4
CD4	35.1±12.7 / 35.8±10.6	39.3±10.6 / 37.7±11.2
CD8	26.9±11.0 / 25.7±11.6	28.9±13.2 / 28.6±12.8
CD19	15.3±8.3 / 16.3±10.1	16.1±7.7 / 15.6±8.4
NK	8.7±6.9 / 7.9±5.6	7.5±7.0 / 7.5±6.2
Active T cell	9.1±9.2 / 11.0±11.0	7.0±5.3 / 7.2±4.8
IFN-gamma	1.5±1.9 / 1.7±2.0	0.9±1.3 / 2.0±2.1

IV-GLN1 / IV-STD1 (basal values); IV-GLN2 / IV-STD2 (after 96 h of supplementation)

Discussion

In this study, we found that the addition of L-alanyl-L-glutamine or standard amino acid solution parenterally to glutamine enriched enteral nutrition had similar effects in terms of lymphocyte subpopulations and cumulative nitrogen balance.

The cellular and humoral immune responses are regulated by cytokines that control two types of helper cells known as Th1 and Th2. Under conditions of metabolic stress. cell-mediated immune responses deteriorate accompanied by a high risk of infection (6). Thus, searching the role of glutamine in Th1/Th2 responses might be helpful in understanding the therapeutic option of glutamine for critically ill patients.

One post hoc finding from the study of glutamine supplementation in ICU patients was that the maximum outcome benefit was obtained with glutamine supplementation over 9 days (7). Also the results of a meta-analysis showed that at least 6 days of parenteral glutamine supplementation was necessary to get the maximum benefit. But in a study conducted on 44 medical and surgical ICU patients, 0.5 g/kg/day alanyl-glutamine given for 8 days either enterally or parenterally did not affect the T-lymphocyte subset (CD-3, CD-4, CD-8) number, gut barrier function or whole-body protein metabolism (8). Also in children with diarrhea, enteral supplementation of glutamine (0.3 g/kg/day) for 7 days did not have any effect on lymphocyte subpopulations (9). In our study, we used 4 days of glutamine supplementation because we thought that it would be difficult to stabilize such severe ICU patients for a longer period of time. This shorter period of supplementation might be an explanation for limited effect of glutamine on lymphocyte subpopulations in our study. There are some recent studies recommending glutamine supplementation for the entire ICU stay (10).

The discrepancies between the studies may result from the duration and dosage of glutamine administered. In a review article it was stated that high-dose parenteral (>0.50 g/kg/day) glutamine had the greatest potential for benefit in critically ill patients (11). In addition, the characteristics and severity of diseases may also play important roles in influencing the efficacy of glutamine supplementation. In one study, postoperatively administered glutamine-supplemented TPN had a beneficial effect on enhancing the immune response but the effect of glutamine administration on improving nitrogen economy was only observed in patients with low APACHE II scores (APACHE II < 6) (12). It was concluded that the amount of glutamine required for reversing the catabolic condition might depend on the characteristics and severity of the diseases. This result may explain the different results obtained in studies with different patient populations.

Although the cumulative nitrogen balance at the end of the study period in our study was lower in the IV-STD group than the IV-GLN group, it did not reflect a statistical significance. This is similar to the results of another study where there is a tendency to have better cumulative nitrogen balance on postoperative days in the glutamine group but no significant difference is

observed between two groups. However, improved nitrogen balances with glutamine supplementation were also reported (13). One possible explanation for a nonsignificant result in our study in terms of nitrogen balance might be the limited number of our patients.

It was stated that since the clearance and the distribution kinetics of glutamine were highly variable, the dosage of glutamine might be guided by determinations of plasma concentration (14). Unfortunately we were not be able to measure plasma glutamine concentrations in this study.

In our study group of critically ill patients, the addition of L-alanyl-L-glutamine or standard amino acid solution parenterally to glutamine enriched enteral nutrition had similar effects in terms of lymphocyte subpopulations. Limitations of the current study are the small sample size and absence of plasma glutamine concentrations. However, this is one of the limited numbers of studies investigating the effects of the addition of parenteral glutamine to enteral glutamine. The optimal dose and route of glutamine supplementation for ICU patients are still to be elucidated.

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