Effects of enteral and parenteral glutamine on intestinal mucosa and on levels of blood glutamine, tumor necrosis factor-alpha, and interleukin-10 in an experimental sepsis model

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ABSTRACT

الأهداف: التحقق من تأثير استخدام الغلوتامين المعوي واللامعوي في تخفيف الانتان لدى مجموعة من الجرذان المصابة به.

الطريقة: أُجريت هذه الدراسة في معامل جامعة اسطنبول للتجارب والأبحاث الطبية، جامعة اسطنبول، اسطنبول، تركيا وذلك خلال الفترة من يونيو إلى سبتمبر 2009م. لقد قمنا بقياس مستويات الغلوتامين في الدم، وعامل تنخر الأورام، وانترليوكين – 10. وقمنا أيضاً بجمع عينات الأنسجة من العقد اللمفية المساريقية، والكبد، والفص الأيمن من الرئة وذلك من أجل تقييم إنتاج البكتيريا. وتم أخذ عينات الأنسجة من الأمعاء الدقيقة من أجل اختبار ضمور الدم والزغابات.

النتائج: أشارت نتائج الدراسة إلى أن تجرثم المجموعة EP قد كان أقل من تجرثم المجموعة C (p=0.007). كما كانت معدلات ضمور الزغابات لكافة المجموعات أقل من المجموعة C ($p<0.05$). وكانت قيم معامل تنخر الأورام وانترليوكين – 10 لدى المجموعتين EP وP أقل من المجموعات الأخرى وذلك بعد مرور 24 و96 ساعة (p=0.000).

خاتمة: لقد وجدنا خلال هذه الدراسة بأن أفضل طرق إعطاء الغلوتامين أثناء علاج الانتان قد كانت الجمع بين الغلوتامين المعوي واللامعوي وذلك بفضل تأثيره في تقليل الغشاء المخاطي، وتقليل إنتاج الجراثيم في مزارع الأنسجة والدم، وتأثيره المعدل مناعياً، بالإضافة إلى أن تكلفته مشابهة تقريباً لتكلفة إعطاء الغلوتامين اللامعوي.

Objectives: To investigate the effects of enteral and parenteral glutamine (Gln) usage on rats in sepsis.

Methods: This study was conducted in Istanbul University Experimental Medical Research Institution (DETAE) laboratory, Istanbul University, Istanbul, Turkey between June and September 2009. The levels of blood Gln, tumor necrosis-alpha (TNF-α) and interleukin (IL)-10 was measured. Samples of tissue were obtained from the mesenteric lymph nodes, liver, and lower lobe of the right lung to evaluate the reproduction of bacteria, and samples of tissue were also obtained from the small intestine to evaluate blood and villus atrophy.

Results: Bacteremia of Group EP (combined group) were found lower than Group C (control) (p=0.007). Villous atrophy rates of all groups were lower than Group C: between Group E (enteral) and C (p=0.003); between Group P (parenteral alanine) and C (p=0.019); and between Group EP and C (p=0.001). The values of serum TNF-α and IL-10 of Group EP and P were lower than the other groups at the 24th and 96th hours (p=0.000).

Conclusion: In this study we found that the most efficient Gln administration technique in sepsis was enteral administration together with parenteral administration due to trophic effect on the intestinal mucosa, decrease of reproduction in tissue and blood cultures, immunomodulator effect, and approximately the same cost as parenteral application.

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Sepsis is defined as a systemic response developed by organism against microorganism and toxins. Effective administration of supportive care in sepsis affects clinical course significantly.[1] Cytokines have important functions in the regulation of immune response in sepsis.[2] Tumor necrosis factor alpha (TNF-α) is the fundamental pro-inflammatory cytokine in the response of human immune system response.[3] Anti-inflammatory cytokines like interleukin-10 (IL-10) were shown to increase together with pro-inflammatory mediators to balance the immune response in sepsis.[4] Glutamine (Gln) is a free, non-essential amino acid, which is found in substantial amount in the human body.[5] The available data support the importance of Gln for human immune system cells.[6] Glutamine that is one of the immuno-nutrients, is an important metabolic substrate of cells growing quickly like intestine cells and lymphocyte.[7] The Gln prevents bacterial translocation, which can lead to septicemia and multiple organ failure by helping the protection of intestine wall unity.[8] The European Society for Clinical Nutrition and Metabolism (ESPEN) Parenteral Nutrition guide suggests with Grade A proof that amino acid solution should contain 0.2-0.4 g/kg/day Gln (for example, 0.3-0.6 g/kg/day Ala-Gln, dipeptide) when there is parenteral nutrition indication in intensive care patients.[9] In 2009, the American Society for Parenteral and Enteral Nutrition/Society of Critical Care Medicine (ASPEN/SCCM) guide suggests addition of Gln to standard enteral formula for intensive care patients with burn, trauma, and combined issues with Grade B proof, while ESPEN enteral nutrition guide suggests it with Grade A proof to patients with burns and trauma. The ESPEN intensive care enteral nutrition guide also suggests enteral nutrition to begin in less than 4 hours for critical patients with Grade C proof.[10] There is a limited number of studies related to Gln supplementation in sepsis patients. Only the effects of parenteral and enteral usage are investigated in these studies. There are no studies in the literature investigating the effect of Gln supplementation by combined enteral and parenteral usage. We planned this study to support the clinical studies performed on this subject, and to find answers to questions such as whether we could provide more bioavailability and low cost with combined usage of enteral and parenteral Gln because their trophic effects on systemic and gastrointestinal system were different in clinics. We analyzed the effects of enteral and parenteral Gln usage on intestinal mucosa, bacteria reproduction, blood Gln, TNF-α and IL-10 levels on rats with developed intra-abdominal sepsis.

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Methods. This study was conducted in Istanbul University Experimental Medical Research Institution (DETAE) laboratory, Istanbul University, Istanbul, Turkey between June and September 2009, on rats taken from DETAE with the approval given by Istanbul University Animal Experiments Local Ethics Committee. A total of 63 female Wistar-Albino 10-week-rats with weights 230 ± 20 g were used in this study. During the operation, humane treatment was applied to rats in accordance with the Guide for the Care and Use of Laboratory Animals.[11] Ketamine hydrochloride (Ketalar® vial, Parke Davis, 50 mg/mL, Eczacibasi, Istanbul, Turkey) 50 mg/kg im (intramuscular), and xylazine hydrochloride (Rompun® vial, 23.32 mg/ml, Bayer, Istanbul, Turkey) 5 mg/kg im were administered during the interventional operations in rats. Required fluid resuscitation was applied with isotonic saline solution (subcutaneous 5-10 mL/day) in addition to nutrition in all rats. Intraperitoneal (ip) live bacteria model was used to develop sepsis in this study.[12] Two pre-study groups and 4 main study groups were formed. Each group was worked on separately, but concurrently within each group. After the study was completed, all the rats were sacrificed with intracardiac puncture under anesthesia.

Prestudy groups: Group I (n=8) - first pre-study group to determine *Escherichia coli* (*E. coli*) strain used to develop sepsis and its amount; Group 2 (n=7) - second pre-study group to prove sepsis is developed.

Main study groups: Group C (control group, n=12) - after development of sepsis, isotonic saline solution is administered with 2 mL/day intravenous (iv) (infusion/60 minutes), and one mL/day 6 catheter diameter size (Ch) orogastric nutrition catheter (Bicakcilar®, Istanbul, Turkey). Group E (Enteral Gln group, n=12) - the group that is given 0.5 g/kg/day Gln (Resource Glutamine®, powder, 20x5 g, Nestle Inc., Istanbul, Turkey) after diluting to one mL with 6 Ch orogastric nutrition catheter, and 2 mL/day iv (infusion/60 minutes) isotonic saline solution, after sepsis is developed; Group P (parenteral alanine [Ala]-Gln group, n=12) - the group where 0.4 g kg/day Ala-Gln (0.27 g/kg/day Gln), (Dipeptiven®, 20% L Ala-L Gln, 20 g [13.46 g L Gln]/100 mL iv solution, Fresenius Kabi, Istanbul, Turkey) iv solution diluted to 2 ml is applied in 60 minutes (infusion/60 minutes) (0.03-0.04 ml/min) with 26 G branule canule or angiocut

(BD Neoflon®, Helsingborg, Sweden) and isotonic saline solution is given with one mL/day orogastric from the tail vein after sepsis is developed; Group EP (combined group, n=12) - combined group where 0.3 g/kg/day Ala-Gln (0.2 g/kg/day Gln) iv solution diluted to 2 mL (infusion/60 minutes), and 0.3 g/kg/day Gln diluted to one mL is applied with orogastric after sepsis is developed.

Prestudy. Group I is formed to determine *E. coli* strain to be used to develop sepsis and its quantity. As a result of study performed on 8 rats, it is concluded to develop sepsis with $5x10^7$ colony forming unit (CFU)/mL *E. coli 1104512* strain. Group 2 was formed with 7 rats to reveal the presence of sepsis, and to show Gln supplementation after sepsis is developed. Basal fever and leucocyte values of the rats in this group were determined normally. Sepsis development is proven with the results of rectal temperature, leucocyte, blood and tissue culture 2 hours after ip one mL $5x10^7$ CFU/mL *E. coli 1104512* injection.

Main study. Intra-peritoneal one mL $5x10^7$ CFU/mL *E. coli 1104512* was injected after the initial blood of all rats in the main study were drawn. Feeding and Gln supplementation of rats began 4 hours after ip *E. coli* was given. Bloods drawn at the beginning of study were registered as basal values. Plasma Gln, serum TNF-α and IL-10 concentrations were monitored in blood samples drawn at the start and at 24th, and 96th hours after nutrition began. Sternotomy and laparotomy were applied in sterile conditions by performing surgical area disinfection to rats after blood samples were drawn at the 96th hour. Approximately 5 ± 1 mL blood drawn by intracardiac puncture was placed in blood culture tube. Approximately one g lung tissue from the lower lobe of the right lung, approximately 2 g liver tissue and mesenteric lymph node were obtained, and placed in different culture plates. Tissue samples of the small intestine (SI) were taken from all rats to determine villus atrophy. The 24th and 96th hour blood values of one rat of Group E that was exitus during anesthesia was applied before the blood drawing process at the 24th and 96th hour blood Gln, the TNF-α, IL-10 values of one rat that became exitus during enteral Gln was given at the 96th hour were included in the analysis with intention-to-treat regression method. Two rats from Group E, one rat from Group P, and one rat from Group EP were not included in the study as they became exitus after anesthesia induction was applied on the first day before blood was drawn.

Microbiological method. Tissue culture samples were transferred to general reproductive media, and kept in an oven at 37°C for 24-48 hours. Endo and chocolate agar passages were taken from the broth medium where reproduction occurred in the first 24-48 hours. Broth mediums with no reproduction after 48 hours was evaluated as "no bacteria reproduced". Samples were taken from bacteria colonies that were reproduced in endo mediums and fall off one by one, and evaluated with API 20E test and *E. coli 1104512* was determined as the reproduced bacteria. Besides *E. coli 1104512* strain applied to develop intraabdominal sepsis, *E. coli* strain, *Bacteroides fragilis* (*B. fragilis*), *Enterobacter*, *Enterococcus*, *Pseudomonas*, and *Staphylococcus* reproductions were investigated. Blood samples obtained for blood culture were inoculated into BACTEC hemoculture tubes, and kept at 37°C in an oven for 48, 72, 96, and 120 hours. At the end of this period, the samples injected by sterile injectors from hemoculture bottles were inoculated into endo and chocolate agar medium with reduction method. These mediums were kept in an oven at 37°C for 24-48 hours. The mediums without reproductions were considered as negative. Gram straining was performed on different colonies that fell separately in mediums with reproduction.

Histopathological method. Approximately 3-5 cm tissue sample was taken from the terminal ileum surface for histopathological investigation. Tissue samples were stained with Hematoxylin-Eosin (H&E), and were evaluated with respect to villous atrophy after being fixed in 10% formaldehyde.

Biochemical methods. White blood cell count. Blood was taken into ethylene diamine tetraacetic acid (EDTA) containing tube, and was measured with blood count device (Abacus, Diatron, Wien, Austria) to determine white blood cell count in this study.

Plasma Gln, TNF-α and IL-10 determination. A 0.6-1 mL blood was taken (at the start, 24th hour, and 96th hour) from the tail vein of rats for plasma Gln levels. Samples were centrifuged at 3000 rpm for 10 minutes (Elektro-mag M4812P, Istanbul, Turkey), and transferred to Eppendorf tubes after plasmas were separated. Samples were stored at -80°C until Gln levels were measured. Plasma Gln levels were determined with Gln kit (Glutamine/Glutamate Determination Kit, GLN-1 SIGMA, Saint Louis, Missouri, USA), and by absorption at 340 nm wavelength with spectrophotometric method (Human Humalyzer 2000, Germany). Serum TNF-α and IL-10 were studied with enzyme-linked immunosorbent assay (ELISA) method in EL x 800 micro ELISA reader, and ELx 800 micro ELISA washer devices (Bio-Tek, Instruments Inc, Winooski, Vermont, USA). Serum TNF-α level and IL-10 level was measured by absorbance at 450 nm with rat TNF-α ELISA kit (rat interleukin [IL]-10,

Invitrogen, ELISA Kit, Catalog KRC0101, CA, USA), and rat IL-10 ELISA kit (Rat IL-10, Invitrogen, ELISA Kit, Catalog KRC0101, CA, USA).

The Statistical Package for Social Sciences for Windows 15.0 (SPSS Inc., Chicago, IL, USA) program was used in the study. Descriptive statistical methods (frequency, percent, average, standard deviation) were used in evaluation of data, and Pearson Chi Square was used to assess data qualitatively. The Kolmogorov-Smirnov distribution test was used to assess the normal distribution. When comparing qualitative data, in case there were more than 2 groups Kruskal-Wallis test was used in comparing intergroup parameters, which were not distributed normally, and Mann Whitney U test was used for determining group causing dissimilarity. Spearman Correlation Analysis was used to compare 2 quantitative data. Friedman test was used in comparison of parameters within groups, and Wilcoxon sign test was used to determine the group causing the difference. Results were assessed in 95% confidence interval (CI) and significance level was evaluated at $p<0.05$.

Results. In Group 2 rats, the average rectal temperature was 39.07 ± 0.34^{0}C, and average leucocyte was 15.21±1.32/mm^3. The *E. coli 1104512* reproduction is determined in all blood and tissue cultures of rats within this group. When the results of culture were evaluated, bacteria reproduction other than *E. coli 1104512* was not determined in tissue and blood cultures. Bacteremia rate was found to be lower in Group EP than Group C (p=0.007). Any significant difference was not determined between the other groups. Bacteremia rate of groups are presented in **Table 1**. When there was reproduction in any of the cultures of tissue and blood, it was assumed as "reproduction +", and when there was no reproduction in any of the cultures of tissue and blood, it was assumed as "reproduction -". Reproduction rate in Group EP was determined to be significantly lower than Group C (p=0.025). The other groups were statistically at equal levels. Reproduction rates of groups are presented in **Table 1**. Villous atrophy rates of Group E (p=0.003), Group P (p=0.019), and Group EP (p=0.001) in ileum were found significantly lower than Group C. The other groups were statistically at equal levels. Villous atrophy rates of groups are presented in **Table 2**. When plasma Gln concentrations were analyzed, no statistical difference was determined between basal Gln values of Group C, Group E, Group P, and Group EP (p=0.745). The Gln values of Group C were determined to be significantly higher than Group E (p=0.000), Group P (p=0.000), and Group EP (p=0.000) at the 24th hour. The Gln values of Group E were significantly higher than Group P (p=0.000) and Group EP (p=0.000). Any statistically significant difference was not determined between Gln values of Group EP and Group P (p=0.622). The Gln values of Group C were significantly lower than Group E (p=0.000), Group P (p=0.000), and Group EP (p=0.001) at the 96th hour. The Gln values of Group E were significantly lower than Group P (p=0.000), and Group EP (p=0.000). The Gln values of Group EP were significantly higher than Group P (p=0.011). Plasma Gln concentrations of groups are presented in **Table 3**.

Table 1 - Bacteremia and reproduction rates of different groups of rats included in a study at Istanbul University Experimental Medical Research Institution laboratory, Istanbul University, Istanbul, Turkey.

Group	n	Bacteremia (+)	Reproduction (+)	*P*-value
C	12	11	12	
E	8	6	8	
P	11	7	10	
EP	11	4*	7†	0.007*, 0.025†

*,†*p*-value for comparison of Groups C (control) and EP (combined group). E - enteral glutamine, P - parenteral alanine

Table 2 - Villous atrophy rates of different groups of rats included in a study at Istanbul University Experimental Medical Research Institution laboratory, Istanbul University, Istanbul, Turkey.

Group	n	Atrophy (+)	*P*-value
C	12	11	
E	8	2*	0.003
P	11	5†	0.019
EP	11	2‡	0.001

*comparison of Groups C and E, †comparison of Groups C and P, ‡comparison of Groups C and EP. C - control, EP - combined group, E - enteral glutamine, P - parenteral alanine

Table 3 - Plasma glutamine concentration of different groups (mmol/L) of rats included in a study at Istanbul University Experimental Medical Research Institution laboratory, Istanbul University, Istanbul, Turkey.

Group	n	Glutamine Average ± standard deviation			*P*-value
		Basal	24th hour	96th hour	
C	12	0.714 ± 0.029	0.865* ± 0.037	0.483* ± 0.046	0.000*
E	10	0.708 ± 0.021	0.796* ± 0.019	0.599* ± 0.010	0.000*
P	11	0.70 ± 0.022	0.725* ± 0.029	0.748* ± 0.051	0.000*
EP	11	0.712 ± 0.016	0.730* ± 0.011	0.779* ± 0.018	0.000*
P-value		0.745	0.000*	0.000	

**p*=000 comparison of average glutamine values within each group at different periods of time. C - control, EP - combined group, E - enteral glutamine, P - parenteral alanine

Variations in plasma Gln concentrations, occurring in time within each group are presented in **Figure 1**. When plasma TNF-α concentrations were evaluated, no statistical difference was found if basal TNF-α values of Group C, Group E, Group P, and Group EP were compared between groups (p=0.906). The 24th hour TNF-α values of Group C were significantly higher than Group E (p=0.015), Group P (p=0.000), and Group EP (p=0.000). The TNF-α values of Group E were significantly higher than Group P (p=0.000) and Group EP (p<0.001). The TNF-α values of Group EP were significantly higher than Group P (p<0.001). On the 96th hour, TNF-α values of Group C were significantly higher than Group E (p=0.002), Group P (p=0.000), and Group EP (p=0.000). The TNF-α values of Group E were significantly higher than Group P (p=0.000), and Group EP (p=0.000). The TNF-α values of Group EP were significantly lower than Group P (p=0.000). Plasma TNF-α concentrations of groups are presented in **Table 4**. Changes in TNF-α concentrations with time within each group are presented in **Figure 2**. When plasma IL-10 concentrations were analyzed, no statistical difference was found if the basal IL-10 values of Group C, Group E, Group P, and Group EP were compared between groups

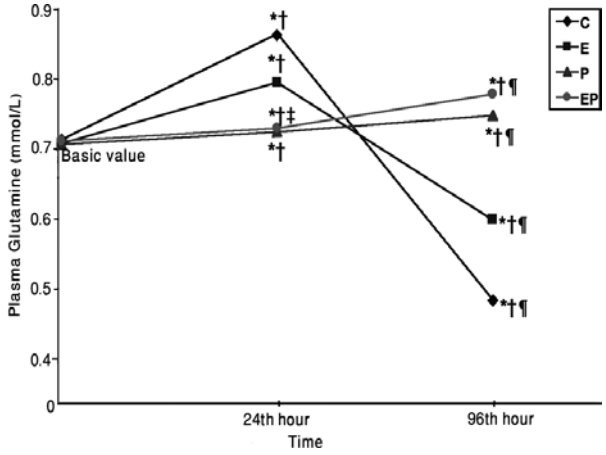

Figure 1 - Variations in plasma glutamine (Gln) concentrations occurring in time within each group. *Group C - p=0.002; Group E - p=0.005; Group P - p=0.003; Group EP - p=0.003 - significant difference is determined between the mean Gln values within the groups at different periods of time, †p=0.000 - significant difference is determined between the mean Gln values of groups at the same period of time, ‡p>0.05 - significant difference is not determined between the mean Gln values of groups EP and P at the 24th and the 96th hours, ¶Group C - p=0.002; Group E - p=0.005; Group P/EP - p=0.003; Group P - p=0.003 - significant difference is determined in the mean increase and mean decrease of Gln values at the 96th hour when compared with the basic values. Groups C - control, EP - combined group, E - enteral glutamine, P - parenteral alanine

Table 4 - Plasma tumor necrosis factor-alpha (TNF-α) concentration of the studied groups (pg/mL).

Group	n	TNF-α Average ± standard deviation			*P*-value
		Basal	24th hour	96th hour	
C	12	8.43 ± 0.35	18.04* ± 1.04	15.63* ± 0.716	0.000*
E	10	8.59 ± 0.56	16.92* ± 0.97	14.32* ± 1.63	0.000*
P	11	8.48 ± 0.18	12.70* ± 0.77	9.95* ± 0.39	0.000*
EP	11	8.42 ± 0.17	13.27* ± 0.66	9.57* ± 0.37	0.000*
P-value		0.906	0.000*	0.000*	

*p=0.000 - comparison of TNF-α values within each group at different periods of time. Groups C - control, EP - combined group, E - enteral glutamine, P - parenteral alanine

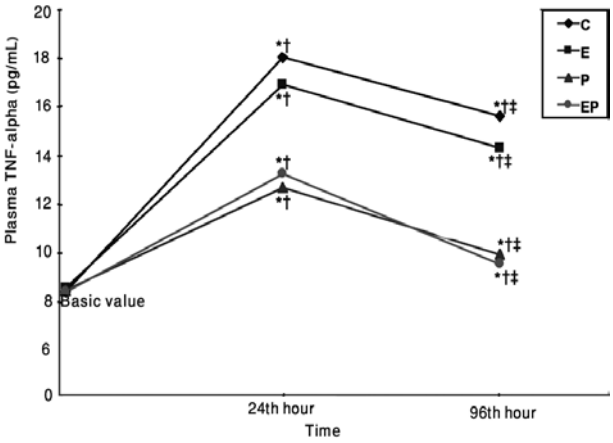

Figure 2 - Changes in tumor necrosis factor (TNF-α) concentrations with time within each group. *Group C - p=0.002; Group E - p=0.005 and p=0.007; Group P - p=0.003; Group EP - p=0.003 - significant difference is determined between the mean TNF-α values within the groups at different periods of time, †p=0.000 - significant difference is determined between the mean TNF-α values of groups at the same period of time, ‡Group C - p=0.002; Group E - p=0.005; Group P/EP - p=0.003; Group P - p=0.003 - significant difference is determined in the mean increase and mean decrease of TNF-α values at the 96th hour when compared with the basic values.Groups C - control, EP - combined group, E - enteral glutamine, P - parenteral alanine

Table 5 - Plasma interleukin-10 concentration of groups (pg/mL).

Group	n	Interleukin-10 Average ± standard deviation			*P*-value
		Basal	24th hour	96th hour	
C	12	13.67 ± 0.85	19.17* ± 1.17	15.34* ± 0.94	0.000*
E	10	13.94 ± 0.55	18.25* ± 0.95	15.08* ± 0.45	0.000*
P	11	13.45 ± 0.63	15.93* ± 0.81	14.49* ± 0.40	0.000*
EP	11	13.18 ± 0.58	15.98* ± 0.43	13.90* ± 0.59	0.000*
P-value		0.067	0.000*	0.000*	

*p=0.000 - comparison of interleukin-10 values within each group at different periods of time. Groups C - control, EP - combined group, E - enteral glutamine, P - parenteral alanine

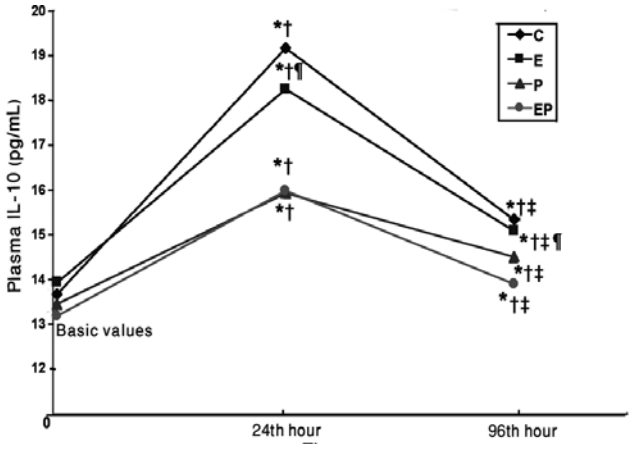

Figure 3 - Changes in interleukin-10 (IL 10) concentrations with time within each group. *Group C - p=0.002; Group E - p=0.005; Group P - p=0.003; Group EP - p=0.003 - significant difference is determined between the mean IL-10 values within the groups at different periods of time, †p=0.000 - significant difference is determined between the mean IL-10 values of groups at the same period of time, ‡p>0.05 - significant difference is not determined between the mean IL-10 values of groups C and E at the 24th and the 96th hours, ¶Group C - p=0.002; Group E - p=0.005; Group P - p=0.003; Group EP - p=0.003- significant difference is determined in the mean increase and mean decrease of IL-10 values at the 96th hour when compared with the basic values. Groups C - control, EP - combined group, E - enteral glutamine, P - parenteral alanine

(p=0.067). The 24th hour, IL-10 values of Group C were significantly higher than Group P (p=0.000) and Group EP (p=0.000). Statistically significant difference was not determined between IL-10 values of Group C and Group E (p=0.121). The IL-10 values of Group E were significantly higher than Group P (p=0.000) and Group EP (p=0.000). Statistically significant difference was not determined between IL-10 values of Group P and Group EP (p=0.895). The 96th hour IL-10 values of Group C were significantly higher than Group P (p=0.009), and Group EP (p=0.001). Statistical significant difference was not determined between IL-10 values of Group C and Group E (p=0.121). The IL-10 values of Group E were significantly higher than Group P (p=0.003) and Group EP (p=0.000). The IL-10 values of Group EP were lower than the values of Group P (p=0.017). Plasma IL-10 concentrations of the groups are presented in **Table 5**. Changes in IL 10 concentrations with time within each group are presented in **Figure 3**. A significant correlation in positive direction was determined between TNF-α and IL-10 values at the 24th (r=0.746; p=0.000), and 96th hours (r=0.591; p=0.000). We determined that TNF-α and IL-10 values at the 24th and 96th hours indicated simultaneous increases.

Discussion. The Gln level of the blood and tissue decreases in patients with sepsis, and this decrease continues until the patient passes to anabolic period. Fastened metabolism in sepsis causes protein catabolism, especially Gln destruction, and depletion.[13] At the same time, while Gln absorption decreases in the intestine, it is used too much in the liver and immune system.[14-16] Low Gln levels are related with immune system dysfunction, deterioration of intestinal unity, and increase in mortality rate.[17,18] The main argument in experimental and clinical studies performed to present the positive effects of Gln used as a nutrition supplementation in sepsis are different in effect, dose, and cost between different usage methods. There is a large number of studies performed with this purpose, regarding enteral and parenteral usage of Gln. However, we could not find any study in literature relating combined Gln usage. Friedland et al[19] determined in their experimental *E. coli* meningitis model performed with rats that leucocyte values increased after the second hour, and this increase occurred simultaneously with TNF-α. High fever and leucocytosis were determined at the fourth hour in rats of Group 2, which was a pre-study group formed to prove sepsis has developed. A study performed by Windmueller[20] showed that Gln is the major energy source of enterocytes. The experimental study performed by Basoglu et al[21] showed that Gln containing nutrition, protected the SI from ischemia and reperfusion injury. In the studies performed to show the trophic effects of Gln on intestinal mucosa, one can see that enteral application apparently decreases intestinal villus atrophy when compared with parenteral application.[22,23]

Intestinal villus atrophy rates decreased significantly in all Gln supplementation given groups than the control groups in this study. We determined that intestinal villus atrophy rates decreased more in enteral application than parenteral application. However, this difference was not significantly important. This result reveals that in cases where enteral supplementation cannot be given, parenteral Gln supplementation can be given to benefit from its intestinal trophic effect, and this is in accordance with the results of the study performed by van der Hulst et al.[8] If one tries only to benefit from the trophic effects of Gln on intestinal mucosa, it is beyond dispute that enteral application is the best way by also taking the costs in account. But as we stated before the importance of immodulator effect in sepsis was a foregone fact. In a study performed by Houdijk et al,[24] bacteremia, pneumonia, and sepsis was observed less when Gln was added to enteral nutrition.

The study revealed that Gln decreased modulator effect on leucocyte and macrophage functions, and therefore decreased the number of bacteria present and infection rate in clinics.[25] Although the studies in literature state that reproduction reducing effect in tissue and blood cells are more pronounced in parenteral Gln administrations, there are no studies comparing combined application with respect to this effect. However, if we consider that parenteral Gln application increases blood Gln level in minutes, it is possible that this effect may be higher with parenteral supplementation in response to endotoxemia of Gln.[26,27] The reduction in reproduction rates in blood and tissue cultures with Gln supplementation was determined, and these results were considered as compatible with literature.[24,25] Reproduction rates of blood and tissue cultures in combined group were significantly lower than the control group. The reason of higher bacteremia rate in enteral group is considered to be the lower systemic effect in enteral group. A more specific decrease in combined group was detected, and this decrease was statistically significant when compared with the control group. Considering these results, immune modulator effect of Gln was observed to be the highest in combined group, and this effect reduced reproduction in blood and tissue cultures significantly. The more immune modulator effect in combined Gln administration than parenteral application, may depend on prevention of cytokine storm by Gln supplementation. The Gln supplementation, especially combined Gln administration reduces the number of bacteria with immune modulator effect, and as a result of this, we suppose the cost of antibacterial specific treatment used in sepsis may be reduced. However, this idea must be supported by further studies.

We observed that skeletal muscle released Gln to plasma until depleting Gln deposits and Gln release from lung decreased as the first metabolic response.[28,29] Austgen et al[30] showed Gln release from the intestine decreased and Gln release from the liver increased in sepsis. Also, Newsholme et al[25] stated that the immune system became a great Gln consumer in sepsis. There is no clear information on the variation of Gln plasma concentrations in sepsis with time. Garrett-Cox et al[27] determined plasma Gln concentrations decreased in the early periods of endotoxemia (the second hour), and approached to basal values at the sixth hour in groups where Gln was not given, and exceeded basal values in group where Gln was given. However, they could not reveal any data regarding levels in the later time periods because 6 hours of blood Gln levels was studied in this study. However, we did not have any data for the first 24 hours as we measured Gln levels at the 24th and 96th hours in our study. Plasma Gln levels in the control group increased with respect to basal values at the 24th hour. This increase at the 24th hour was considered to depend on Gln discharge from skeletal muscle as a result of sepsis.[28] The decrease of plasma Gln levels below basal values in this group at the 96th hour showed Gln need could not be satisfied in sepsis by organism. The result obtained from the control group showed there was a requirement for Gln supplementation to supply increased need in sepsis. When Gln levels were evaluated at the 24th hour, these results were interpreted as enterally administered Gln could not inhibit Gln discharge from muscular tissue since the effect of enterally administered Gln on plasma Gln concentration would appear later. Plasma Gln levels in parenteral and combined group at the 24th hour was significantly lower than the control and enteral group. We concluded that this result was based on the rapid increase of plasma level of parenterally administered Gln, and the inhibition of Gln discharge from muscular tissue. When plasma Gln levels were evaluated at the 96th hour, Gln levels in the combined group were significantly higher than the other groups. We determined that plasma Gln concentrations in parenteral group increased more than the enteral group in line with the study performed by Luo et al,[31] but combined Gln application was the most effective way to keep plasma Gln levels at optimum concentrations.

Opinions stating that TNF-α is a mediator which plays the main role in septic shock and endotoxemia, depend on a series of studies.[32] An increase in TNF-α level is determined in healthy individuals after endotoxin injection within 60-90 minutes.[32] This may lead to positive effects at low concentrations and fatal effects at high concentrations.[33] Casey et al[34] showed high level of TNF-α, a proinflammatory cytokine in patients with sepsis might be used as an indicator for clinical course, and that serial measurements might be useful. The IL-10 plays a critical role in organ failure in sepsis and protection from death of host, and is an important anti-inflammatory cytokine, which is at balance between immunoregulator cytokines.[35,36] Oliveira et al[37] stated that IL-10 levels increased in bronchoalveolar lavage and peritoneum fluid in the study conducted related to decrease of lung and distal organ damage by parenteral Gln in experimental abdominal sepsis model. However, it was not possible to determine whether this increase was related to Gln supplementation, or was induced by sepsis. Garrett-Cox et al[27] showed that parenteral Gln supplementation induced endotoxemia in sepsis and

suppressed TNF-α and IL-10 increase measured at the 2nd and 6th hours in the study.

In our study, we presented that increase of TNF-α and IL-10 was reduced with Gln supplementation. In this study, the cytokine levels were evaluated at the 24th and 96th hours. While cytokine levels measured at the 2nd and 6th hours were higher than the basal values in the study performed by Garrett-Cox et al[27] cytokine levels measured in this study at the 24th and 96th hours were 2-3 times the basal values. As there was no clear information regarding the course of cytokines, blood levels should be measured more frequently to reveal this course. Although cytokine increase was suppressed in all Gln supplementation given groups, this effect is mostly observed in combined group.

In summary, suppression effect on TNF-α levels was detected most definitely in parenteral group. This situation was interpreted as early response of Gln to endotoxemia would be most effective by providing blood Gln concentration in the early period at required amounts. The TNF-α levels at the 96th hour were significantly higher in the control than the other groups. The TNF-α levels of enteral group at the 96th hour were significantly higher than the parenteral and combined groups (p<0.05). This result can be attributed to concentrations of blood Gln levels, which could not be maintained in sufficient levels in accordance with the investigation performed by Melis et al.[26] The TNF-α level in combined group at the 96th hour was significantly lower than the parenteral group. We concluded the reason for this situation was the higher blood Gln concentration of the combined group than the parenteral group at the 96th hour. The suppression effect on TNF-α levels at the 96th hour was most apparent in the combined group. This situation showed that suppression effect increased simultaneously with increase of blood Gln concentration in sepsis with time. Basal values, increases and decreases in TNF-α levels at the 24th and 96th hours were statistically significant (p<0.05) when compared within groups.

Serum levels of anti-inflammatory cytokines in sepsis, increase in parallel with pro-inflammatory mediators. Due to this, anti-inflammation shows its development at the same time with hyper-inflammation. Although high levels of anti-inflammatory mediator presence was in company with mortality of these patients, clinical role of cyclic change between hyper-inflammation and hypoinflammation have not been explained clearly.[38] When IL-10 levels was evaluated in our study, it was observed that IL-10 levels showed similarity with TNF-α, and that they had positive correlation (24th hour; r=0.746, p<0.01, and 96th hour; r=0.591, p<0.01). Steinhauser et al[4] stated that lL-10 also provided bacterial clarity. When the serum IL-10 levels of parenteral and combined groups at the 24th and 96th hours were evaluated, they were determined to be lower than the values of enteral and control groups (p<0.05). This result showed that immune suppression effect of enteral application was inadequate. Ertel et al[39] showed in the experimental study that the body in sepsis tried to decrease inflammation with compensation mechanism to delay tissue necrosis and multiple organ failure of the body in sepsis, and it provided this by keeping IL-6 and TNF-α blood levels low. The TNF-α levels in sepsis group decreased below basal values at the 24th hour in this study. Similar results were obtained in the experimental sepsis model performed by Karaca et al.[40] We observed that cytokines in the control group had a tendency to decrease. However, TNF-α levels at the 24th hour were 2-3 times the basal values, and although TNF-α levels at the 96th hour tend to decrease, they never decrease below the basal values. This diversity in our study might be based on virulence of microorganism, and on the variability of host response. This compensation mechanism ensured by keeping cytokine levels low in sepsis appeared more definitely with Gln supplementation, and especially with combined Gln supplementation. In other words, Gln supplementation made a big contribution to this compensation mechanism developed by the organism in sepsis to protect itself. If blood Gln and cytokine levels could be measured more frequently through the first 24 hours period, we could obtain more clear findings that would reveal the effects of blood Gln concentrations in sepsis on cytokine levels. We consider that the complicated course of immune response in sepsis could be clarified with the experimental studies that would be performed for this purpose. When a 7-day cost analysis was conducted with doses used for adult patients (70 kg) in this study, the following results were obtained: Gln enteral ($151.64); Gln parenteral ($356.40), and Gln combined ($358.28). We consider that combined Gln may be preferred due to its higher bioavailability, although combined Gln cost was almost equal to the cost of parenteral application. However, extensive clinic studies covering hospitalization duration of patients are required for more clear data regarding this subject.

This study is limited only with the effect of glutamine supplementation in sepsis, and with presentation of the difference between effects of various administration methods.

In conclusion, as a result, we determined that the best availability from excellent candidate, Gln with numerous

biological features, for nutritional supplementation in sepsis is possible with combined application due to trophic effect to intestinal mucosa, immune modulator effect, and because it decreases reproduction in blood and tissue cultures by keeping plasma Gln concentration at the effective level. Besides, we suppose that to prefer Gln used in low doses via combined application in clinics, is not inconvenient because its cost is near to the cost of parenteral application along with the positive effects stated above. However, we consider that our hypothesis should be developed with advanced studies. The implications of the findings in the present study for future research are : to present the effects of glutamine added to standard treatment that is used to support the treatment in sepsis and to implement this to clinical practice; and to present cytokine responses in the early periods with glutamine supplementation in sepsis.

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