The effects of combined use of glutamine and growth hormone on the bacterial translocation associated with obstructive jaundice

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ABSTRACT

الأهداف: التحقق من آثار الاستعمال المتحد لعقار جلوتامين وهرمونات النمو على تحول البكتريا المرتبطة باليرقان الانسدادي.

الطريقة: أجريت هذه الدراسة بمختبرات قسم الفسيولوجية بجامعة أتاتورك التعليمية – تركيا، في الفترة ما بين 15 يونيو 2007م وحتى 15 سبتمبر 2007م. شملت الدراسة 40 جرذ في خمس مجموعات، تكونت كل مجموعة من 8 جرذان. في مجموعة الدراسة تم ربط القناة الصفراء للحصول على اليرقان الانسدادي. أعطيت الجرذان في مجموعة التحكم (CG) كلوريد الصوديوم (NaCI)، وفي مجموعة جلوتامين (GLG) أعطيت عقار جلوتامين، وفي مجموعة هرمونات النمو (GHG) أعطيت الجرذان هرمونات النمو، وفي مجموعة جلوتامين+هرمونات النمو (GLGHG) أعطيت الجرذان الجلوتامين مع هرمونات النمو بجرعات متساوية بنفس الطريقة. تم الحصول على عينات من الدم والطحال والكبد والعقد اللمفاوية والأعوري. تم تقييم أنشطة البيلوربين الكامل (TB)، وفوسفات الكالين (ALP)، وتحول جلوتامين جاما (GGT).

النتائج: في مجمعة التحكم (CG)، كان محتوى الأعوري أعلى من مجموعة الجلوتامين (GLG)، وكان البيلوربين الكامل (TB) أعلى من مجموعة هرمونات النمو (GHG) ومجموعة الجلوتامين مع هرمونات النمو (GLGHG). كان معدل البيلوربين الكامل (BT) أقل في مجموعة الجلوتامين وهرمونات النمو (GLGHG)، مع اختلاف عند الحدود مع قيم مجموعة الجولتامين (GLG) ومجموعة هرمونات النمو (GHG).

خاتمة: وجدنا في البيلوربين الكامل الوقائي (BT)، أن الاستعمال المتحد للجلوتامين وهرمونات النمو كانت أكثر فعالية من استعمال كل من هذه العوامل على حده.

Objective: To investigate the effects of combined use of glutamine and growth hormone on bacterial translocation.

Methods: The study was performed at the laboratories of the Department of Physiology at Ataturk University Medical School, Erzurum, Turkey between June and September 2007. Forty rats divided into 5 groups of 8, were included in the study. In the study groups, the common bile duct was ligated to obtain obstructive jaundice. The rats in the control group (CG) were given sodium chloride, in the glutamine group (GLG) they were given glutamine, in the growth hormone group (GHG), growth hormone, and in the glutamine + growth hormone group (GLGHG) glutamine + growth hormone at equal doses by the same methods. Blood, spleen, liver, lymph node, and cecal content samples were obtained. Total bilirubin (TB), alkaline phosphates (ALP), and gamma glutamine transferase (GGT) activities were evaluated.

Results: In the CG, cecal contents were higher than in the GLG, and cecal contents and BT were higher than in the GHG and the GLGHG. The BT rate was the lowest in the GLGHG, with a borderline difference with the values of the GLG and GHG.

Conclusion: We found that in preventing BT, combined use of glutamine, and growth hormone was more effective than using each of these agents alone.

Many diseases such as obstructive jaundice, sepsis, acute pancreatitis, and multiorgan failures disturb the gastrointestinal mucosa barrier, and lead to the transfer of bacteria and toxins into systemic circulation and other tissues from the intestinal flora.[1] The dispersion of the bacteria and toxins passing through the mucosa barrier via lymphatic and ductus

thoracicus to the circulation and other organs is known as bacterial translocation (BT).[2] Glutamine (GL), the most abundant free amino acid in the circulation, is the primary fuel for cell division and proliferation of the intestine and plays a key role in the transport of nitrogen between organs.[3,4] Growth hormone (GH), a strong anabolic hormone, has been reported to provide the prevention of nitrogen loss and the protein sparing effect when it was administered to the patients with trauma, injury, burn, or the patients after operation.[5,6] Various experimental studies have been conducted to evaluate the BT process and the effects of different agents on BT. To this end, some studies have used GL and GH. However, the effects of combined use of these agents are not known. Thus, in this experimental study, the efficiency of combined use of GL and GH in prevention of BT developing after experimental obstructive jaundice was investigated.

Methods. All the experiments in this study were carried out after ethical approval of the Local Ethics Committee of the Experimental Studies at Ataturk University. The study was performed at the laboratories of the Department of Physiology at Ataturk University Medical School, Erzurum, Turkey between June and September 2007. The study involved 40 Wistar Albino rats weighing 250-300 gr. The rats were divided into 5 groups of 8 in each. After 12-hour fasting period, median incisions were inflicted on the rats under ketamine HCL anesthesia (35 mg/kg subcutaneously [sc]). In the sham group (SG), the common bile duct (CBD) was detected and the procedure was ended. In the other groups, the CBD was tied with 4/0 silk suture and cut. Starting from the first postoperative day, for 7 days, the control group (CG) received sodium chloride (NaCl) (1.5 ml sc); the glutamine group (GLG) received glutamine (1 mg/kg/day oral); the growth hormone group (GHG) received growth hormone (1 mg/kg/day sc), and glutamine + growth hormone group (GLGHG) received equal doses of glutamine and growth hormone via the same route. On the eighth day after the procedure, blood samples from the vena porta, lymph node samples from the terminal ileum mesentery, and samples of the spleen, liver, and cecal were obtained under sterile conditions from each of the subjects and the subjects were sacrificed with ether anesthesia. Two ml of the blood sample of each subject was inoculated for blood culture evaluation. Inoculations were performed on diphasic blood culture plates (Diomed Ltd, Cambridge, UK), incubated at 36°C, and observed for 2 weeks. The blood samples obtained for biochemical analyses were transferred to glass tubes and centrifuged. Then, their plasmas were isolated and kept in covered plastic tubes at -80°C until the day of evaluation when total bilirubin (TB), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT) values were determined with auto-analyzer. The samples obtained for evaluation of BT were transferred to the laboratory in a mean time of 30 minutes. The samples of the spleen, lymph node, and liver were homogenized by adding one ml of 0.9% NaCl. From each suspension, 0.2 ml was taken and inoculated onto the blood agar and McConkey plates. Upon completion of all of the procedures, cecal content was diluted 10 times with 0.9% NaCl with the help of a serological pipe, 0.01 ml of this mixture was collected and inoculated on the same plate. The cultures were incubated for 48 hours at 36°C. Then, the microorganisms were isolated and classified through classical methods. The cultures were evaluated macroscopically using gram stained preparations and passages in blood agar plate. In cecal cultures, the bacterial colonies were multiplied with 1000, and thus the colony count in one ml of feces (CUF) was calculated.

Statistical analysis. All of the statistical analyses were performed by the Statistical Package for Social Sciences (SPSS) version 10.0, (SPSSFW, SPSS Inc., Chicago, II., USA). Descriptive statistics were given as mean ± standard deviation. Since the sample sizes of the groups were too small, we used Kruskal-Wallis test for more than 2 groups. The differences between the 2 groups were investigated with Mann-Whitney U test (with Bonferroni correction). *P*-values less than or equal to 0.05 were considered statistically significant.[7]

Results. In CG and GHG, one subject died (total: 2 subjects). Thus, the evaluations were made in 7 subjects only. The values for all the variables in SG were lower than those of the other groups ($p=0.000$). No statistically significant differences were detected between the values of the GLG and GHG. The BT was detected most frequently in the lymph nodes, followed by the liver. *Escherichia coli (E. coli)* was the most common microorganism in bacterial translocation (**Figure 1**). Bacterial translocation rates into the liver, spleen, and the lymph node were the lowest in the GLGHG, with a slight difference between the values of the glutamine group and growth hormone group ($p=0.01$). No statistically significant differences were detected between the values of the GLG and GHG. The BT rate in the CG were higher than in the GLGHG ($p<0.001$). The CG had a higher rate of BT in the liver and spleen than GHG and GLG ($p=0.000$). The distribution of bacterial colonies for all the groups is presented in **Figure 2**. The cecal content of the CG had the highest counts of colonies. The CG had a higher level of cecal content than GLG and GHG ($p=0.000$). Similarly, cecal content in the CG were higher than in the GLGHG ($p=0.000$). No statistically significant

differences were detected between the values of the GLG and GHG. There were no statistically significant differences for blood culture growth between the groups (*p*=0.490). Liver function test results are presented in **Table 1**. The ALP of the CG was lower than that of the other groups, while TB and GGT values were higher than those of the other groups (*p*=0.000). In the study group, the lowest ALP value was detected in the GLG, and the lowest TB, and GGT values were detected in the GLGHG. The CG had a higher level of GGT activity than GLG. It also had higher levels of GGT and

Figure 1 - The distribution of bacterial translocation according to groups.

Figure 2 - The distribution of cecal content.

ALP than GHG (*p*=0.000). Similarly, GGT activity in the CG was higher than in the GLGHG (*p*=0.000). No statistically significant differences were detected between the values of the GLG and GHG. In the GLGHG, the mean TB and GGT values were lower than in the GLG and GHG. However, only the GGT levels significantly differed (*p*=0.000).

Discussion. Despite all the preventive measures, surgical interventions of obstructive jaundice result in high morbidity and mortality rates. This is usually more common in elderly patients in whom postoperative septic complications or renal failure are the most important factors for morbidity and mortality.[8,9] Various studies have shown that bacteria in the intestinal flora are the source of septic complications and BT and endotoxemia rates increase in patients with obstructive jaundice.[10,11] The BT associated with obstructive jaundice is due to immune depression, excessive increase of the bacteria in the intestinal lumen, and disruption of the mucosal barrier resistance in the intestines.[12,13] In our study, BT and liver dysfunction rate were higher in the control group than in the others. While the BT rate of GLGHG was slightly different from that of GLG and GHG, no BT was detected in SG. In addition, *E. coli* was the most common pathogen detected in BT, which is compatible with the earlier reports.

Glutamine, either in enteral or parenteral form, increases the intestinal villous height, maintains the mucosal integrity, and reduces BT.[14] Studies have shown that GL enriched diets show a decreased BT and improvement in the ability to kill translocated *E. coli*.[3,4,15] In our study, however, single GL treatment was not as effective as the combined treatment of GL and GH or only GH treatment for the prevention of BT in rats with obstructive jaundice. Although the BT rate in the GLG group was lower than in the CG, it was still higher than the rate of BT in the group with combined use of glutamine and growth. In contrast to GL there are some controversies concerning the effect of GH on BT. While some studies reported that GH decreased the BT in animal studies, this was not the case in another study.[16-19] In our study, individual use of glutamine and growth hormone yielded similar results. Although both
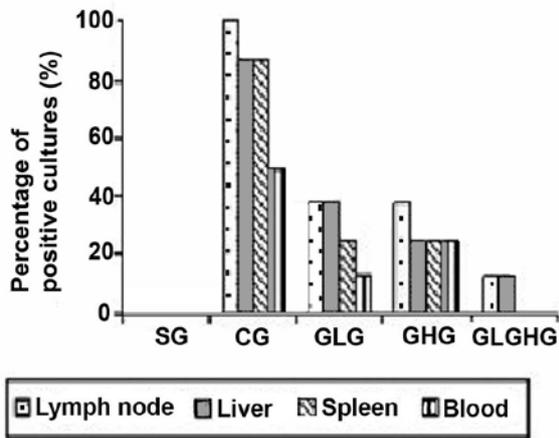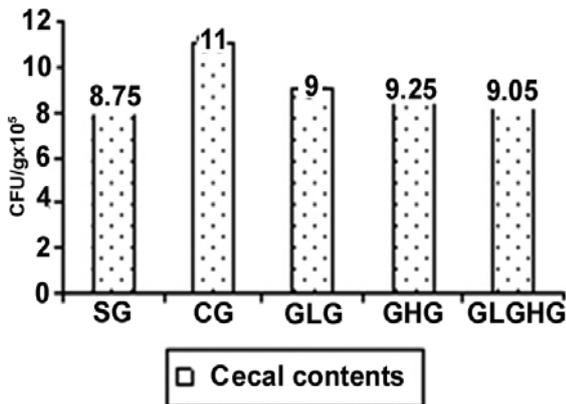
Table 1 - The liver function test results of the groups (expressed as mean ± SD)

Test	SG	CG	GLG	GHG	GLGHG
ALP (U/l)	254 ± 50	361 ± 75	371 ± 75	513 ± 25	449 ± 62
TB (mg/dl)	0.3 ± 0.18	8.0 ± 0.18	6.0 ± 0.18	5.7 ± 0.18	4.5 ± 0.18
GGT (U/l)	8.5 ± 1.07	29.37 ± 1.07	16.37 ± 1.07	16.25 ± 1.07	9.00 ± 1.07

TB - total bilirubine, ALP - alkaline phosphatase, GGT - gamma-glutamyltransferase, SG -sham group, CG - control group, GLG - glutamine group, GHG - growth hormone group, GLGHG - glutamine + growth hormone group

of these agents decreased the rate of BT in GLG and GHG groups compared to CG, they did not provide the desired results.

Several studies showed that the combination of both GL and GH treatment improved the intestinal absorption in short bowel syndrome,[20] and increased the intestinal GL uptake in sepsis or trauma.[21] It is known that sodium-dependent GL transport by enterocyte decreased in sepsis due to decrease of circulating substrates for synthesis of carrier proteins by enterocytes.[22-24] The GH in contrast, appears to increase the intestinal GL uptake in part, by an increase in GL carrier capacity and to induce hepatic release of GL in short bowel syndrome or sepsis.[22-27] Similar to literature findings, BT was most frequently detected in the liver and lymph nodes. We showed that the combined treatment of GL and GH was the most effective in prevention of BT. Some studies reported that the combined treatment of GL and GH initially improves the absorption of proteins, increase body-weight and lean body mass, decrease body fat, and decrease stool output in patients with short bowel syndrome.[20] Some other studies, however, reported results that are inconsistent with these findings.[28-30] We think that the synergistic effect of the combination of GL and GH shown in this study may be due to the mechanism described above. Despite septic conditions, gut GL uptake would increase markedly if GH was given simultaneously with the administration of GL. Unneberg et al[21] reported a similar result in an animal trauma model.

Literature reveals low rates of blood culture positivity. In our study, this rate was very low as well. The low rates in GLG, GHG, and GLGHG might have been due to positive effects of the agents used. In our study, except for ALP levels, the results for the mean liver functions and cecal content of GLGHG were much better than the other groups. Although it is not clear why ALP level was lower in CG, the high levels of ALP in the other groups may have been associated with the agents used. The GL or GH only use has decreased BT rate compared to the rate in CG, however, by using both of these agents concomitantly, we obtained better results than obtained in CG, the results of which were similar to those of SG. The underlying mechanism may be the same as the one described above.

In the light of our findings, it can be said that combined use of GL and GH is more effective in prevention of BT than individual use of these agents. Clinically, the combined use of these agents would be useful in obstructive jaundice patients, in whom sepsis would complicate the course of the medical and surgical treatment. However, to provide more definitive conclusions, further clinical and experimental studies are needed.

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