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Effect of beta-glucan in preventing bacterial translocation in a model of experimental obstructive jaundice

Deneysel tıkanma sarılığı modelinde beta-glukanın bakteriyal translokasyonu önlemedeki etkinliği

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Abstract

Aim. Sepsis is the major cause of post-operative morbidity and mortality in obstructive jaundice as a result of bacterial translocation from the gut. This study was conducted to investigate the effect of beta-glucan in preventing bacterial translocation in an animal model where obstructive jaundice was developed by common bile duct ligation. **Methods.** Forty-five Wistar-albino rats were divided into three groups of fifteen animal each. Only laparotomy was administered to the first group. Bile duct ligation was administered to the second group. Bile duct ligation and oral beta-glucan for ten days were administered to the third group. The animals were sacrified at the end of the tenth day. Blood, liver, spleen and mesenteric lymph nodes were cultured. The samples taken from terminal ileum and liver were examined histopathologically. Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin, direct bilirubin and C-reactive protein (CRP) analyses were done on the blood samples taken from the rats. **Results.** In the first group bacterial translocation was observed in one animal whereas bacterial translocation was observed in twelve animals in II'nd group. In third group bacterial translocation was determined in six animals that received beta-glucan. The differences between these three groups were statistically significant ($p<0.05$). According to biochemical data only the decrease of ALP and total bilirubin values between group two and three were statistically significant ($p<0.05$). After the histopathological examination of liver and terminal ileum, no significant difference between group two and three was observed ($p>0.05$). **Conclusion.** The result of this study showed that beta-glucan, as a natural immune system activator, has an effect in preventing bacterial translocation in obstructive jaundice.

Keywords: Obstructive jaundice, bacterial translocation, beta-glucan

Özet

Amaç. Sepsis, barsaktan kaynaklanan bakteriyel translokasyonun bir sonucu olarak tıkanma sarılığında post-operatif morbidite ve mortalitenin en önemli sebebidir. Bu çalışma, ortak safra kanalı ligasyonu yoluyla tıkanma sarılığı geliştirilen bir hayvan modelinde bakteriyel translokasyonun önlenmesinde beta-glukanın etkisi araştırmak için yapılmıştır. **Yöntem.** Kırk beş adet Wister albino rat, her birinde onbeş hayvanın bulunduğu üç gruba ayrıldı. I.gruba sadece laparatomi yapıldı. II. gruba safra kanalı ligasyonu yapıldı. III. gruba ise safra kanalı ligasyonu ve on gün beta-glukan oral verildi. Onuncu günün sonunda hayvanlar sakrifiye edildi. Kan, karaciğer, dalak ve mezenter lenf nodu kültürleri ekildi. Terminal ileum ve karaciğerden alınan örnekler histopatolojik olarak incelendi. Ratlardan alınan kan örneklerinden aspartate aminotransferaz (AST), alanine aminotransferaz (ALT), alkalin fosfatase (ALP), laktat dehidrogenaz (LDH), total bilirubin, direk Bilirubin ve C-reaktif protein (CRP) çalışıldı. **Bulgular.** Birincigrupta 1 hayvanda bakteriyel translokasyon gözlenirken, II. grupta bakteriyel translokasyon oniki hayvanda gözlendi. III. grupta beta-glukan verilen hayvanların altı tanesinde bakteriyel translokasyon tespit edildi. Bu üç grup arasındaki fark istatistiki açıdan anlamlıydı ($p<0,05$). Biyokimyasal değerler açısından grup II ile III arasında sadece ALP ve total bilirubin değerlerindeki azalma istatistiki olarak anlamlıydı ($p<0,05$). Terminal ileum ve karaciğerin histopatolojik incelenmesi sonucu II ve III. gruplar arasında anlamlı bir farklılık gözlenmedi ($p>0.05$). **Sonuç.** Elde edilen sonuçlar göstermiştir ki, tıkanma sarılığında gelişen bakteriyel translokasyonu önlemede doğal bir immun sistem aktivatörü olan beta glukanın etkinliği vardır.

Anahtar sözcükler: Tıkanma sarılığı, beta-glukan, bakteriyal translokasyonu

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Introduction

Patients suffering from obstructive jaundice are typically sensitive to septic complications and renal failure. This situation makes contribution to high post-operative morbidity and mortality after surgery despite of the developing surgery techniques and antibiotics. It is known that bacterial translocation and endotoxemia play great role on the appearance of these complications [1].

Bacterial translocation can be defined as the spreading of particularly the gram-negative bacteria from the intestinal lumen to mesenteric lymph nodes (MNL), liver, spleen and systemic circulation [2]. There are three main etiological factors for bacterial translocation; 1: Change of intestinal mucosal barrier, 2: Change of flora of the intestinal bacteria and 3: Change of the immune defense mechanism against the host [1, 2].

Reticuloendothelial system (RES) plays a central role on the prevention of bacterial dissemination. RES cleans the microorganisms and endotoxins form the blood and defend the body against the infections. The functions of the hepatic macrophages which are important parts of the RES system are restraint during obstructive jaundice [2].

Beta-glucan is a natural polysaccharide which is obtained from the baker's yeast *Saccharomyces cerevisiae* and reinforces the immune system. It increases the proliferation, adhesion ability, chemotactic activity and cytotoxic features of the macrophages [3]. The effect of beta-glucan in preventing bacterial translocation in model of experimental obstructive jaundice is researched during this study.

Material and method

This experimental study was performed at the laboratory of the Department of Pharmacology of the Medical Faculty in Süleyman Demirel University in 2009. Adult, Wister-albino male rat species of 150-200 grams each are used at this study. A hundred mg/kg of Ketamine and 25mg/kg of Xylazine was used for general anesthesia during all surgical operations. The abdominal region of all animals was shaved and the regional disinfection is made by using 10% povidone iodine. They were separated into groups randomly.

Group I: Control group (n=15): Laparotomy was applied and the common bile duct was only mobilized.

Group II: Obstructive jaundice Group (no=15). Obstructive jaundice was created by ligated the common bile duct during laparotomy.

Group III: Obstructive jaundice + beta-glucan Group (no=15) Obstructive jaundice was created by ligated the common bile duct during laparotomy and cure with 10 mg/kg beta-glucan orally for 10 days.

All groups were allowed to feed with standard rat feed and water until sampling. No cure was applied to Group I and II. Ten mg/kg of beta-glucan was applied to Group three for 10 days after the operation by making gastric intubation with feeding tube. At the end of 10 days, all rates were sacrificed.

Sampling procedures were performed under general anesthesia. After the laparotomy made by using the old incision scar, 1 cc of blood was taken from portal vein for blood culture. Additionally 3 cc of blood was taken from inferior vena cava for biochemical studies. Samples were taken from the mesentery, lymph node, spleen and liver for analysis. Liver sample and distal terminal ileum was taken for pathological analysis.

The blood taken for analysis was put into Pedi-Bac T aerobic culture flasks. These flasks contain brain-heart infusion completed to 2 mL by adding haemin, menadrin and pyridoxine and sodium polianetol sulphonate (SPS). Tissues of the MLN, spleen and liver were homogenized by pressed with double knife method and placed onto EMB and Blood agar plates. The tissue samples under pathological analysis were flicked with %10 formalin. Bloods taken for biochemical examination were centrifuged for 3 minutes under 4000 rpm and stored under -20^{o}C until the date of experiment after separated from the serum.

Assessment: The microbiological assessment was made by assessing the samples taken from the culture flasks; in the BactT/Alert automate blood culture system. The spreaded flasks are incubated at 37^{o}C and continuously shook and monitored in 10 minute intervals. The CO_2 generation in the liquid culture flasks is measured according to colorimeter principle. When positive flask is detected, the positivity is indicated both visually and sound. From these positive detected flasks, Blood agar and EMB agar were passed and incubated for 24 hours at 37^{o}C. The isolated microorganisms were defined by using the standard microbiological methods. The samples not indicating any positivities were considered negative. The samples which were passed to the EMB agar and blood agar stock were incubated for 72 hours at 37^{o}C. The isolated microorganisms were defined by using the standard microbiological methods.

Histopathological assessment; the tissue samples of the flicked liver and terminal ileum were divided into 0,5 cm pieces and sections with the thickness of 5 micrometer were prepared by embedding into paraffin for histopathological examination and stained with Hematoxylin and Eosine. Liver preparations of all groups were examined under light microscope and the changes on the hepatocyte and portal structures were assessed. The preparations of terminal ileum of all groups were examined under light microscope and mucosal damage, changes on the lamina propria layer PNL infiltration and villus were assessed. Biochemical assessment; the total bilirubin, direct bilirubin, ALT, AST, ALP and LDH levels were measured.

Mann-Whitney U test was applied at the end of the experiment for comparing the microbiological, biochemical and histopathological data obtained from the groups. $P<0.05$ values were considered reasonable. The statistical analysis was made by using SPSS software.

Results

Microbiological studies: The blood, MLN, liver and spleen cultures from all groups were assessed. No growth observed on blood cultures taken from the group I. On the groups which were caused to happen obstructive jaundice by common bile duct ligation; bacterial growth observed in 11 cultures in group II while no growth occurred in 4 cultures. In group III, growth observed in 4 cultures and no growth was observed in other 11 cultures. As the result of the assessment of the MLN cultures, growth was observed in 1 sample in the control group and no growth was observed in other 14 samples. In group II, growth was observed in 12 samples while no growth was observed in other 3 samples. In group III on the other hand, growth was observed in 6 samples while no growth was observed in other 9 samples.

As the result of the assessment of the tissue cultures of liver; no growth observed in control group. In group II, growth was observed in 12 samples while no growth was observed in other 3 samples. In group III on the other hand, growth was observed in 4

samples while no growth was observed in other 11 samples.

As the result of the assessment of the tissue cultures of spleen; growth was observed in 1 sample in the group I and no growth was observed in other 14 samples. In group II, growth was observed in 12 samples while no growth was observed in other 3 samples. In group III on the other hand, growth was observed in 6 samples while no growth was observed in other 9 samples (Table 1). The bacteria isolated in all cultures were *Escherichia coli* mainly and also *Enterobacter cloacae* and *Enterococcus faceum*. As the result of the assessment performed after these results, it was accepted that there is bacterial translocation in rats if growth exists from any of 4 different cultures and no bacterial transformation is considered if there is no growth. Accordingly, bacterial translocation was detected in 1 sample in the group I, 12 samples in group II and 6 samples in group III (Figure 1).

Table 1. Blood, MLN, Liver and Spleen culture results for the groups.

	Blood		Mesenteric lymph nodes		Liver		Spleen	
	Negative	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**
Control	15	0	14	1	15	0	14	1
Group I	4	11	3	12	3	12	3	12
Group II	11	4	6	9	11	4	6	9

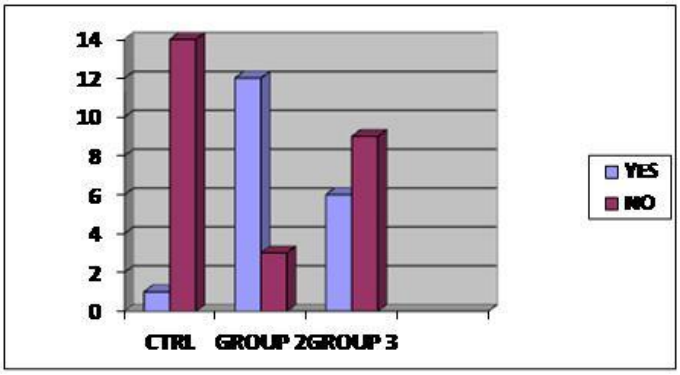

Figure 1. Bacterial translocation according to the groups.

As the result of the statistical assessment, the results of both groups II and III in which obstructive jaundice was created by making common bile duct ligation were pretty different and statistically significant ($p<0.05$) in terms of blood, MLN, liver and spleen culture results when compared to the group I. As the result of comparing the obstructive jaundice groups with each other, which is more important; the growth rate decreases at all sample to which beta-glucan cure is applied and this make sense statistically ($p<0.05$). As the result of the bacterial translocation, the results of both groups (group II and III) in which obstructive jaundice was created by making common bile duct ligation were pretty different and statistically significant ($p<0.05$) when compared to the control group again there is a decrease in bacterial translocation in the cultures which is applied beta-glucan cure is applied and this make sense statistically ($p<0.05$). When assessed in terms of CRP levels, no statistically significant result was found between all three groups.

Biochemical studies: When the groups were assessed in terms of serum LDH, AST, ALT, Total biluribin and Direct Bilirubin levels, it was detected that the results of both groups (group II and III) in which obstructive jaundice was created, were higher when compared to the control group (Table 2). These results were statistically significant ($p<0.05$). When the groups (group II and III) in which obstructive jaundice was created, were assessed between each other, it was detected that the arithmetic average of the biochemical values of the group which was applied beta-glucan cure (group III)

decreased when compared to group II. These results were statistically significant ($p<0.05$).

Table 2. Biochemical results of the control group and groups 2 and 3.

	Control	Group II	Group III
LDH	1695.86±181.18	2146.45±314.45	2103.53±472.52
AST	153.13±21.6	397.2±134.17	383±83.69
ALT	44.46±8.4	62.53±19.4	55.53±9.22
ALP	465±92.11	658.33±54.86	580.8±85.06
T.Bilirubin	0.4±0.12	20.35±353	17.55±4.15
D.Bilirubin	0.26±0.12	16.63±3.19	14.04±3.36

Histopathological studies: As the result of the examination of the liver samples; when the group I and two groups (group II and III) in which obstructive jaundice was created, it was observed that cholestasis, sinusoidal dilatation, parenchymal PNL increase, portal PNL infiltration and ductuler proliferation have prominently increased. These results were statistically significant ($p<0.05$). When the obstructive jaundice groups were compared in consideration of these criteria, no difference was observed except the slight increase observed in group which was cured with beta-glucan (group III). These results were statistically significant ($p<0.05$).

As the result of the examination of the terminal ileum samples, the decrease in the mucosal damage, PNL infiltration in lamina propria layer, lymphoid follicular hyperplasia and goblet cells between three groups were not found significant ($p>0.005$). There is a significant difference between the obstructive jaundice groups and the group I in terms of capillary dilatation, mucosal lymphatic dilatation and villus changes, however no significant difference was observed between the obstructive jaundice groups.

Discussion

The findings of this study show that, beta-glucan which is a natural immune system activator is effective on prevention of bacterial translocation emerged during the obstructive jaundice. Clinical and experimental studies make us think that, the major bacteria source related to the endotoxemia, sepsis and multi organ failure pathogenesis is enteric [4]. The events underlying the bacterial translocation are: the deformation of intestinal barrier function, intraluminal excessive bacterial growth with the deformation of endogenous flora and the decreasing of the host immunity [4, 5]. The studies made for determining if the physical damage in the intestinal mucosa causes the translocation of endogenous bacteria show that, the physical damages resulting with the elimination of the obstacles between the intestine lumen and lamina propria causes the translocation of bacteria thorough the gastrointestinal tract [6]. As the result of the studies, bacterial translocation was observed 7 days after the obstructive jaundice on rats [4, 7, 8]. Again, the hypothesis that the intestinal damage in rats having jaundice mainly occurs at terminal ileum, were justified with these studies. The studies supporting intraluminal excessive bacterial growth with the deformation of endogenous flora mainly focus on the non presence of bile in the intestine system and its effects. It is known that the bile inhibits intestinal bacteria development by lythic effects on the cell wall. This situation makes contribution to regulation of endogenous intestinal micro flora [9, 10]. Additionally, the bile salts inactivate endotoxins by creating non-absorbable complexes with endotoxins [9, 10]. Since the bile is not in the intestinal system on obstructive jaundice, the bacterial translocation occurs as the result of excessive bacterial reproduction and endotoxemia. The participation of mucosal, cell-mediated and humoral immunity exists on the prevention of the bacterial translocation through the gastrointestinal tract [11, 12]. The clinical and experimental studies indicate that the specific and non-specific immunity becomes weaker during obstructive jaundice. These studies show that, RES function, macrophage activity, kupfer cell function and natural killer (NK) cell activity decrease [1, 11].

The effect of the immune system activation on the bacterial translocation in obstructive jaundice was researched in this study and beta-glucan was used as an immune system activator. Beta-glucan is a totally natural substance which is obtained from the baker's yeast (*Saccharomyces cerevisiae*) and reinforces the immune system. It increases the proliferation, adhesion ability, chemotactic activity and cytotoxic features of the macrophages. It is reported that beta-glucan has antioxidant, hematopoietic and radio-protective effects [3].

When the group I and obstructive jaundice group was compared during our study, bacterial translocation was observed in 12 out of 15 rats in the obstructive jaundice according group the literature. This result is statistically significant when compared to the group I and not in accordance with the literature ($p<0.05$). Bacterial transformation was observed in 6 rats in the group which was applied beta-glucan for curing the obstructive jaundice. This decrease on bacterial translocation observed as the result of beta-glucan treatment, is statistically significant ($p<0.05$).

There is a study related to the changes on the parameters in obstructive jaundice and its reasons. Yasuda et al. [12], and Muriel et al. [13], detected increase in ALT, AST and ALP values in the plasma compared to the group supplemented with vitamin E, in their experimental obstructive jaundice model created by making common bile duct ligation,. In our study, the biochemical parameters of the obstructive jaundice group which was not cured and the group cured with beta-glucan, were compared. The parameters of the group cured with beta-glucan were found decreasing reasonably in terms of statistics ($p<0.05$). We think that such decreases can be explained by antioxidant and free radical scavenger function of beta-glucan.

With regards to the histopathological changes in liver in our study; the most tangible information known is that the obstructive jaundice may proceed to liver damage and parenchymal failure in the later stages. The increase of the pressure in the bile channels and the bile stasis are the most important reasons of these histopathological changes [14, 15]. We classified that the changes in liver during the earliest stages of the obstructive jaundice during our study as the changes in the hepatocyte structures and changes in the portal structures. When we compared the group I and obstructive jaundice group in terms of these changes; it was observed that cholestasis, sinusoidal dilatation, kupffer cell increase, parenchymal PNL increase, portal edema and dilatation, portal PNL infiltration and ductal proliferation increased significantly. This was statistically significant as well ($p<0.05$) and not in concordance with the literature in terms of the histopathological findings detected [16-18]. When obstructive jaundice groups were compared in consideration with these criteria on the other hand, no difference observed except the slight increase observed in group which was cured with beta-glucan (group III). These results were not statistically significant ($p>0.05$). The failure of beta-glucan, identified as the immune system activator, to cause significant increase in the chuffer cells seems contrary however, since the phagocytic index of the existing cells were not assessed, we think that it is not possible to make a certain judgment.

During the studies made with obstructive jaundice, when the histological changes were identified in the structure of the intestine wall, it was seen that the terminal ileum is the region which was affected every time. No significant changes observed histopathologically in terminal ileum between all three groups ($p<0.05$). It is not in contrary with the literature that finding the beta-glucan cure reduces the bacterial translocation while makes nearly no positive change in the terminal ileum. In this study in which Aldemir et al. [19] conducted on the effect of immune system activation on bacterial translocation in obstructive jaundice, it has been detected that polyclonal immunoglobulin's reduce the bacterial translocation by neutralizing the endotoxins [18].

As a result, Beta-glucan reduces the bacterial translocation in obstructive jaundice. It decreases the biological parameters in statistically significant levels. No positive effects

on the histopathological findings in the liver and ileum have been detected. In case that phase III study results are the same in light with the results obtained, it is thought that beta-glucan can be used routinely in a clinic and be effective on decreasing the morbidity and mortality rates of the patients suffering from obstructive jaundice.

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