EXPERIMENTAL STUDY

The protective role of probiotics in sepsis-induced rats

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ABSTRACT

BACKGROUND: Probiotic ingestion is associated with an increase in intestinal flora of useful bacteria, which contributes to the known protective effects it has on the intestinal barrier and thereby reducing infection. The present study aims to investigate the protective effect of Lactobacillus rhamnosus gg (LGG) as an important probiotic with gastrointestinal barrier strengthening effect in sepsis.

METHODS: Our study was conducted in the Animal Experiments Laboratory after obtaining ethicalapproval to conduct this study. Twenty-four rats were randomly divided into threegroups and group 1 (control group n=8), group 2 (sepsis group, n=8), group 3 (sepsis + probiotic group, n=8) were planned as double-blind. LGG was used as a probiotic. For the sepsis model, *E. coli* (0111: B4) was injected intraperitoneally, and the rats were sacrificed 48 hours after treatment. Blood samples were collected from all animals before sacrification and sent to the biochemistry laboratory to evaluate oxidant and antioxidant parameters.

RESULTS: CRP values of Group 1 were significantly lower than Group 2, and CRP values of Group 3 were significantly lower. While total thiol levels of Group 2 were significantly lower than Group 1, total thiol levels of Group 3 were significantly higher than Group 2. There was no statistically significant difference between the groups for eNOS, GPX, PON1 and MDA levels.

CONCLUSION: Prophylactic use of probiotics, such as LGG to reduce bacterial translocation and strengthen the immune system, is an inexpensive and effective method of treatment, and we recommend the repetition of studies supported by prospective clinical trials.

Keywords: eNOS; MDA; probiotic; sepsis.

INTRODUCTION

Sepsis is a systemic inflammatory response to infection, often not compatible with age, affecting approximately 31.5 million patients worldwide.[1,2] Sepsisis thought to account for about 17% of hospital mortality.[3] Recently, a multinational intensive care unit (ICU) study, which involvedmore than 14,000 patients in more than 1200 ICUs, found that 51% of patients were infected, and 71% were on antibiotics in the survey. In this study, antibiotics used to treat infections ledto loss of gastrointestinal (GI) microbiota and potentially to the overgrowth of pathogens.The disruption of the intestinal barrier with flora wasthe main factor in the development of sepsis.[4]

In recent years, randomized, double-blind studies have shown that probiotics administered to patients reduce infection rates.[5,6] The degradation of the intestinal barrier and increased permeability in sepsis is considered one of the most important factors that worsen the situation and probiotics are believed to be protective.[7] Lactobacillus rhamnosus GG (LGG) is one of the most studied probiotics on this subject.[8]

Investigating the protective effect of LGG, which is an important probiotic with gastrointestinal barrier strengthening effect on sepsis in prophylactic use, was this study's aim.

For this, we have shown that sepsis-induced C reactive protein (CRP) as an inflammatory indicator, Malondialdehyde (MDA)and endothelial nitric oxide synthase (eNOS) as a marker of oxidation, and we measured the total thiol level of antioxidant molecules, glutathione peroxidase (GPx) and PON1 activity from the 3 Paraoxonases (PON) gene family to evaluate the efficacy of probiotics.

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MATERIALS AND METHODS

After obtaining the ethical approval from Adnan Menderes University Animal Experiments Ethics Committee, our study was conducted in the animal experiments laboratory. Twenty-four young, healthy male Wistar-Albino rats weighing between 250–300 grams were used in thisstudy. The rats were kept in wire cages, 12 hours of light and 12 hours of dark circadian rhythm before the experiment and their temperature was kept at 20–25°C, and the rats were randomized inthreegroups.

Group1. (Control group, n=8): Rats were given standard feed and water for 10 days. At the end of the procedure, blood was taken for biochemical examination.

Group 2 (Sepsis group, n=8): Rats were fed with standard feed and water for 10 days. Experimental sepsis with *E. coli* was induced and the rats were kept for twodays, and at the end of twodays, blood was taken for biochemical examination, and rats were sacrificed.

Group 3 (Sepsis + Probiotic group, n=8): Standard feed and water were given to rats for 10 days with LGG orogastric tube at a dose of 1x10-7 CFU/day. Experimental sepsis with *E. coli* was then induced and rats were kept for twodays, and at the end of twodays, blood was taken for biochemical examination, and rats were sacrificed.

Blood samples were centrifuged in biochemical tubes and eNOS, CRP, GPX, total thiol, MDA, PON1 tests were studied in each group and compared statistically.

Sepsis Model

Lipopolysaccharide derived from *E. coli* 0111: B4 serotype was injected intraperitoneally at a dose of 15mg/kg for 48 hours.

RESULTS

When the groups' CRP values were examined, the average value of Group 1 was 34.10, the average value of Group 2 was 70.40, and the average value of Group 3 was 39.90. While the Group 1's value was considerably lower than Group 2's value, Group 3's CRP values were considerably lower than Group 2's CRP values (p=0.00), Gpx values were 61.95 for Group 1, 63.27 for Group 2, and 78.77 (pg/ml) for Group 3. No statistically significant difference was found between the groups (Fig. 1). Althoughthe mean values of eNOS were 55.70, 59.40 and 53.60 for all threegroups, no statistically significant difference was found between them. Malondialdehyde (MDA) values were 15.60 in Group 1 and 28.50 and 23.82 in Group 2 and 3, respectively (p=0.44). Total thiol values were 786.58 in Group 1, 589.30 in Group 2 and 870.96 in Group 3. While the Group 2's values decreased significantly compared to Group 1, Group 3's values increased considerably compared to Group 2 (p=0.037) (Fig. 2). PON1 values were 0.29, 0.39, and 0.60 in all threegroups, but there was no statistically considerable difference was among the groups (Fig. 3).

DISCUSSION

According to 2016 data of the 3rd International Sepsis consensus, sepsis causing multiple organ failure continues to be

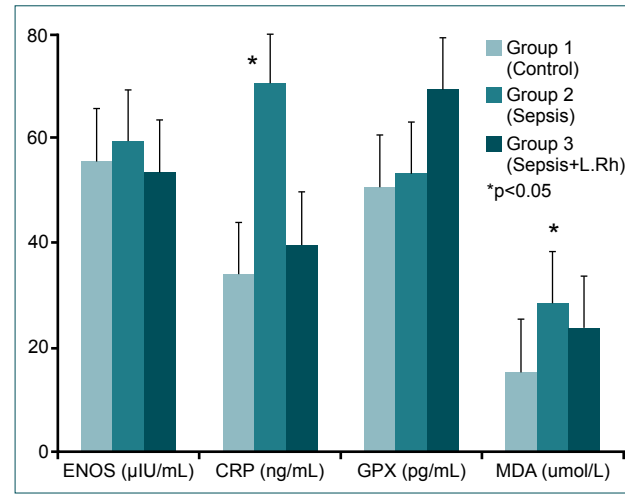

Figure 1. eNOS, CRP, GPX, MDA changes in groups. *P<0.05.

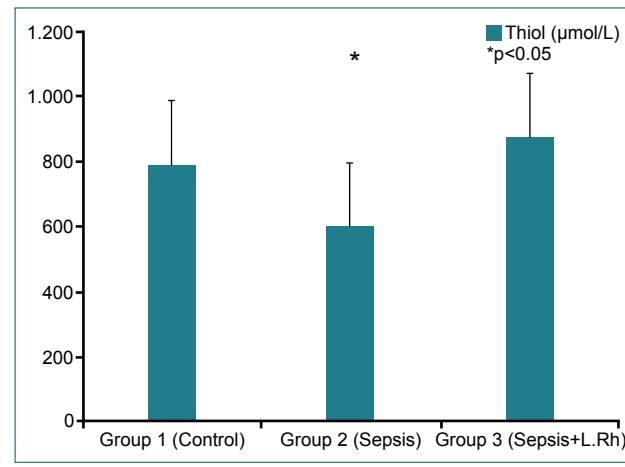

Figure 2. Total thiol (SH) µmol/l changes in groups. *P<0.05

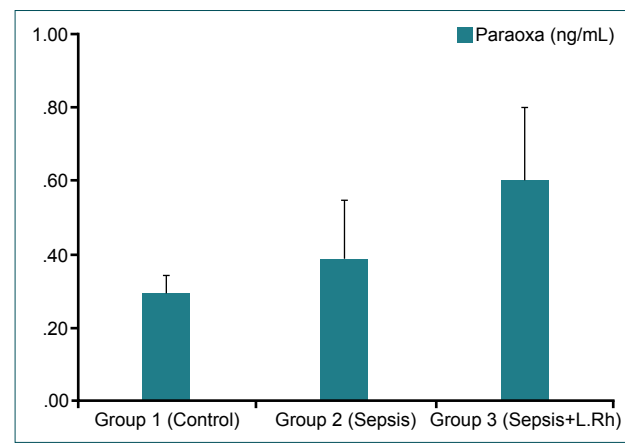

Figure 3. Paraoxa (ng/mL) changes in groups. *P>0.05.

a severecause of mortality in patients who still have an infection in hospitals. Giventhe difficulties and complications of sepsis treatment, it is clear that preventive measures should be taken for patients at risk of sepsis. Strengthening the intestinal flora with probiotic support of the patients in the risk group and may reduce sepsis occurrence and possible complications.

Popular studies are currently being conducted with many agents on sepsis, one of which is on probiotics that ensure the integrity of the intestinal barrier and strengthen the host immune mechanism.[9,10] In the study conducted by Panpetch et al.,[11] it is reported that LGG had a positive effect on sepsis, especially by reducing bacterial translocation. Mailänder-Sánchez et al.[12] reported that LGG reduces nosocomial candida infections and thus sepsis, while Kane et al.[13] reported that bacterial permeability and necrotizing enterocolitis risk in neonatal infants.[1,12] In our study on this probiotic, which has an important effect, we compared its effectiveness using various markers and found that it provides significant protection between the probiotic given before sepsis and the sepsis group. We think that this protection obtained in these rats under probiotic prophylaxis may be due to the strengthening effects of the intestinal barrier in parallel with other studies.

The use of prophylactic antibiotics to prevent infection in many pre-operative or intensive care patients is still widespread.[4] However, with this method, intestinal flora of many patients who already have nutritional problems will be further deteriorated, and barrier function will be weakened. This will increase the patient's progression to sepsis ata predictable rate. However, initiation of probiotic supplementation in patients in the risk group whose ICU or oral intake is impaired will strengthen intestinal flora and intestinal barrier function and at least reduce the course of sepsis.In another study of Ávila et al.,[14] it was shown that LGG administered before sepsis formation plays a protective role in rats. In the same study, it was concluded that early initiation of LGG, administeredbetween 7 and 15 days before sepsis formation, has more positive results. In our experimental study, we demonstrated that prophylactic use of LGG, parallel to findings of Ávila's study, mightbe protective against sepsis. In our study, we demonstrated that prophylactic use of LGG mightbe protective due to sepsis.

CRP is a rising protein in stress, chronic inflammation and sepsis. In the sepsis group, CRP significantly increased, and in the prophylactic group, there was a decrease in CRP value.[15] In the study conducted by Rabha et al.,[16] they induced experimental sepsis in rats and treated them with an agent called Kaempferol. MDA and eNOS were high in sepsis groups and low in treatment groups and reported that the agent was effective in treatment. In our study, these markers, which wereoxidative stress markers, were higher in the sepsis group and low in the prophylactic probiotic group. This finding suggeststhat the protective effect of LGG in sepsis provides the level of protection that can be achieved with treatment in some studies.

Although PON1 activity was found to contribute to the antioxidant system by reducing the lipidperoxide content of macrophages and increasing the level of glutathione,[17] we did not find such a relationship in our study. GPx is considered to be an indirect marker of tissue antioxidant capacity.[18] GPX values are expected to be lower in sepsis-generated groups. In our study, GPx levels were higher in Group 3. This result may suggest that there is an increase in the antioxidant capacity of the tissue with the given LGG and that the tissue is protected against oxidant stress markers.

It is said that probiotics have antioxidant effects, and can therefore, be used to treat many inflammatory diseases.[19–21] Total thiol is an important antioxidant marker of sulfhydryl group.[22] In a retrospective pilot study conducted by Molina et al.,[23] Thiol levels were higher in the control group compared to the septic group. In our study, the other antioxidative stress markers of control and prophylactic groups were higher compared to the sepsis group. This shows that the protective effects of LGG in sepsis.

As a result, sepsis still appears to be a public health problem with high mortality in intensive care units and hospitals. Treatment is both difficult and costly. Prophylactic use of probiotics, such as LGG, is an effective and cost-effective treatment modality.We believe that these studies should be repeated and supported by prospective clinical studies.

Ethics Committee Approval: Adnan Menderes University Animal Experiments Local Ethics Committee granted approval for this study (date: 20.02.2018, number: 64583101/2018/032).

Peer-review: Internally peer-reviewed.

Authorship Contributions: Concept: M.Y., A.O.E.; Design: M.Y., A.O.E.; Supervision: A.O.E.; Data: A.O.E.; Analysis: M.Y.; Literature search: M.Y.; Writing: M.Y., A.O.E.; Critical revision: M.Y., A.O.E.

Conflict of Interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZET*

Sepsis oluşturulan sıçanlarda probiyotiklerin koruyucu rolü

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AMAÇ: Oral alınan probiyotikler ile bağırsaklarda faydalı bakterilerin artırıldığı ve bu sayede bağırsakların enfeksiyonlar için koruyucu bariyer gücünün arttığı bilinmektedir. Bağırsak bariyer gücünün azalması ise oluşacak sepsiste tablonun kötüleşmesinde en önemli parametrelerden birisi olduğuna inanılmaktadır. Bağırsak bariyer gücünün artırılmasında birçok probiyotiğin etkili olduğu gösterilmiştir. Lactobacillus rhamnosus GG (LGG) bu konuda çalışılmış probiyotiklerden biridir. Bu çalışmanın amacı sepsis üzerine gastrointestinal bariyer güçlendirici etki için önemli bir probiyotik olan LGG'nin koruyucu etkisini araştırmaktır.

GEREÇ VE YÖNTEM: Çalışmamız Adnan Menderes Üniversitesi Hayvan Deneyleri Etik Kurulu onayı alındıktan sonra hayvan deneyleri laboratuvarında yapıldı. Çalışmada ağırlıkları 250–300 gram arasında değişen 24 adet genç sağlıklı erkek Wistar-Albino sıçan kullanıldı. Sıçanlar, deney öncesi tel kafeslerde, 12 saat aydınlık 12 saat karanlık sirkadiyen ritimde ve sıcaklığı 20–25 °C'de tutuldu. Yirmi dört sıçan rastgele olarak üç gruba ayrıldı ve Grup 1 (kontrol grubu, n=8), Grup 2 (sepsis grubu, n=8), Grup 3 (sepsis + probiyotik grubu, n=8) olarak çift kör şeklinde planlandı. Probiyotik olarak LGG 1x10–7 CFU/gün kullanıldı. Sepsis modeli için *E. coli*'nin serotiplerinden (0111: B4) ekstrakte edilen lipopolisakarit, 15 mg/kg'lık bir dozda intraperitoneal olarak enjekte edildi ve sıçanlar işemden 48 saat sonra sakrifiye edildi. Sakrifikasyon öncesi tüm hayvanlardan kan örnekleri alındı ve bu örnekler biyokimya laboratuvarına oksidan ve antioksidan parametreler değerlendirilmek üzere gönderildi.

BULGULAR: Grup 1'in CRP değeri Grup 2'den, Grup 3'ün de CRP değerleri Grup 2'den anlamlı olarak düşük saptandı, Grup 2'nin total tiyol seviyeleri Grup 1'e göre anlamlı derecede düşük iken, Grup 3'ün total tiyol seviyeleri Grup 2'den anlamlı derecede yüksek saptandı. eNOS, GPX, PON1 ve MDA düzeyleri için ise gruplar arasında istatistiksel olarak anlamlı bir fark yoktu.

TARTIŞMA: Bakteriyel translokasyonu azaltmak ve bağışıklık sistemini güçlendirmek için LGG gibi probiyotiklerin proflaktik olarak kullanımı, tedavi için ucuz ve etkili bir yöntemdir ve bu çalışmaların tekrarlanarak ileriye yönelik klinik çalışmalarla desteklenmesini öneriyoruz.

Anahtar sözcükler: eNOS; MDA; probiyotik; sepsis.

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