Kafkas Univ Vet Fak Derg
26 (6): 801-806, 2020
DOI: 10.9775/kvfd.2020.24504

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

The Protective Effect of Lactoferrin on Adenosine Deaminase, Nitric Oxide and Liver Enzymes in Lipopolysaccharide-Induced Experimental Endotoxemia Model in Rats [1]

Canan GULMEZ [1,a] Kezban YILDIZ DALGINLI [2,b] Emine ATAKISI [3,c] Onur ATAKISI [4,d]

[1] *This work was supported by Kafkas University Scientific Research Projects Coordinator (Grand number: 2017-FM-79), Turkey*

[1] Department of Pharmacy Services, Tuzluca Vocational High School, Igdir University, TR-76000 Igdir - TURKEY
[2] Department of Chemistry and Chemical Processing Technologies, Kars Vocational High School, Kafkas University, TR-36000 Kars - TURKEY
[3] Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, TR-36000 Kars - TURKEY
[4] Department of Chemistry, Faculty Science and Letter, Kafkas University, TR-36000 Kars - TURKEY
ORCIDs: [a] 0000-0003-3253-1407; [b] 0000-0002-1483-348X; [c] 0000-0002-5685-1870; [d] 0000-0003-1183-6076

Article ID: KVFD-2020-24504 Received: 30.04.2020 Accepted: 12.10.2020 Published Online: 13.10.2020

How to Cite This Article

Gulmez C, Yildiz Dalginli K, Atakisi E, Atakisi O: The protective effect of lactoferrin on adenosine deaminase, nitric oxide and liver enzymes in lipopolysaccharide-induced experimental endotoxemia model in rats. *Kafkas Univ Vet Fak Derg,* 26 (6): 801-806, 2020. DOI: 10.9775/kvfd.2020.24504

Abstract

In this study, the effect of lactoferrin (LF) on adenosine deaminase (ADA) activity, nitric oxide (NO) and liver enzyme levels were investigated in lipopolysaccharide (LPS)-induced experimental endotoxemia model in rats. Forty Sprague Dawley female rats were divided into four groups as control, LF (20 mg/kg, i.p.), LPS (20 µg/kg, i.p.) *(E. coli* type 0111:B4) and LF+LPS (20 mg/kg LF+20 µg/kg LPS, i.p.). For a week, LF was given daily, while LPS was given a single dose. Liver adenosine deaminase, nitric oxide and liver enzymes (aspartate aminotransferase 'AST', alanine aminotransferase 'ALT' and gamma glutamyltranspeptidase 'GGT') levels of animals sacrificed six hours after LPS injection were determined. It was found that ADA activity, considered to be a marker of cellular immunity, and NO levels increased in LPS-induced endotoxemia and LF supplementation decreased these levels significantly (P<0.001). There was no statistically significant difference between liver ALT, AST and GGT activity levels of all groups, but serum AST and GGT activity levels were significantly higher (P<0.001 and P<0.05, respectively) in the LPS group than in the other groups, and LF supplementation significantly reduced these enzyme levels. As a result, it can be said that LPS increases ADA synthesis and NO release and LF acts as an anti-inflammatory and immunosuppressor in stimulating immune response.

Keywords: *Lactoferrin, Lipopolysaccharide, Endotoxemia, Adenosine deaminase, Nitric oxide*

Ratlarda Lipopolisakkarit İndüklü Deneysel Endotoksemi Modelinde Adenozin Deaminaz, Nitrik Oksit ve Karaciğer Enzimleri Üzerine Laktoferrinin Koruyucu Etkisi

Öz

Bu çalışmada, ratlarda lipopolisakkarit (LPS) ile indüklenen deneysel endotoksemi modelinde laktoferrin (LF)'in adenozin deaminaz (ADA) aktivitesi, nitrik oksit (NO) ve karaciğer enzim düzeyleri üzerine etkisi araştırılmıştır. Çalışmada 40 adet Sprague Dawley dişi rat kontrol, LF (20 mg/kg, ip), LPS (20 µg/kg, ip) *(E. coli* tip 0111: B4) ve LF+LPS (20 mg/kg LF+20 µg/kg LPS, ip) olmak üzere dört gruba ayrıldı. Bir hafta boyunca LF hergün verilirken, LPS tek doz uygulandı. LPS enjeksiyonundan 6 saat sonra sakrifiye edilen hayvanların karaciğer ADA aktivitesi, NO düzeyleri ve karaciğer enzim (aspartat aminotransferaz 'AST', alanin aminotransferaz 'ALT' ve gama glutamiltranspeptidaz 'GGT') aktiviteleri belirlendi. Hücresel immunitenin bir belirteci olan ADA aktivitesi ve NO düzeyleri LPS indüklü endotoksemide arttı ve LF takviyesi bu düzeyleri önemli şekilde azalttı (P<0.001). Tüm grupların karaciğer ALT, AST ve GGT aktivite düzeyleri arasında istatistiksel olarak önemli bir fark görülmezken, LPS grubunda serum AST ve GGT aktivite düzeyleri diğer gruplara kıyasla önemli şekilde daha yüksekti ve laktoferrin takviyesi bu düzeyleri önemli ölçüde azalttı (sırasıyla P<0.001 ve P<0.05). Sonuç olarak, LPS'nin ADA sentezini ve NO salımını arttırdığı ve LF'nin immün yanıtı uyarmada antienflamatuar ve immün baskılayıcı görev gördüğü söylenebilir.

Anahtar sözcükler: *Laktoferrin, Lipopolisakkarit, Endotoksemi, Adenozin deaminaz, Nitrik oksit*

Correspondence
+90 476 2230017/3426 Fax: +90 476 2230010
canan_glm@hotmail.com, canan.gulmez@igdir.edu.tr

INTRODUCTION

Lactoferrin (LF) is an iron-binding single-chain glycoprotein of the transferrin gene family, weighing 80-kDa [1]. Lactoferrin is a multifunctional protein that plays a role in many physiological processes such as regulation of iron metabolism, defense against a wide range of microbial infections, regulation of cell growth and differentiation, cancer prevention and metastasis [1,2]. Also, it is used in both health and industry because of its antiinflammatory, antifungal, antibacterial, antimicrobial, immunomodulatory, antioxidant and even antineoplastic properties.

Systemic status of bacterial infection is called sepsis and gram (-) bacterial endotoxin in circulation is called endotoxemia. As a systemic inflammatory response to infection, sepsis describes a complex clinical syndrome. The sepsis progressively results in septic shock, multiple organ failure, and ultimately death. Bacteria, viruses, fungi and parasites can cause septic shock and result in a mortality rate of around 20-80%. Lipopolysaccharide (LPS) is a group of substances in the glycolipid structure obtained from the cell wall of gram-negative bacteria, used in experimental animal septic shock models [3].

Lactoferrin binds to endothelial cells with specific affinity and crosses from the apical to the abluminal surface without being degraded, possibly via caveolae. Although the effect of LF on endothelial cells is not yet known, LF increases the production of nitric oxide, a vasodilating agent produced from endothelial cells, from macrophages. Adenosine deaminase (ADA), a key enzyme in purine metabolism, is considered to be a marker of cellular immunity. ADA activity has been demonstrated during the activation period of autoimmune diseases and has been reported to be associated with highly impaired cellular and humoral immunity, developmental retardation and serious infections [4,5]. LF can prevent the development of tissue damage caused by inflammation and the release of pro-inflammatory cytokines and reactive oxygen species, thanks to its antimicrobial activity and the ability to bind components of bacterial cell walls (LPS) or their receptors [6]. The mechanism of interaction between LF and LPS has not yet been understood. New studies are needed to better understand its activity and interactions to enable safe use of LF in clinical and veterinary medicine. In this study, it was aimed to investigate the effect of LF on adenosine deaminase, nitric oxide and liver enzymes in LPS-induced experimental endotoxemia model in rats.

MATERIAL and METHODS

Animal

The required permission for the study was obtained by the Local Ethics Committee of Kafkas University Animal Experiments (Approval No: KAU-HADYEK/2015-018). Housing, maintenance, and experimental procedures were carried out at Kafkas University Experimental Animals Production and Experimental Research Center. During the experiment, the subjects were placed in cages in a 12-h light and 12-h dark cycle and to reach food and water whenever they wanted. The rats were housed in plastic cages with chip shafts and five rats in each cage during the trial period.

Experimental Design

Forty Sprague Dawley female 6 months old rats were used in the study. Subjects were randomly distributed to four groups and rats were weighed for dose calculations. The rats were as follows: Control (n=10), LF (n=10), LPS (n=10), and LF + LPS (n=10) groups. During the experiment, saline was administered intraperitoneally to the control group daily for one week. Group II was given LF (20 mg/kg i.p.) once a day for one week. Group III received LPS (20 µg/kg i.p. *(E. coli* type 0111:B4)) single dose. Group IV was given LF (20 mg/kg i.p.) once a day for one week with lipopolysaccharide (20 µg/kg i.p.) single dose. Six hours after a single dose of lipopolysaccharide injection, animals were sacrificed under anesthesia and the intracardiac blood samples from animals were centrifuged at 3000 rpm and +4°C for 15 min to separate the serum. Liver tissues were stored to be used in subsequent analyzes.

Determination of Nitric Oxide Level

Nitric oxide concentrations in liver tissue were determined using a spectrophotometer (PowerWave XS, BioTek, Vermonts, USA) by the method of Miranda et al.[7]. The samples were de-proteinized with 10% zinc sulphate. Total NO (nitrate and nitrite) concentrations were measured colorimetrically by acidic Griess reaction via reaction involving reduction of nitrate to nitrite by vanadium (III) chloride.

Adenosine Deaminase Activity Assay

Adenosine deaminase activity in liver samples was performed according to the method of Giusti and Galanti [8]. Adenosine, used as a substrate, was incubated with the sample at 37°C for 30 min. The ammonia formed forms blue indophenol in the presence of sodium hypochlorite and phenol in alkaline medium. In this experiment where sodium nitroprusside has a catalyst effect, the ammonia concentration is directly proportional to the absorbance of indophenol. Briefly, 1 mL of phosphate buffer and 50 µL of distilled water into the reagent blind tube, 1 mL ammonium sulfate and 50 µL distilled water into the standard tube, 1 mL adenosine solution and 50 µL sample into the sample tube, 1 mL of adenosine solution was added to the sample blind tube. After the test tubes were closed, they were incubated in a 37°C incubator for 1 h and 3 mL of phenol/nitroprusside solution was added. After adding 0.05 mL of sample to the sample blind tube, 3 mL of alkaline hypochlorite solution was added to all tubes. The tubes were closed again and incubated at 37°C for 30 min. After incubation, the absorbance of the

tubes against the blind was read at 625 nm. One unit of ADA activity was defined as the amount of enzyme that releases 1 µmol of ammonia from adenosine per minute. ADA activities of the groups were expressed as U/mg protein, divided by the amount of protein.

Determination of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Gamma Glutamyltranspeptidase (GGT) Activities

The serum and liver aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyltranspeptidase (GGT) activities were determined colorimetrically using commercial kits (ERBA Diagnostics, Miami, Florida, ABD).

Determination of Total Protein

Liver total protein concentration was determined by the Bradford method using bovine serum albumin (BSA) as the standard at 595 nm.

Statistical Analysis

Statistical analysis of the data obtained from the study was done using SPSS Windows 16.0 package program (SPSS Inc.). Mean values between groups were determined by one-way analysis of variance (ANOVA) and differences between groups by Duncan test. Data (n=10) are expressed as mean±SD values.

RESULTS

Liver NO levels increased significantly in LPS group compared to other groups (P<0.001) *(Fig. 1)*. The NO levels in control, LF and LF+LPS groups were statistically similar. Liver ADA activity levels, considered to be a marker of cellular immunity, increased significantly in LPS group compared to other groups (P<0.001) *(Fig. 2)*. The ADA activity levels of other groups were statistically similar.

Serum AST and GGT activity levels were significantly higher in the LPS group than in the other groups, and LF supplementation significantly reduced these enzyme levels (P<0.001 and P<0.05, respectively). Serum ALT activity level was significantly higher (P<0.05) in the LPS group, but it was not statistically significant even though ALT activity decreased with lactoferrin supplementation. AST, ALT and GGT activities were significantly lower in the LF group compared to the LPS group *(Fig. 3-A)*. When liver ALT, AST

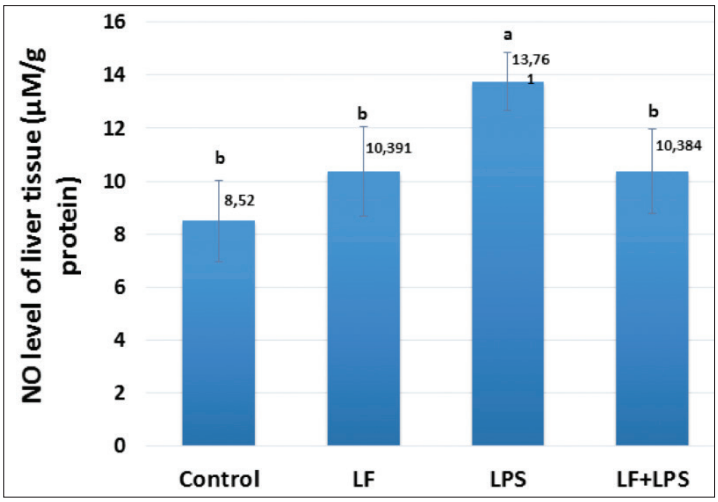

Fig 1. The effects of lactoferrin on liver nitric oxide levels in LPS-induced experimental endotoxemia model. Data (n=10) are expressed as mean±SD values. The difference between the groups indicated by different letters (a and b) is statistically significant (P<0.001)

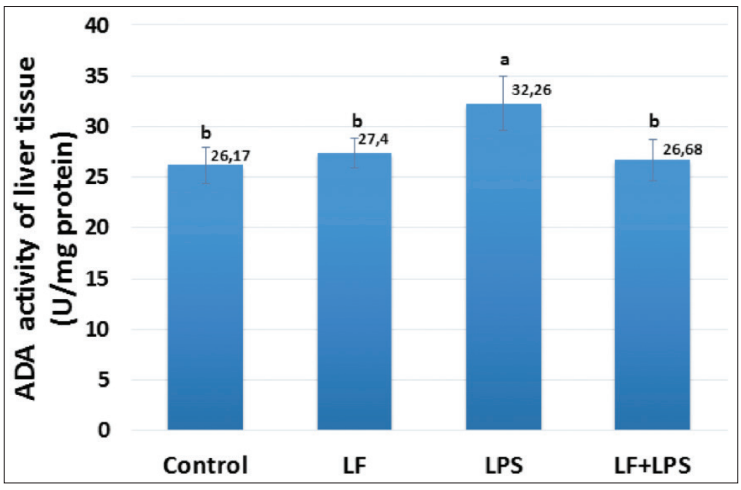

Fig 2. The effect of lactoferrin on liver adenosine deaminase activity levels in LPS-induced experimental endotoxemia model. Data (n=10) are expressed as mean±SD values. The difference between the groups indicated by different letters (a and b) is statistically significant (P<0.001)

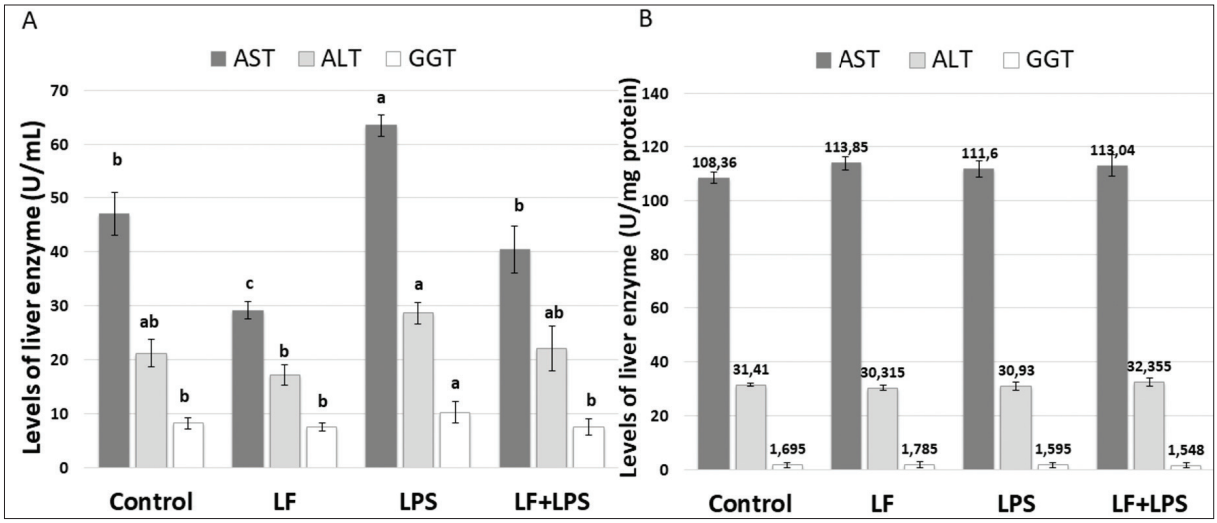

Fig 3. The effect of lactoferrin on AST, ALT and GGT activity levels in LPS-induced experimental endotoxemia model. **A:** Serum AST, ALT and GGT activity levels (P<0.05), **B:** Liver AST, ALT and GGT activity levels (Non-significant). Data (n=10) are expressed as mean±SD values. The diff erence between the groups indicated by different letters (a, b and c) is statistically significant

and GGT activity levels of all groups were compared, it was not statistically significant *(Fig. 3-B)*.

DISCUSSION

Lipopolysaccharide, the main outer membrane component of gram-negative bacteria, causes many pathophysiological processes such as fever, hypotension, disseminated intravascular coagulation, and multiple organ failure. When the history of sepsis is examined, there are many diagnostic approaches from microbiological evaluation of infection to the use of prognostic biomarkers. The main therapeutic approach to sepsis in recent years is intravenous immunoglobulin therapy and appropriate antibiotic use. LF binding to LPS causes bacterial surface damage and the binding to free LPS neutralizes its pro-inflammatory effects. Thus, LF is a potential adjunctive agent for inflammation and endotoxemia/sepsis [9]. LF, a major component produced from LF neutrophils, increases in bacterial infection sites [10,11]. Studies have reported that there is a direct binding between LF and LPS in LF-mediated LPS inhibition based on molecule-molecule interactions [12-15]. LF has been reported to interfere with intracellular events leading to nuclear factor kappa B (NF-κB) activation, inhibiting cytokine production and inhibiting LPS-induced cytokine gene expression within 2 h by inhibiting NF-κB binding to DNA [3]. LF from porcine-20 (LF-20), one of the anti-microbial peptides containing 20 amino acids, has been reported to inhibit the response to LPS-induced inflammation by inhibiting MyD88/NF-κB and MyD88/MAPK signaling pathways [16]. Although LF is a glycoprotein that plays an important role in immunomodulation, its mechanism of action has not been fully explained yet. In this study, the effect of LF on ADA activity, NO and liver enzyme levels were investigated in an experimental endotoxemia model in rats.

Adenosine deaminase, a key enzyme in purine metabolism, is an enzyme that catalyzes the conversion of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxynosine, respectively. ADA activity in humans is higher in thymus, spleen, lymph node, bone marrow and peripheral blood lymphocytes. ADA1, which has a low molecular weight of two isoforms, is found in the thymus, erythrocyte and heart, while ADA2 in high molecular weight is found in the liver, kidneys and intestines. ADA2 is also the dominant enzyme form in human blood plasma. ADA activities detected in T lymphocytes are higher than B lymphocytes, but there is a significant increase in ADA activity during T cell differentiation [17]. The ADA reaction is of great importance in controlling the intracellular concentration of adenosine. Since this reaction catalyzed by ADA is irreversible, it constitutes the control step in the destruction of adenosine. Increasing intracellular levels of adenosine and deoxyadenosine is toxic, and ADA plays an important role in regulating the intracellular levels of these nucleosides [18]. It is known that ADA activity increases during the mutagenic defense of the cell and is responsible for the proliferation and differentiation of lymphocytes and monocytes. ADA inhibition has been associated with lower plasma-mediated reactive oxygen species (ROS) production. In one study, incubation of neutrophils in plasma has been reported to increase total ADA activity 10 times from 1.3 U/mL to 12 U/mL [19]. ADA activity has been demonstrated in the autoimmune disease period such as typhoid, infectious mononucleosis, brucellosis, acute pneumonia, tuberculosis, sarcoidosis, liver diseases, acute leukemia, cancer, rheumatoid arthritis, systemic lupus erythematosus, psoriasis and Behçet disease [4]. ADA deficiency has been reported to be associated with lymphopenia, severely impaired cellular and humoral immunity, developmental retardation, and severe infections [5].

Nitric oxide is a signaling molecule that acts as an endocrine molecule in recent years [20]. During inflammation, NO

is increased by inducible nitric oxide species (iNOS) and activated by cytokines and reacts with superoxide anions leading to formation of peroxinitrite radical [21] and free radicals [22]. In a study conducted on CaCo-2 and RAW 246.7 cell lines, it was reported that recombinant LF (rLF) and its hydrolysates were effective in decreasing interleukin 8 and production of ROS in relieving the response to LPS-induced inflammation. Also, rLF did not affect the NO content in CaCo-2 monolayers [19]. The studies reported here indicate that recombinant human LF is effective in modulating the inflammatory responses of CD14+ and CD16+ macrophages during low and high stimulating conditions [23]. NO levels and ADA activity were increased in patients with *Entamoeba coli*, *Enterobius vermicularis*, *Giardia intestinalis*, *Demodex spp.*, hydatid cyst and *Toxoplasma gondii* serum positive compared to control [24].

Adenosine and its receptors have been reported to increase nitric oxide production in LPS -treated RAW 264.7 cells. Increased NO production by adenosine was inhibited by adenosine uptake inhibitors, such as dipyridamole, S(4-nitrobenzyl)-6-thioinosine and S(4-nitrobenzyl)-6-thio-guanosine [25]. In doxorubicin-induced cardio toxicity, ADA and NO levels were observed to be significantly higher than control. Erdosteine, which is given as a protector in cardiac toxicity, did not change the ADA activity, but significantly decreased the NO level in rats [26]. Akinyemi et al.[27] show that significantly increased adenosine deaminase activity with cadmium-induced renal toxicity in rats decreased curcumin. It was observed that cadmium toxicity decreased kidney nitric oxide level and this level increased with curcumin supplementation. The ADA activity and NO levels were examined by giving glutathione to rabbits at certain hours (0. 3. 6. and 12.) and no change was observed in plasma ADA levels. However, NO levels decreased significantly at 3. and 6. hours with significant glutathione supplementation [28]. In the study, it was found that ADA activity and NO levels increased in LPS-induced endotoxemia model and LF supplementation decreased these levels significantly ($P<0.001$). It can be said that LPS increases ADA synthesis and NO release and LF acts as an anti-inflammatory and immunosuppressor in stimulating immune response. Excessive amounts of neutrophils, macrophages, lymphocytes and eosinophils are produced as a response to microorganisms [20]. LPS stimulates immune responses and proinflammatory mediator secretion by monocytes, macrophages, and neutrophils, which are recruited into specific host tissues by LPS exposure [29,30]. The levels of TNF-α, IL-1b, IL-6, IL-8 and NO have been shown to increase during infection [20]. In a previous study, it was found that total oxidant capacity and NO was higher after the injection of LPS and total antioxidant capacity was demonstrated with a reduction in response to LPS in mice [31].

The alanine aminotransferase 'ALT', alkaline phosphatase (ALP), and GGT enzymes are used to evaluate the functions of the liver and high levels of these enzymes increase risk of disease and all-cause mortality. In clinical practice, they are used as an indicator of damage in a spectrum ranging from inflammation to necrosis rather than indicating liver function [32]. In the presented study, there was no statistically significant difference between liver ALT, AST and GGT activity levels of all groups, but serum AST and GGT activity levels were significantly higher in the LPS group than in the other groups, and LF supplementation significantly reduced these enzyme levels. AST, ALT, and GGT activities were significantly lower in the LF group comparing to the LPS group.

As a result, LF showed a protective effect by reducing ADA synthesis and NO release in the LPS-induced experimental endotoxemia model. LPS increases ADA activity and NO levels and LF plays a complex role in modulating the inflammatory response. The results showed that ADA and NO may have the potential to become a marker in endotoxemia.

Author Contribution

O.A and E.A. conceived the original idea, designed and supervised the project. C.G. and K.Y.D performed experiments. All authors wrote the manuscript.

Conflict of Interest

All authors declare that there is no potential conflict of interest.

Funding

This work was supported by Kafkas University Scientific Research Projects Coordinator (Grand number: 2017-FM-79), Turkey.

REFERENCES

1. Iglesias-Figueroa BF, Espinoza-Sánchez EA, Siqueiros-Cendón TS, Rascón-Cruz Q: Lactoferrin as a nutraceutical protein from milk, an overview. *Int Dairy J*, 89, 37-41, 2019. DOI: 10.1016/j.idairyj.2018.09.004

2. Moreno-Expósito L, Illescas-Montes R, Melguizo-Rodríguez L, Ruiz C, Ramos-Torrecillas J, de Luna-Bertos E: Multifunctional capacity and therapeutic potential of lactoferrin. *Life Sci*, 195, 61-64, 2018. DOI: 10.1016/j.lfs.2018.01.002

3. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL: Sepsis and septic shock. *Nat Rev*, 2:16045, 2016. DOI: 10.1038/nrdp.2016.45

4. Pormohammad A, Riahi SM, Nasiri MJ, Fallah F, Aghazadeh M, Doustdar F, Pouriran R: Diagnostic test accuracy of adenosine deaminase for tuberculous meningitis: A systematic review and meta-analysis. *J Infect*, 74 (6): 545-554, 2017. DOI: 10.1016/j.jinf.2017.02.012

5. Flinn AM, Gennery AR: Adenosine deaminase deficiency: A review. *Orphanet J Rare Dis,* 13:65, 2018. DOI: 10.1186/s13023-018-0807-5

6. Adlerova L, Bartoskova A, Faldyna M: Lactoferrin: A review. *Vet Med*, 53 (9): 457-468, 2008. DOI: 10.17221/1978-VETMED

7. Miranda KM, Espey MG, Wink DA: A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide.* 5, 62-71, 2001. DOI: 10.1006/niox.2000.0319

8. Giusti G, Galanti B: Colorimetric method. **In,** Bergmeyer HU (Ed): Methods of Enzymatic Analysis. Weinheim: Verlag Chemie, 315-323, 1984.

9. Esposito S, De Simone G, Boccia G, De Caro F, Pagliano P: Sepsis and septic shock: New definitions, new diagnostic and therapeutic approaches. *J Glob Antimicrob Resist*, 10, 204-212, 2017. DOI: 10.1016/j.jgar. 2017.06.013

10. Martins CA, Fonteles MG, Barrett LJ, Guerrant RL: Correlation of lactoferrin with neutrophilic inflammation in body fluids. *Clin Diagn Lab Immunol*, 2 (6): 763-765, 1995.

11. Wright DG, Gallin JI: Secretory responses of human neutrophils: Exocytosis of specific (secondary) granules by human neutrophils during adherence *in vitro* and during exudation *in vivo*. *J Immunol*, 123 (1): 285-294, 1979.

12. Caccavo D, Afeltra A, Pece S, Giuliani G, Freudenberg M, Galanos C, Jirillo E: Lactoferrin-lipid A-lipopolysaccharide interaction: Inhibition by anti-human lactoferrin monoclonal antibody AGM 10.14. *Infect Immun*, 67 (9): 4668-4672, 1999.

13. Elass-Rochard E, Legrand D, Salmon V, Roseanu A, Trif M, Tobias PS, Mazurier J, Spik G: Lactoferrin inhibits the endotoxin interaction with CD14 by competition with the lipopolysaccharide-binding protein. *Infect Immun*, 66 (2): 486-491, 1998.

14. van Berkel PHC, Geerts EJM, van Veen AH, Mericskay M, de Boer AH, Nuijens HJ: N-terminal stretch Arg2, Arg3, Arg4 and Arg5 of human lactoferrin is essential for binding to heparin, bacterial lipopolysaccharide, human lysozyme and DNA. *Biochem J*, 328, 145-151, 1997. DOI: 10.1042/bj3280145

15. Na YJ, Han SB, Kang JS, Yoon YD, Park SK, Kim HM, Yang KH, Joe CO: Lactoferrin works as a new LPS-binding protein in inflammatory activation of macrophages. *Int Immunopharmacol*, 4 (9): 1187-1199, 2004. DOI: 10.1016/j.intimp.2004.05.009

16. Zong X, Song D, Wang T, Xia X, Hu W, Han F, Wang Y: LFP-20, a porcine lactoferrin peptide, ameliorates LPS-induced inflammation via the MyD88/NF-κB and MyD88/MAPK signaling pathways. *Dev Comp Immunol*, 52 (2): 123-131, 2015. DOI: 10.1016/j.dci.2015.05.006

17. Balis ME: Adenosine deaminase and malignant cells. *Ann N Y Acad Sci*, 451, 142-149, 1985. DOI: 10.1111/j.1749-6632.1985.tb27105.x

18. Sauer A, Brigida I, Carriglio N, Aiuti A: Auto immun edysregulation and purine metabolism in adenosine deaminase deficiency. *Front Immunol*, 3:265, 2012. DOI: 10.3389/fimmu.2012.00265

19. Kälvegren H, Fridfeldt J, Bengtsson T: The role of plasma adenosine deaminase in chemoattractant-stimulated oxygen radical production in neutrophils. *Eur J Cell Biol*, 89 (6): 462-467, 2010. DOI: 10.1016/j.ejcb.2009.12.004

20. Cyr AR, Huckaby LV, Shiva SS, Zuckerbraun BS: Nitric oxide and endothelial dysfunction. *Crit Care Clin*, 36, 307-321, 2020. DOI: 10.1016/j.ccc.2019.12.009

21. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA: Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A*, 87, 1620–1624, 1990. DOI: 10.1073/pnas.87.4.1620

22. Pryor WA, Squadrito GL: The chemistry of peroxynitrite: A product from the reaction of nitric oxide with superoxide. *Am J Physiol*, 268, L699-L722, 1995. DOI: 10.1152/ajplung.1995.268.5.L699

23. Hwang SA, Kruzel ML, Actor JK: Recombinant human lactoferrin modulates human PBMC derived macrophage responses to BCG and LPS. *Tuberculosis*, 101, S53-S62, 2016. DOI: 10.1016/j.tube.2016.09.011

24. Kiran TR, Karaman U, Arici YK, Yildiz S: Comparison of malondialdehyde, nitric oxide, adenosine deaminase and glutathione levels in patients with *Entamoeba coli, Enterobius vermicularis, Giardia intestinalis, Demodex spp*. positive, hydatid cyst and *Toxoplasma gondii* serum positive. *Ann Med Res*, 26 (7): 1420-1425, 2019. DOI: 10.5455/annalsmedres.2019.05.236

25. Min HW, Moochhala S, Eng KH: Adenosine and its receptor agonists regulate nitric oxide production in RAW 264.7 macrophages via both receptor binding and its downstream metabolites-Inosine. *Life Sci*, 66 (19): 1781-1793, 2000. DOI: 10.1016/s0024-3205(00)00502-6

26. Fadillioglu E, Yılmaz HR, Erdogan H, Sogut S: The activities of tissue xanthine oxidase and adenosine deaminase and the levels of hydroxyproline and nitric oxide in rat hearts subjected to doxorubicin: Protective effect of erdosteine. *Toxicology*, 191 (2-3): 153-158, 2003. DOI: 10.1016/s0300-483x(03)00258-0

27. Akinyemi AJ, Onyebueke N, Faboya OA, Onikanni SA, Fadaka A, Olayide I: Curcumin inhibits adenosine deaminase and arginase activities in cadmium-induced renal toxicity in rat kidney. *J Food Drug Anal*, 25 (2): 438-446, 2017. DOI: 10.1016/j.jfda.2016.06.004

28. Atakisi O, Erdogan HM, Atakisi E, Citil M, Kanici A, Merhan O, Uzun, M: Effects of reduced glutathione on nitric oxide level, total antioxidant and oxidant capacity and adenosine deaminase activity. *Eur Rev Med Pharmacol Sci*, 14, 19-23, 2010.

29. Rietschel ET, Brade H: Bacterial endotoxins. *Sci Am*, 267 (2): 54–61, 1992. DOI: 10.1038/scientificamerican0892-54

30. Morrison DC, Ryan JL: Endotoxins and disease mechanisms. *Annu Rev Med*, 38, 417-432, 1987. DOI: 10.1146/annurev.me.38.020187.002221

31. Ilgun T, Yıldız Dalgınlı K, Gulmez C, Atakisi O: Changes in the levels of liver HSP70, plasma nitric oxide, and the antioxidative system in an experimentally induced endotoxemia mouse model and the role of reduced glutathione. *Turk J Biol*, 40, 1272-1277, 2016. DOI: 10.3906/biy-1601-75

32. Turati F, La Vecchia C: Liver enzymes and all-cause mortality: Open issues. *Liver Int,* 39 (8): 1389-1390, 2019. DOI: 10.1111/liv.14099