



# Effects of erythropoietin on bacterial translocation in a rat model of experimental colitis

Türkmen Arıkan<sup>1</sup>, Alper Akcan<sup>1</sup>, Yunus Dönder<sup>2</sup>, Zeki Yılmaz<sup>1</sup>, Erdoğan Sözüer<sup>1</sup>, Bahadır Öz<sup>1</sup>, Mehmet Baykan<sup>3</sup>, Mustafa Gök<sup>1</sup>, Bilgehan Poyrazoğlu<sup>4</sup>

<sup>1</sup> Department of General Surgery, Erciyes University School of Medicine, Kayseri, Turkey

<sup>2</sup> Clinic of General Surgery, Kilis State Hospital, Kilis, Turkey

<sup>3</sup> Clinic of General Surgery, Melikgazi Private Hospital, Kayseri, Turkey

<sup>4</sup> Clinic of General Surgery, Magnet Private Hospital, Kayseri, Turkey

## ABSTRACT

**Objective:** In this experimental study, it was aimed to assess the effects of erythropoietin (EPO) on bacterial translocation in a rat model of colitis.

**Material and Methods:** The rats were randomly assigned into control, colitis and EPO-treated groups (n= 8 in each group). Saline solution (NS) was administered to control rats via rectal route. A trinitrobenzene sulfonic acid and ethanol mixture (TNBS-E) was used to induce colitis in the experiment groups. No treatment was administered to colitis group after induction. Starting at one day after induction of colitis with TNBS-E, EPO (1000 IU/kg) was administered subcutaneously for three days to the rats in the EPO-treated group. Colonic inflammation was assessed by gross and microscopic examination on day five. Blood samples were obtained to evaluate bacterial translocation while hepatic, mesenteric tissue samples and mesenteric lymph node (MLN) samples were collected for tissue culture. Tissue myeloperoxidase (MPO) levels, and tumor necrosis factor alpha (TNF- $\alpha$ ) and endotoxin levels in the sera were studied.

**Results:** Significant gross and microscopic differences were found in the comparison between colitis and EPO-treated groups ( $p < 0.05$ ). MPO level was significantly lower when compared to the colitis group ( $p < 0.05$ ). Serum TNF- $\alpha$  and plasma endotoxin levels were significantly lower in the EPO-treated group than the colitis group ( $p < 0.05$ ). Bacterial translocation was lower in the liver, spleen, MLNs and systemic blood in the EPO-treated group when compared to the colitis group ( $p < 0.05$ ).

**Conclusion:** In TNBS-E-induced rat model of colitis, EPO significantly decreased inflammation and bacterial translocation based on histopathological, biochemical and microbiological parameters.

**Keywords:** Erythropoietin, experimental colitis, bacterial translocation

## INTRODUCTION

Inflammatory bowel diseases (IBD) is a group of diseases progressing with chronic inflammation in the gastrointestinal system (GIS). Ulcerative Colitis (UC) is a recurrent non-transmural inflammatory disorder confined to the colon while Chorn Disease (CH) is a recurrent disorder that is associated with the transmural inflammation of the whole gastrointestinal tract. Current hypothesis about the etiology of IBD postulates that the disease stems from immune system dysregulation in the gastrointestinal tract in patients with genetic predisposition (1). In patients with genetic predisposition, together with environmental factors, the excessive inflammation without control leads to the intestinal migration of the inflammatory cells, and progressive tissue damage develops due to the effects of cytokines in the gastrointestinal tract (GIT) (2).

IBD is associated with an increase in epithelial permeability resulting in chronic stimulation of the mucosal immunity by bacterial products. It is suggested that the increase in mucosal permeability may be the primary defect in IBD (3). Intestinal epithelial cells involve control mechanisms to inhibit inappropriate immune response activation. However, the bacterial products passing across the mucosal barrier directly contact with immune cells, promoting a classical immune response. The impaired response by mucosal immunity and cytokine release result in chronic mucosal damage (1). This may lead passage of enteric bacteria and bacterial substances into sterile extraintestinal sites through the intestinal wall.

Erythropoietin (EPO) is a glycoprotein growth hormone with a molecular weight of 34 kDa which stimulates the growth of erythroid progenitor cells (4). It is primarily produced in kidneys (4). It is released from the kidneys as a response to hypoxia and

**Cite this article as:** Arıkan T, Akcan A, Dönder Y, Yılmaz Z, Sözüer E, Öz B, Baykan M, Gök M, Poyrazoğlu B. Effects of erythropoietin on bacterial translocation in a rat model of experimental colitis. Turk J Surg 2019; 35 (3): 202-209.

### Corresponding Author

Türkmen Arıkan

E-mail: turkmenarikan@gmail.com

Received: 30.08.2018

Accepted: 08.10.2018

Available Online Date: 23.09.2019

© Copyright 2019 by Turkish Surgical Society Available online at www.turkjsurg.com

DOI: 10.5578/turkjsurg.4272

stimulates the hematopoietic system. EPO is produced in mammalian cell culture by recombinant DNA technology and is used for the treatment of anemia caused by renal failure or cancer chemotherapy (5).

Erythropoietin is a growth hormone which primarily stimulates the growth of erythroid progenitor cells. It has been shown that EPO decreases apoptosis in neurons in rat models (6). In experimental colitis models, EPO has been shown to decrease the effects of inflammatory bowel disease (7-9).

EPO has also been determined to decrease bacterial translocation in rats with induced obstructive jaundice (10). Another study in rat models has demonstrated that EPO administration after hemorrhagic shock enhances barrier function of the mucosa and reduces bacterial translocation (11).

However, there are no studies in the literature investigating EPO effects on bacterial translocation in experimental colitis model. Our study aimed to assess the effects of EPO on bacterial translocation in IBD, and anti-inflammatory effects of erythropoietin based on colitis pathophysiology.

## MATERIAL and METHODS

### Animals

The present study was conducted on 24 male Wistar-Albino rats aged 28-32 weeks (weighing 220-280 g). The rats were acclimatized over a week before the experiments. All rats were kept at 21°C and fed with standard rat chow. Prior to the experiments, the rats were fasted for 12 hours with only water allowed. This experimental study was conducted at the Hakan Çetinsaya Experimental and Clinical Research Center (ECRC) of Erciyes University School of Medicine. The study was approved by the Medical Faculty Ethics Committee.

### Experimental Design

The animals were randomly assigned into three groups (eight animals per group):

- Group 1 (control group): the rats in this group received saline solution (SS) via rectal route.
- Group 2 (colitis group): colitis was induced by TNBS-E administration and no treatment was given.
- Group 3 (EPO-treated group): EPO was administered after induction of colitis.

### Experimental Procedure

In all rats, sedation was achieved by halothane, and spontaneous respiration was maintained throughout the procedure. The animals were weighed after sedation. In order to induce colitis, a 3.5-F plastic cannula was inserted 5 cm into the rectum. Colitis was triggered by slow instillation of trinitrobenzene sulfonic acid (TNBS) (50 mg/kg) in 0.25 mL ethanol (50%) into the colon through this cannula. Then, the rats were placed in supine position until recovery from sedation in order to prevent leakage.

The control group received only 0.9% NaCl solution through the rectal cannula. The colitis group was administered TNBS (50 mg/kg) in 0.25 mL (50%) ethanol intracolonicly to induce colitis. In the EPO-treated group, starting at one day after the induction of colitis with intracolonicly administered TNBS-E, Erythropoietin Beta (Neorecormon, Roche, Mannheim, Germany) 1000 IU/kg was administered subcutaneously for three days. Identical amounts of subcutaneous sterile saline solution were simultaneously administered to the controls and rats in the colitis groups.

All injections were administered upon cleansing of the injection site with a 10% povidone-iodine antiseptic solution. All rats received standard rat pellets for five days. On day five after colitis induction, anesthesia was achieved with 50 mg/kg ketamine and 8 mg/kg xylazine via intraperitoneal route. A midline incision was made after removal of abdominal hair and skin preparation with 10% povidone-iodine. Blood sampling was performed from vena cava (3 mL) and portal veins (2 mL) for tumor necrosis factor alpha (TNF- $\alpha$ ), and plasma endotoxin assays and blood culture tests. Using sterile forceps, the liver, spleen and mesenteric lymph nodes were crushed followed by homogenization and transfer into a medium to isolate bacteria immediately. Then the colon was removed from mid-transverse segment to rectum. The rats were sacrificed by ether inhalation at lethal doses.

### Serum TNF- $\alpha$ Assay

The sera were obtained by centrifugation 4000 rpm over 10 minutes. Sera were stored in Eppendorf tubes at -80°C until assays. Serum TNF- $\alpha$  level measurement was performed using a Rat TNF- $\alpha$  Elisa kit (Rat TNF- $\alpha$  Invitrogen ELISA Kit, USA). TNF- $\alpha$  level is expressed as pg/mL.

### Plasma Endotoxin Assay

Plasma endotoxin measurement was performed in samples obtained from the portal vein using modified (quantitatively) Limulus-amebocyte-lysate test (Sunred). Plasma endotoxin level is given as pg/mL.

### Determination of Bacterial Translocation

Blood culture tests were performed using an automated blood culture system. The blood samples were transferred to BacT/Alert culture bottles. After inoculation, the bottles were incubated at 35-37°C. During incubation, the bottles were mixed by shaking and controlled at 10-minute intervals throughout incubation. CO<sub>2</sub> production in fluid media was continuously measured by colorimetric principle and monitored by a reflectometer available in the system. The system provided a visual and audio signal if a positive result was detected. The samples found positive were inoculated onto blood agar and eosin methylene blue agar (EMB agar) and were incubated for 24 hours at 37°C. After incubation, microbial identification was performed according to conventional methods. After seven days, samples with no positive signal were accepted as negative. Tissue samples were inoculated into sterile bottles containing brain-heart infusion broth which were then

incubated over 24-to-48 hours at 37°C. Then the samples were incubated in blood agar and EMB agar broths over hours at 37°C. Microbial identification was performed according to conventional methods.

**Evaluation of Colonic Damage**

The distal colon obtained from rats was opened in a longitudinal manner. The colon was washed out by using normal saline in order to remove feces. Then the colon segments were examined by a pathologist.

**Gross Examination of Colon**

Gross changes in the colon mucosa were assessed in five categories previously defined by Campos et al. (12). Macroscopic morphology scores are shown in Table 1.

**Microscopic Examination of the Colon**

Microscopic evaluation of the colonic mucosa was performed as described by Yamamoto et al. After fixation with a 10% formaldehyde solution for 24 h, the tissue samples were placed into ethanol (70%). Then the sections (4 µm in thickness) prepared from paraffin-embedded tissue samples were stained by H&E. The sections were examined by a pathologist blinded to the groups. Microscopic changes were graded from 0-3 (Table 2) (13).

**Measurement of Tissue Myeloperoxidase Activity**

In the colon mucosa, MPO activity was measured after preparation of the colon homogenates via Elisa method by a Rat MPO ELISA Kit (Cell Sciences, USA) as defined by Krawisz et al. (14). Briefly, the colon mucosa sample underwent homogenization using ice-cold solution containing PBS (PH: 6.0) and hexadecyl trimethyl ammonium bromide. After triple freeze-thaw cycles, the homogenate was centrifuged at 40.000 rpm for 15 minutes at 4°C. Then O-Dianisidine -H<sub>2</sub>O<sub>2</sub> buffer was mixed to the supernatant. The absorbance (λ= 460 nM) was measured over 2 minutes to detect changes. MPO activity was defined as amount of H<sub>2</sub>O<sub>2</sub> (1 mMol) degraded in one minute. The result is given as unit per gram tissue weight (U/g tissue).

**Table 1.** Classification of macroscopic findings in the colonic mucosa

Grade	Finding
0	Preserved mucosal layer normal in appearance
1	Erythematous areas without edema, congestion or superficial ulceration
2	Linear superficial ulcerations (7-15 mm); with depressed, erythematous or darker mucosal, granular bottom
3	Ulcerated area (15-45 mm) with irregular bottom; granular with rounded shaped island of normal or edematous mucosa among ulcerated mucosa
4	Extensive, irregular and multiple ulcers (> 45 mm); thinned intestinal wall; granular irregular bottom

**Table 2.** Classification of microscopic findings in the colonic mucosa

Grade	Finding
0	Normal epithelium, no cellular swelling, normal crypt appearance, low monocyte infiltration, low or absent neutrophil infiltration
1	Indicates loss of single epithelial cells. Moderate epithelial swelling, single inflammatory cell infiltration of crypts, mild monocyte-neutrophil infiltration
2	Multiple epithelial cell loss, epithelial flattening, cryptitis and moderate monocyte-neutrophil infiltration
3	Marked epithelial ulceration, crypt abscesses and a marked increase in monocytes and neurophils

**Statistical Analysis**

Data analysis was performed by SPSS Statistics 21.0 statistical software package (IBM,Corp., Armonk,NY,USA). Normality was tested using Shapiro-Wilk normality in numeric variables. One-way analysis of variance was used to compare the variables with normal distribution among the groups. Multiple comparisons with the Student-Newman-Keuls test were used for the groups showing a difference in the result of one-way analysis of variance. Kruskal-Wallis test was used for variables with skewed distribution. Multiple comparisons with the Student-Newman-Keuls test were used for the groups showing a difference in Kruskal-Wallis analysis. A two proportions test was used to evaluate bacterial translocation. Summary of the statistics of the variables with normal distribution was presented as mean ± SD, while the summary of the statistics of the variables without normal distribution was expressed as median (minimum-maximum) values. A p<0.05 value was set for statistical significance.

**RESULTS**

**Colonic Damage**

**Macroscopic Evaluation Results**

In this study, colonic macroscopic damage score values were statistically expressed as median (min-max) values: 3.0 (3.0-4.0) in the TNBS-E-induced colitis group, 2.0 (2.0-3.0) in the EPO + colitis (treatment) group and 0.0 (0.0-1.0) in the control group, showing significant differences among groups (p< 0.001). Normal colonic epithelium was observed in the control group. While widespread irregular ulcers and transmural inflammation were observed in the colitis group, only minimal damage and mild inflammation were found in the surface epithelium in the EPO-treated group.

**Microscopic Evaluation Results**

In this study, colonic microscopic damage score values were statistically expressed as median (minimum-maximum) values: 2.0 (2.0-3.0) in the TNBS-E-induced colitis group, 1.0 (1.0-2.0) in the EPO + colitis (treatment) group and 0.0 (0.0-1.0) in the con-

trol group ( $p < 0.001$ ). Normal colonic epithelium was observed in the control group. While widespread mucosal damage and transmural infiltration of neutrophils, monocytes, and lymphocytes were observed in the colitis group, mild epithelial damage and inflammation were observed in the EPO-treated group. Microscopic images are shown in Figure 1.

### Serum TNF- $\alpha$ Results

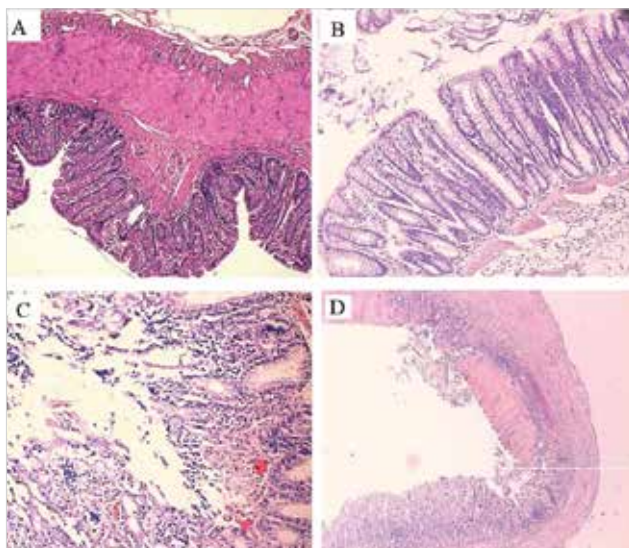
Plasma TNF- $\alpha$  value is expressed as mean  $\pm$  SD. TNF- $\alpha$  value was  $27.91 \pm 2.19$  pg/mL in the EPO-treated group,  $33.55 \pm 1.95$  pg/mL in the colitis group, and  $23.88 \pm 2.07$  pg/mL in the control group. It was found to be significantly lower in the EPO-treated group than the colitis group ( $p < 0.001$ ). These values are shown in Figure 2.

### Plasma Endotoxin Results

Plasma endotoxin value is expressed as mean  $\pm$  SD. Endotoxin level was  $20.15 \pm 1.33$  pg/mL in the EPO-treated group,  $26.45 \pm 2.55$  pg/mL in the colitis group, and  $15.88 \pm 2.07$  pg/mL in the control group. It was found to be significantly lower in the EPO-treated group than the colitis group ( $p < 0.001$ ). These values are shown in Figure 3.

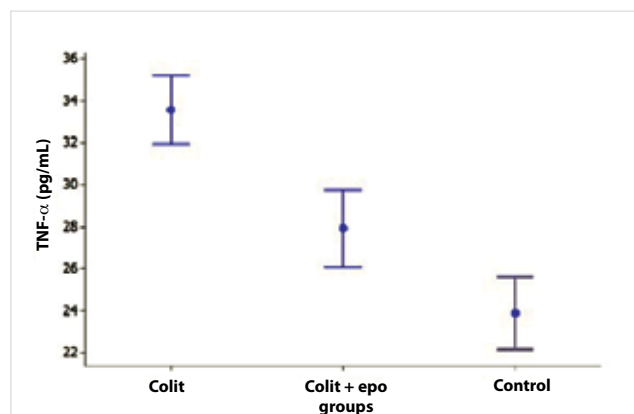
### Myeloperoxidase Results

Tissue MPO level is expressed as median (min-max). MPO level was 24.1 (20.9-27.5) U/gr in the EPO-treated group, 57.6 (24.1-88.6) U/gr in the colitis group and 15.5 (5.1-26.2) U/gr in the control group. It was found to be significantly lower in the

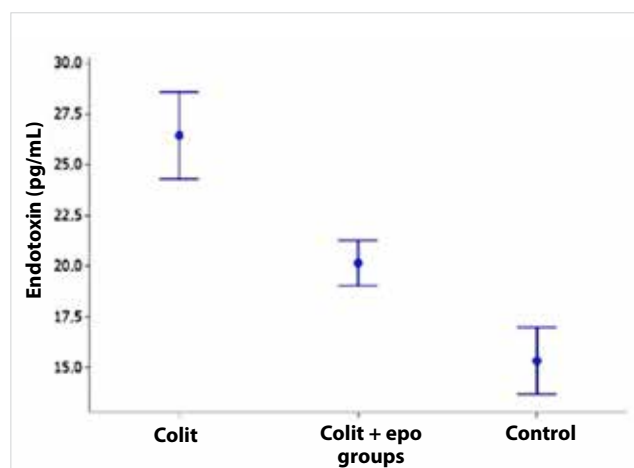


**Figure 1.** A. Normal colonic epithelium appearance from the control group (Grade 0). B. In the treatment group, moderate edema in the epithelium, mild inflammation and neutrophil infiltration in the lamina propria (Grade 1). C. In the colitis group, epithelial cell loss, cryptitis, erosion, appearance of the lymphoid follicle and mucosal ulceration (Grade 2). D. In the colitis group, prominent epithelial ulceration, crypt abscesses and significant increase of monocytes and neutrophil levels (Grade 3).

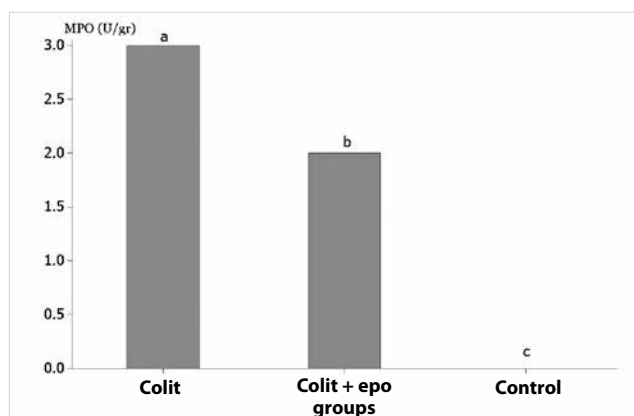
EPO-treated group when compared to the colitis group ( $p = 0.021$ ). There was no significant difference between the treatment and control groups. These values are shown in Figure 4.



**Figure 2.** Comparison of the groups in terms of TNF- $\alpha$  values.



**Figure 3.** Mean endotoxin values (pg/mL) and confidence intervals by groups ( $p < 0.001$ ).



**Figure 4.** MPO values by groups (U/gr) ( $p < 0.05$ ).

**Table 3.** Bacterial translocation by groups. A two proportions test was used for the comparison. The number of positive tests was tested for all animals (8 animals in each group) ( $p < 0.05$ )

Tissue	Control group	Colitis group	Colitis + Epo group	p
MLN	0	8	3	< 0.001
Spleen	0	8	2	< 0.001
Liver	0	7	3	0.002
Systemic blood	0	5	0	0.004

MLN: Mesenteric lymph nodes, Epo: Erythropoietin.

### Bacterial Translocation Results

It was failed to detect positive growth in the control group. There was a significant increase in the frequency of bacterial translocation to the liver, spleen, MLNs and blood in the group with trinitrobenzene sulfonic acid-ethanol-induced colitis ( $p < 0.05$ ). However, there was significant decrease in bacterial translocation to the liver, spleen, MLN and blood by treatment with EPO when compared to colitis group. Bacterial translocation incidences in different groups are shown in Table 3. The bacteria included *Escherichia coli*, *Enterococcus* spp., *Proteus* spp. and *K. pneumoniae*.

### DISCUSSION

Inflammatory bowel diseases (IBD), namely Crohn's Disease and Ulcerative Colitis, are chronic inflammatory diseases involving gastrointestinal (GI) tract. Defective immune system regulation due to environmental and genetic factors, abnormal GI luminal factors associated with microorganisms in the GI lumen flora and antigens in dietary intake, and a defective GI barrier that allows the penetration of colonic mucosa by GI luminal factors are among the factors that underlie the etiology of IBD (15).

TNBS-induced colitis model is a chronic inflammation and ulceration model that occurs after delayed hypersensitivity induced by the haptene. The mucosal barrier is disrupted by ethanol, leading to ulceration and inflammation in a dose-dependent manner. It was shown that the colitis in the TNBS model is similar to the colitis in humans, and it was especially pointed out as a convenient model for the development and trial of new drugs for the treatment of the disease (16).

In the literature, experimental colitis was induced by different doses of TNBS in a 5% ethanol solution. Doses of 5, 50, 100, and 150 mg/kg TNBS in 0.25 mL ethanol were tested, and the optimal dose for colitis induction was found to be 50 mg/kg TNBS in a 0.25 mL 50% ethanol solution (17). In this study, we used 50 mg/kg TNBS in a 0.25 mL 50% ethanol solution to induce colitis, based on this information. Togashiet al., in their study, have measured in vivo mucosal sulfhydryl compounds in a TNBS-E-induced experimental colitis model and has shown that advanced mucosal damage occurs one or two days after induction (18). Three days after TNBS administration, inflammation and ulceration of the colon were demonstrated endoscopically (19).

Several protective effects of EPO against ischemia were demonstrated in cell cultures and animal models, including anti-apoptotic, antioxidant, and neuroprotective effects (20,21). There are studies indicating that EPO decreases the damage caused by ischemia/reperfusion in kidneys (22) and colon (23). Large amounts of EPO receptors were identified on macrophages. In experimental colitis models, EPO has been shown to inhibit NF- $\kappa$ B activation and pro-inflammatory gene expression of myeloid cells in the lamina propria, leading to a decrease in the severity of colitis (9).

Taşdemir et al., in their study investigating the effects of melatonin and erythropoietin in a DNBS-induced experimental colitis model, have administered subcutaneous EPO on the 2<sup>nd</sup> and 3<sup>rd</sup> day following rectal DNBS administration, and the animals have been examined under anesthesia on the 4<sup>th</sup> day (24). In a study by Cuzzocrea et al., investigating the effects of EPO on inflammatory bowel disease, colitis was induced by dinitrobenzene sulfonic acid (DNBS), EPO (1000 IU/kg) was administered daily as SC bolus injections from day 2 onwards and the effects of EPO were evaluated on the 4<sup>th</sup> day (9).

In our study, after induction of colitis with TNBS, we administered EPO (1000 IU/kg/day s.c.) from the 2<sup>nd</sup> to the 4<sup>th</sup> day, and on the 5<sup>th</sup> day, we collected tissue and blood samples from the rats under anesthesia.

In order to evaluate the effects of EPO on pro-inflammatory cytokines, blood TNF- $\alpha$  levels and tissue MPO levels were measured in this study. TNF- $\alpha$  is one of the major cytokines implicated in IBD and TNF- $\alpha$  levels were found to be elevated in IBD (25). Hence, it was demonstrated that TNF- $\alpha$  monoclonal antibodies are effective in the treatment of IBD by preventing the binding of TNF- $\alpha$  to its receptor, and they are used as therapeutic agents for the treatment of patients non-respondent to current treatment (26).

In the study by Küçük et al., bacterial translocation has been assessed in an experimental model of colitis regarding the effects of Met-RANTES. They have demonstrated that TNF- $\alpha$  levels were increased by increasing inflammation and Met-RANTES treatment resulted in decreased serum TNF- $\alpha$  levels and bacterial translocation (27). Another study by Nairiz et al. investigating the effects of EPO on experimental colitis has demonstrated that in inflamed colons of TNBS-subjected mice, there was a significant decrease

in *Nos2*, *TNF- $\alpha$* , *IL-6*, *IL-12p35* and *IL-23p19* mRNA expression levels associated with EPO treatment. Furthermore, the supernatants of colon samples from TNBS-subjected mice receiving EPO had significantly reduced levels of *TNF- $\alpha$* , *IL-6*, *IL-12p70* and *IL-23* (9). In this study, we compared the colitis and EPO-treated groups in terms of serum *TNF- $\alpha$*  levels. We found significantly lower levels of *TNF- $\alpha$*  in the EPO-treated group than those in the colitis group ( $p < 0.05$ ) (Graph 3). High *TNF- $\alpha$*  level is a common feature of all colitis models in rats. Our study demonstrates that severe inflammation and increased bacterial translocation were in parallel with elevated *TNF- $\alpha$*  levels in the colitis group. The detection of low *TNF- $\alpha$*  levels in the EPO-treated group shows the anti-inflammatory effect of EPO.

MPO is a lysosomal enzyme found in phagocytic cells. It is abundant in the azurophilic granules of polymorphonuclear leukocytes and activated leukocytes entering the tissue release enzymes, including MPO, elastase, protease, and lactoferrin. Therefore, MPO activity may be used as a quantitative indicator of neutrophil sequestration. Neutrophil activity has been shown to increase in IBD and elevated MPO levels have been considered as an indicator of neutrophil migration (28).

Cuzzocrea et al., in their study investigating the effects of erythropoietin on experimental colitis, have shown that EPO treatment decreases inflammation in the colon by microscopic examination of the colonic tissue. MPO activity in the inflamed colon has been measured and significant differences found between the colitis and EPO groups (8).

Consistent with the literature, we also found that MPO levels were significantly higher in rats with induced colitis when compared to the controls ( $p < 0.05$ ). Tissue MPO levels were significantly lower in the group receiving EPO treatment in comparison to the colitis group ( $p < 0.05$ ) and the values were higher in the EPO-treated group when compared to the control group but the difference was not significant (Figure 4).

Histopathologically, there were macroscopic and microscopic improvements in the EPO-treated group when compared to the colitis group. These results demonstrate that EPO reduces mucosal damage by means of its anti-inflammatory effect.

There are some defense mechanisms that prevent the passage of bacteria in the normal intestinal flora across the intestinal mucosa. These include the physical barrier function of the mucosal epithelium, the mucus layer between bacteria in the lumen and the intestinal epithelium, blockage of epithelial adhesion sites on the bacterial wall by secreted IgA and the presence of intestinal peristalsis. These local defense mechanisms maintain the normal intestinal flora (29). Under normal conditions, normal flora bacteria in the intestinal lumen cannot pass the mucosal barrier. Intestinal bacterial translocation is defined as the passage of gastrointestinal micro-flora to local mesenteric lymph

nodes (MLN) through lamina propria and to other organs such as the liver and spleen thereafter (30).

In cases such as IBD, acute pancreatitis, severe burns, intestinal obstruction and shock, bowel barrier function and mucosal integrity are impaired, which causes the bacteria to be released from the intestine and spread to other organs (31).

The presence of bacterial translocation in IBD has also been shown in experimental colitis models. Halaçlar et al., in their study, have investigated the effects of glucagon-like peptide 2 (GLP 2) on bacterial translocation in an experimental colitis model. In this study, bacterial translocation to the liver, spleen, MLNs and systemic blood has been investigated by culturing tissue and blood samples after the induction of colitis with TNBS. *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Enterobacter agglomerans* have been identified as the translocated bacteria (32).

Kao et al. have investigated the effect of EPO on intestinal bacterial translocation in a case of shock. After inducing hemorrhagic shock in rats, intravenous rHuEPO (1000U/kg) have been administered together with saline solution, and bacterial translocation has been assessed by cultured mesenteric lymph nodes. It has been reported that EPO reduced bacterial translocation associated with ischemic-reperfusion damage by reducing the tissue damage caused by hemorrhagic shock and saline resuscitation (11).

We also performed blood and tissue cultures to determine bacterial translocation in the course of colitis. TNBS-E-induced colitis caused a significant increase in the frequency of bacterial translocation in our study while EPO treatment reduced bacterial translocation. The translocated bacteria and translocation frequencies in each group are shown in Table 3.

Endotoxin is a component of the lipopolysaccharide structure present in the cell walls of all gram-negative bacilli. Rather than through its direct effect, endotoxin triggers a strong response by stimulating hormonal and chemical mediators. Endotoxemia originating from the intestine is particularly observed in patients with IBD, burns, trauma, liver failure or acute pancreatitis (33). In our study, plasma endotoxin levels were significantly elevated in the rats with induced colitis compared to the control group. The endotoxin level was shown to be significantly lower in the EPO-treated group ( $p < 0.05$ ) (Figure 3).

The effects of EPO in the experimental colitis model were investigated and the following findings were obtained. A significant improvement in macroscopic scores and histopathological appearance was found in the EPO-treated group ( $p < 0.05$ ). There was a significant increase in tissue MPO levels increased in the colitis group whereas a significant decrease in the EPO-treated group ( $p < 0.05$ ). Plasma endotoxin levels increased in the EPO-treated group while they significantly decreased in the

EPO-treated group ( $p < 0.05$ ). TNF- $\alpha$  levels were elevated in the EPO-treated group while they significantly decreased in the EPO-treated group ( $p < 0.05$ ). Bacterial translocation observed in the colitis group was significantly reduced in the EPO-treated group ( $p < 0.05$ ).

## CONCLUSION

In light of these results, the decrease in bacterial translocation in the EPO-treated group could be explained not only by the anti-inflammatory effect of EPO, but also by its protective effect on the intestinal mucosa and its decreasing effect on intestinal permeability. EPO treatment, if supported by further experimental and clinical studies, may become an alternative approach in IBD.

**Ethics Committee Approval:** The study was approved by the Medical Faculty Ethics Committee (Date: 14.11.2012 No: 12/112).

**Informed Consent:** Due to the retrospective design of the study, the informed consent was not obtained.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept - T.A., A.A., Z.Y., E.S.; Design - T.A., A.A.; Supervision - T.A., A.A., Y.D., Z.Y., E.S.; Resource - T.A., Y.D., B.P., M.B.; Materials - T.A., Y.D., M.G.; Data Collection and/or Processing - T.A., Y.D., M.B., M.G., B.P.; Analysis and Interpretation - T.A., Z.Y., E.S., B.Ö., M.B., M.G., B.P.; Literature Search - T.A., B.Ö., E.S.; Writing Manuscript - T.A., A.A., Y.D., B.Ö., E.S.; Critical Reviews - T.A., A.A., Y.D., B.Ö., E.S., Z.Y., M.B., B.P., M.G.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study has received no financial support.

## REFERENCES

- Podolsky DK. Inflammatory bowel. *N Engl J Med* 2002; 347: 417-29. [CrossRef]
- Loftus EV Jr, Sandborn WJ. Epidemiology of inflammatory bowel disease. *Gastroenterol Clin North Am* 2002; 31: 1-20. [CrossRef]
- Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 2003; 3: 521-33. [CrossRef]
- Xing X, Zhang W, Sun H. Role of erythropoietin in renal anemia therapy. *Tropical Journal of Pharmaceutical Research* 2016; 15: 1083-8. [CrossRef]
- Agapidou A, Vakalolopoulou S, Papadopoulou T, Chadjiaggelidou C, Garypidou V. Successful treatment of severe anemia using erythropoietin in a Jehovah witness with non-Hodgkin lymphoma. *Hematol Rep* 2014; 6: 5600. [CrossRef]
- Jiang CJ, Wang ZJ, Zhao YJ, Zhang ZY, Tao JJ, Ma JY. Erythropoietin reduces apoptosis of brain tissue cells in rats after cerebral ischemia/reperfusion injury: a characteristic analysis using magnetic resonance imaging. *Neural Regen Res* 2016; 11: 1450-5. [CrossRef]
- Mateus V, Rocha J, Alves P, Mota-Filipe H, Sepodes B, Pinto RM. Anti-inflammatory effect of erythropoietin in the TNBS-induced colitis basic. *Clin Pharmacol Toxicol* 2017; 120: 138-45. [CrossRef]
- Cuzzocrea S, Mazzon E, Di Paola R, Patel NS, Genovese T, Muià C, et al. Erythropoietin reduces the development of experimental inflammatory bowel disease. *J Pharmacol Exp Ther* 2004; 311: 1272-80. [CrossRef]
- Nairz M, Schroll A, Moschen AR, Sonnweber T, Theurl M, Theurl I, et al. Erythropoietin contrastingly affects bacterial infection and experimental colitis by inhibiting nuclear factor-kappaB-inducible immune pathways. *Immunity* 2011; 34: 61-74. [CrossRef]
- Onder A, Kapan M, Yuksel H, Tekin R, Kele A, Evliyaoglu O, et al. The effects of erythropoietin on bacterial translocation and inflammation in rats with obstructive jaundice. *Ann Ital Chir* 2014; 85: 159-65.
- Kao NR, Xenocostas A, Driman DK, Rui T, Huang W, Jiao X, et al. Recombinant human erythropoietin improves gut barrier function in a hemorrhagic shock and resuscitation rat model. *J Trauma* 2011; 71: 456-61. [CrossRef]
- Campos FG, Waitzberg DL, Logulo AF, Cukier C, Soares SR, Oliveira TS, et al. Technical and histological standardization of experimental colitis with trinitrobenzenesulfonic acid (TNBS). *Rev Hosp Clin Fac Med Sao Paulo* 1997; 52: 180-6.
- Yamamoto M, Yoshizaki K, Kishimoto T, Ito H. IL-6 is required for the development of Th1 cell-mediated murine colitis. *J Immunol* 2000; 164: 4878-82. [CrossRef]
- Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology* 1984; 87: 1344-50. [CrossRef]
- Rahimi R, Mozaffari S, Abdollahi M. On the use of herbal medicines in management of inflammatory bowel diseases: a systematic review of animal and human studies. *Dig Dis Sci* 2009; 54: 471-80. [CrossRef]
- Jurjus AR, Khoury NN, Reimund JM. Animal models of inflammatory bowel disease. *J Pharmacol Toxicol Methods* 2004; 50: 81-92. [CrossRef]
- Motavallian-Naeini A, Andalib S, Rabbani M, Mahzouni P, Afsharipour M, Minaian M. Validation and optimization of experimental colitis induction in rats using 2, 4, 6-trinitrobenzene sulfonic acid. *Res Pharm Sci* 2012; 7: 159-69.
- Togashi H, Oikawa K, Adachi T, Sugahara K, Ito J, Takeda T, et al. Mucosal sulfhydryl compounds evaluation by in vivo electron spin resonance spectroscopy in mice with experimental colitis. *Gut* 2003; 52: 1291-6. [CrossRef]
- Brenna O, Furnes MW, Drozdov I, van Beelen Granlund A, Flatberg A, Sandvik AK, et al. Relevance of TNBS-colitis in rats: a methodological study with endoscopic, histologic and Transcriptomic [corrected] characterization and correlation to IBD. *PLoS One* 2013; 8: e54543. [CrossRef]
- Agnello D, Bigini P, Villa P, Mennini T, Cerami A, Brines ML, et al. Erythropoietin exerts an anti-inflammatory effect on the CNS in a model of experimental autoimmune encephalomyelitis. *Brain Res* 2002; 952: 128-34. [CrossRef]
- Dmytriyeva O, Pankratova S, Korshunova I, Walmod PS. Epobis is a nonerythropoietic and neuroprotective agonist of the erythropoietin receptor with anti-inflammatory and memory enhancing effects. *Mediators Inflamm* 2016; 2016: 11. [CrossRef]
- Banaei S, Ahmadiasl N, Alihemmati A. Combination anti-apoptotic effect of erythropoietin and melatonin on ischemia reperfusion-induced renal injury in rats. *Acta Med Iran* 2016; 54: 624-30.
- Mori S, Sawada T, Okada T, Kubota K. Erythropoietin and its derivative protect the intestine from severe ischemia/reperfusion injury in therat. *Surgery* 2008; 143: 556-65. [CrossRef]
- Tasdemir S, Parlakpınar H, Vardi N, Kaya E, Acet A. Effect of endogenous melatonin and erythropoietin on dinitrobenzene sulfonic acid-induced colitis. *Fundam Clin Pharmacol* 2013; 27: 299-307. [CrossRef]

25. Murch SH, Lamkin VA, Savage MO, Walker-Smith JA, MacDonald TT. Serum concentrations of tumour necrosis factor alpha in childhood chronic inflammatory bowel disease. *Gut* 1991; 32: 913-7. [CrossRef]
26. Hoentjen F, van Bodegraven AA. Safety of anti-tumor necrosis factor therapy in inflammatory bowel disease. *World J Gastroenterol* 2009; 15: 2067-73. [CrossRef]
27. Kucuk C, Sozuer E, GURSOY S, Canoz O, Artis T, Akcan A, et al. Treatment with Met-RANTES decreases bacterial translocation in experimental colitis. *Am J Surg* 2006; 191: 77-83. [CrossRef]
28. Wallace JL, MacNaughton WK, Morris GP, Beck PL. Inhibition of leukotriene synthesis markedly accelerates healing in a rat model of inflammatory bowel disease. *Gastroenterology* 1989; 96: 29-36. [CrossRef]
29. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 2005; 307: 1915-20. [CrossRef]
30. O'Boyle CJ, MacFie J, Mitchell CJ, Johnstone D, Sagar PM, Sedman PC. Microbiology of bacterial translocation in humans. *Gut* 1998; 42: 29-35. [CrossRef]
31. Balzan S, Almeida C, Cleva R, Zilberstein B, Ceconello I. Bacterial translocation: overview of mechanisms and clinical impact. *J Gastroenterol Hepatol* 2007; 22: 464-71. [CrossRef]
32. Halaçlar B, Ağaç Ay A, Akcan AC, Ay A, Öz B, Arslan E. Effects of glucagon-like peptide-2 on bacterial translocation in rat models of colitis. *Turk J Gastroenterol* 2012; 23: 691-8. [CrossRef]
33. Casey LC, Balk RA, Bone RC. Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. *Ann Intern Med* 1993; 119: 771-8. [CrossRef]



### ORJİNAL ÇALIŞMA-ÖZET

Turk J Surg 2019; 35 (3): 202-209

## Sıçanlarda oluşturulan deneysel kolit modelinde eritropoietinin bakteriyel translokasyon üzerine etkisi

Türkmen Arıkan<sup>1</sup>, Alper Akcan<sup>1</sup>, Yunus Dönder<sup>2</sup>, Zeki Yılmaz<sup>1</sup>, Erdoğan Sözüer<sup>1</sup>, Bahadır Öz<sup>1</sup>, Mehmet Baykan<sup>3</sup>, Mustafa Gök<sup>1</sup>, Bilgehan Poyrazoğlu<sup>4</sup>

<sup>1</sup> Erciyes Üniversitesi Tıp Fakültesi, Genel Cerrahi Anabilim Dalı, Kayseri, Türkiye

<sup>2</sup> Kilis Devlet Hastanesi, Genel Cerrahi Kliniği, Kilis, Türkiye

<sup>3</sup> Özel Melikgazi Hastanesi, Genel Cerrahi Kliniği, Kayseri, Türkiye

<sup>4</sup> Özel Magnet Hastanesi, Genel Cerrahi Kliniği, Kayseri, Türkiye

### ÖZET

**Giriş ve Amaç:** Bu çalışmanın amacı kolit sürecinin patofizyolojisinden yola çıkarak, ratlarda oluşturulan deneysel kolit modelinde eritropoietinin (EPO) bakteriyel translokasyonu üzerine etkisini araştırmaktır.

**Gereç ve Yöntem:** Gruplar randomize olarak her biri sekiz rattan oluşan üç gruba (kontrol, kolit ve tedavi ) ayrıldı. Kolit grubuna trinitrobenzen-sülfonik asit ve etanol karışımı (TNBS-E) rektal yoldan verilerek kolit oluşturuldu. Tedavi grubuna ise TNBS-E ile kolit oluşturulduktan bir gün sonra başlanarak üç gün boyunca günde bir defa subkutan olarak 1000 IU/kg EPO verildi. Beşinci gün kolondaki inflamasyon makroskopik ve mikroskopik olarak değerlendirildi . Bakteriyel translokasyonu değerlendirmek için kan alındı ve doku kültürü amacıyla karaciğer, dalak, mezenterik lenf nodlarından (MLN) doku örneklemeleri yapıldı. Doku düzeyinde miyeloperoksidaz (MPO), serumda ise tümör nekroz faktörü-alfa (TNF-α), endotoksin düzeylerine bakıldı.

**Bulgular:** Kolit ve tedavi grupları karşılaştırıldığında gruplar arasında makroskopik ve mikroskopik açıdan belirgin farklılık mevcuttu ( $p < 0.05$ ). Tedavi grubunda ölçülen MPO düzeyleri kolit grubuna göre anlamlı düzeyde düşüktü ( $p < 0.05$ ). TNF-α ve plazma endotoksin düzeyleri tedavi grubunda kolit grubuna göre belirgin şekilde düşüktü ( $p < 0.05$ ). Kolit grubuyla karşılaştırılınca, tedavi grubunda karaciğer, dalak, MLN'ler ve sistemik kanda bakteriyel translokasyon düşüktü ( $p < 0.05$ ).

**Sonuç:** Deneysel olarak TNBS-E ile oluşturulmuş kolit modelinde uygulanan EPO, histopatolojik, biyokimyasal, mikrobiyolojik parametrelere bakıldığında inflamasyonu ve bakteriyel translokasyonu belirgin düzeyde azaltmıştır.

**Anahtar Kelimeler:** Eritropoietin, deneysel kolit, bakteriyel translokasyon

**DOI:** 10.5578/turksurg.4272