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Original Article

Effects of erythropoietin on renal tissue in an experimental rat model of septic shock

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Abstract

Aim: To examine the effect of erythropoetin on renal function and histological findings in experimental septic shock model, 24 Sprague-Dawley rats were used.

Material and Methods: Rats were divided into control, septic shock, and erythropoetin-treated septic shock groups. Femoral vein and artery catheterization were performed on all rats. Rats in the control group underwent laparotomy and catheterization; in the test groups, cecal ligation-perforation and bladder cannulation were added. Rats in the treatment group received a single intraperitoneal (IP) injection of 250 mg/kg erythropoetin 60 minutes after cecal ligation perforation. Rats were monitored for blood pressure, heart rate, and body temperature to assess the postoperative septic response. The body fluids were replaced as necessary. At the end of 24 hours, rats were sacrificed and renal samples were collected.

Results: In our study, although there were lessstudies about septic shock model with erythropoietin, we have achieved similar results as the data in the available literatures. WBC, fever, urinary volume, urinary creatinin, BUN, blood creatinin values were significantly better in sepsis treated by erythropoietin group than non-treated sepsis group (p<0,001, table 1)Significant improvements were observed in histological findings in rats treated with erythropoetin, compared to rats with untreated.

Discussion: Our findings demonstrate that erythropoetin has antioxidant effects of sepsis as seen in other studies. We conclude that erythropoetin may be an effective treatment for oxidative damage due to renal tissue perfusion defects in cases of septic shock.

Keywords: Erythropoetin; Septic Shock; Rat, Kidney.

INTRODUCTION

Sepsis is a systemic response that develops against infection and inflammation. Aggressive treatment with specific antibiotics and other pharmacological agents are currently used to treat septic shock and multiple organ dysfunction syndrome due to sepsis. These disorders remain the most important cause of mortality in intensive care units. In addition septic shock is one of the most common reasons for intensive care unit admissions [1-3]. Sepsis and septic shock mortality ranges from 30 to 90 % (1).

EPO is a molecular weight of 30.4 kDa glycoprotein hormone in mammals and are known to play a role in the regulation of erythropoiesis (4).

It is stimulating progenitor cells in the bone marrow to induce the differentiation of the erythroid cells and increases stimulating oxygen-carrying capacity. In recent studies generally it is evaluated as a protective agent due to anti-apoptotic and anti-ischemic properties of the tissue (5).

Several studies has been demonstrated the efficacy of the erythropoetin in brain, kidney, liver and heart protection for such as ischemia-reperfusion injury (6).

In the present study, we used an experimental model of sepsis and septic shock in rats to investigate the effects of erythropoetin on oxidative stress and kidney histology due to hypoperfusion.

MATERIAL and METHODS

This animal study was approved by the Ethics Comittee and approval date and number was written on title page.

Rats and diet:
Male and female Sprague-Dawley rats weighing an average of 200-300 grams were purchased from

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Experimental Research Centre for Medical and Surgical Investigation. Animals were housed at room temperature under controlled laboratory conditions during the experiment and were fed standard rat chow and tap water.

Experimental protocol

Rats were anaesthetized using 60 mg/kg ketamine hydro sterilized conditions, inguinal incision was performed following 10% povidone iodine cleaning; all rats were catheterized, isolating the femoral artery and vein by pigtail 60(polyethylene) catheters. The femoral vein catheter was advanced to the vena cava. Cannulas were washed with saline containing 100 IU heparin before and after cannulation, and blockage of cannula was prevented. Closed catheters were removed from the back of the neck through the subcutaneous tunnel. All surgeries were performed on a surgical table heated to 36.6 °C to avoid hypothermia during surgery and follow-up.

Experimental groups consisting of 8 rats underwent laparotomy and cecal ligation to induce septic shock, or received bladder cannulation alone (control group), under anesthesia. Urine follow-up, invasive blood pressure measurement, and ECG recordings were started in the 14th postoperative hour and recorded for every 5 minutes. At first indication of septic shock – defined as invasive blood pressure measurements – rats received 250 mg/kg erythropoetin, in saline. Baseline and outcome body temperatures were measured by rectal probe. After 24 hours, rats were sacrificed using excessive ketamine, and kidney specimens were removed for histopathological investigation.

Blood samples (5 ml) were obtained for hemogram, and biochemical measurements (BUN and Cr) before catheterization and at the end of the experiment. Arterial pressure was measured by pressure transducer (Transpac IV, USA). Invasive arterial pressure and heart rate were monitored by Data Equationsystem (MP 100 Biopac, USA). Hemogram and biochemical measurements were performed using standart methods in our hematology and biochemical departments.

Histological methods

Kidney specimens were fixed in 10% formalin for 48 hours, washed with tap water for 3-4 hours, and dehydrated in increasing concentrations of alcohol (70%, 80% and 90%). To make pellucid, specimens were incubated in xylol twice for 20 minutes and embedded in 3 paraffin incubators. Tissue was sectioned by microtome, and incubated 1 hour in a 37°C water bath prior to staining with hematoxylin-eosin dual stain for 2 and 10 minutes, respectively. Sections were washed, dehydrated in increasing alcohol concentrations, washed twice in xylol for 30 minutes, and mounted on slides using entellan. Sections were imaged on an Olympus BH-2 microscope and an Olympus DP-70 digital camera.

Statistical analysis

Data were evaluated with SPSS 13.0 and Sigma Stat 3.1 software One-way analysis of variance was performed for normally distributed variables with nonparametric post-hoc Tukey HSD and Fisher LSD tests. The paired t test was used for binary comparisons of before and after variables (Pairt Samples Statistics). Non-normally distributed variables were analyzed by Kruskal-Wallis one-way analysis of variance on ranks; binary comparisons of before and after variables were analyzed by using Wilcoxon signed ranked test.

RESULTS

Table 1. shows hemoglobin (HB1), hematocrit (HCT1), white blood cell (WBC1), platelets (PLT1), and fever (FEVER1) values at the start of the experiment, and hemoglobin (HB2), hematocrit (HCT2), white blood cell (WBC2), platelets (PLT2), and fever (FEVER2) values at the end of the experiment was taken. Also urinary volume, urinary creatinin 1 (start) – 2 (end), blood urine nitrogen (BUN) 1 and 2, blood creatinin 1-2 and creatinin clearance.

HB and HCT values did not change significantly during the course of experiment. All biochemical values tested were the same among the groups at the start of the experiment. At the end of the experiment WBC, fever, urinary volume, urinary creatinin, BUN, blood creatinin values were significantly beter in sepsis treated by erythropoietin group than non-treated sepsis group (p<0,001, table 1)

Histological findings

Renal corteks, corpuscles in the renal cortex (Malpighian corpuscle and Bowman capsule), and renal tubule structures were histologically normal in control group rats (Figure 1). Also renal medulla and medullar tubule structures were histologically normal. Intensive degenerations and inflammation in medullar tubules, degenerations in renal corpuscules, dilatations in cortical tubules, necrotic areas and epithelial loss in tubules were observed in the kidneys of rats in the untreated septic shock group (Figure 2).

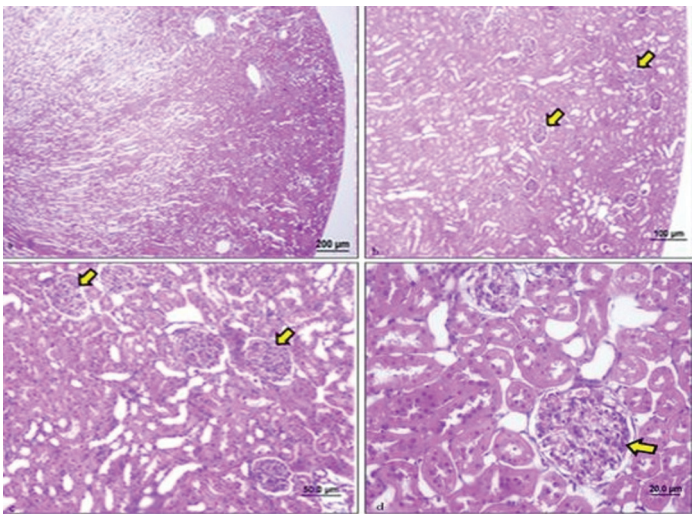

Figure 1. Control Group; a) Kidney histology was normal in control group animals (bar:10 µm, HE). b,c,d) Kidney tubule and malpighian corpuscle was normal in control group animals (arrow head) (bar:10 µm, bar:20 µm, bar:40 µm, HE)

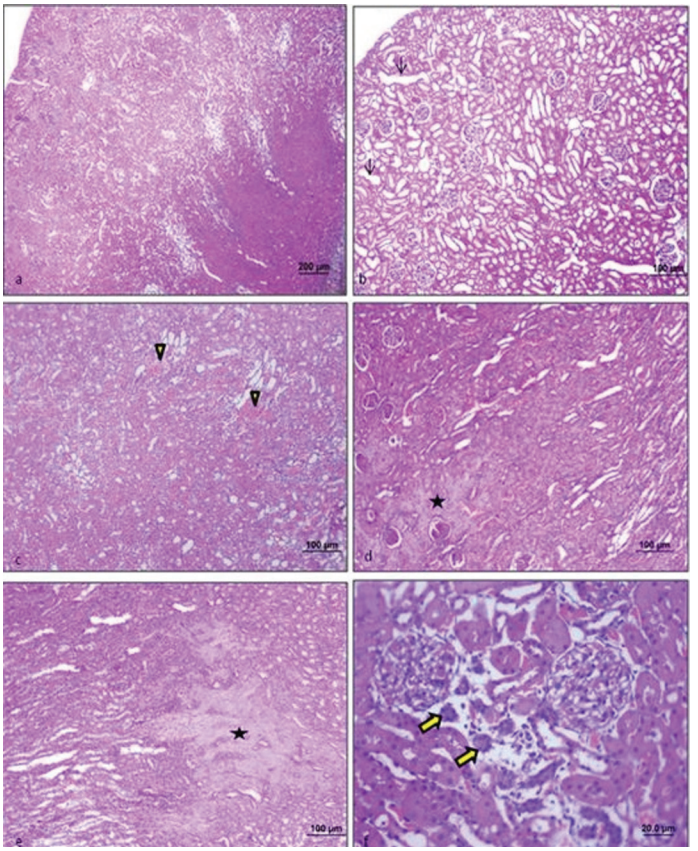

Figure 2. Sepsis Group; Rats exposed to septic shock by cecal ligation. When examined kidney tissue in the light microscopy; (a) Extensive degeneration was observed in renal tubular structures in the cortex and medulla (bar: 4µm, HE) (b) tubular dilatation (arrow) (bar:10 µm, HE), (c) interstitial hemorrhage (arrow) (bar:10µm, HE), (d,e) necrotic areas (*)(bar:10µm, HE), (f) tubular atrophy (thick arrow) (bar:40µm, HE)

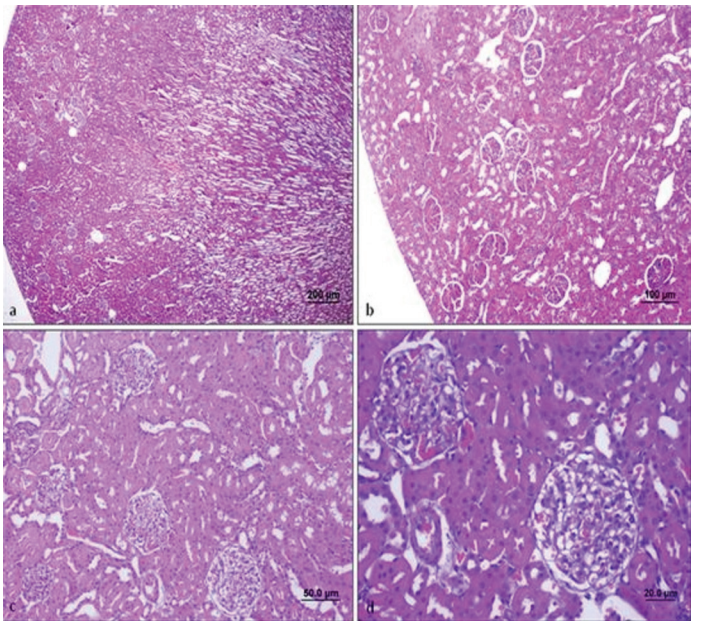

Figure 3. Treatment Group (septic shock + erythropoetin): When examined kidney tissue in the light microscopy: Rats treated with erythropoetin exhibit a marked decrease tubular damage and hemorrhage compared to rats with untreated septic shock, (a) tubular damage and hemorrhage is reduced, and near-normal appearance structure of the renal cortex and medulla. (bar: 4µm, HE), (b,c,d) Kidney tubule and malpighian corpuscle (bar:10 µm, bar:20 µm, bar:40 µm, HE)

Rats exposed to septic shock and treated with erythropoetin exhibited decreased degeneration and inflammation into the tubule structures, compared to the sepsis group; the kidneys of rats in this group had near-normal histological structure (Figure 3). Statistically significant improvements were observed in histological findings in rats treated with erythropoetin, compared to rats with untreated.(Table 2).

Table 1. Comparison of haematologic parameters, urine parameters and fever of rats after the study

	Group 1	Group 2	Group 3	P
HGB1	11,3625±1,11732	11,6250±1,27139	11,7350±1,01101	P≥ 0,05
HTC1	34,6500±3,5605	34,4625±3,75421	34,7000±3,4222	P≥ 0,05
WBC1	7256,24±894,21	7246,25±1339,65	7770,00±1441,41	P≥ 0,05
PLT1	502250±174044	480250±140212,8	554000,00±186673,8	P≥ 0,05
FEVER1	36,53±0,2875	36,51±0,3399	36,53±0,2973	P≥ 0,05
HGB2	10,36±0,789	10,03±0,870	10,81±0,798	P≥ 0,05
HTC2	31,21±2,18	30,47±2,04	32,63±2,07	P≥ 0,05
WBC2	11703,75±2347	17657,5±1902,16	16652,5±1509,20	P≤0,001
PLT2	450000±106426,36	475500±101843,01	454875±118500,67	P≥ 0,05
FEVER2	36,52±0,281	38,32±0,503	38,01±0,476	P≤0,001
URINE VOLUME	14,68±2,60	6,12±1,24	10,12±0,99	P≤0,001
U-CREATININ	43,62±1,92	15,87±2,35	24,25±4,06	P≤0,001
BUN1	22,25±3,49	25±3,16	24,37±3,85	P≥0,05
BUN2	35,25±4,09	95,62±10,37	56,62±6,98	P≤0,001
B.CREATININ1	0,46±0,18	0,55±0,28	0,45±0,15	P≥0,05
B.CREATININ2	0,57±0,21	0,95±0,24	0,7±0,21	P≤0,05
CREATIN CLEARANCE	1±0,26	0,11±0,09	0,56±0,14	P≤0,001

Table 2. The findings of histological evaluations of all groups at the end of the study

Kidney	Glomerular Damage	Tubular atrophy	Necrotic areas	Inflammation	Hemorrhage
Control 1	-	-	-	-	-
Control 2	-	-	-	-	-
Control 3	-	-	-	-	-
Control 4	-	-	-	-	-
Control 5	-	-	-	-	+
Control 6	-	+	-	-	-
Control 7	-	-	-	-	-
Control 8	-	-	-	-	-
Sepsis Group 1	-	++	+++	-	+
Sepsis Group 2	-	+++	-	-	-
Sepsis Group 3	+	+++	+++	-	+++
Sepsis Group 4	+	+++	-	-	+
Sepsis Group 5	++	++	-	-	-
Sepsis Group 6	+	++	++	-	-
Sepsis Group 7	++	++	-	-	++
Sepsis Group 8	-	+++	-	-	++
Sep-sis+Erythropoetin Group 1	-	-	-	-	+
Sep-sis+Erythropoetin Group 2	-	-	-	-	-
Sep-sis+Erythropoetin Group 3	-	++	-	-	-
Sep-sis+Erythropoetin Group 4	-	-	-	-	-
Sep-sis+Erythropoetin Group 5	-	-	-	-	-
Sep-sis+Erythropoetin Group 6	+	-	-	-	+
Sep-sis+Erythropoetin Group 7	-	-	-	-	-
Sep-sis+Erythropoetin Group 8	+	-	-	-	+

(Histological findings: absent (–); mild (+); moderate (++); severe (+++).)

DISCUSSION

Currently, although new antibiotics, drugs and treatment protocols for immunomodulation started to be used, septic shock continues to be a serious health problem with 30-90% mortality rate (1,7). Although clinical experimental studies about septic shock accelerated, a variety of patients in these studies, different etiological causes of septic shock, etc. different treatment modalities is caused to not produce the desired results (8). Today, sufficiency of experimental models of septic shock is still being debated. In the present study we aimed to investigate the effects of erythropoetin on kidney histology and oxidative damage due to septic shock.

First, EPO (erythropoietin) has been discovered as a glycoprotein hormone to provide the extramedullary and bone marrow erythropoiesis but in recent years the general tissue protective properties of this hormone has been demonstrated. EPO is a growth hormone and has useful significant effects on angiogenesis and mitosis etc. in clinical use (9,10). Studies demonstrate that EPO increase exercise tolerance, increase cardiac output to regulate the peripheral vascular resistance, decrease myocardial ischemia, decrease ventricular hypertrophy (11). In addition studies also demonstrate that EPO increase the brain functions, decrease tendency of uremic bleeding, increase platelet function, increase sexual function, regulate endocrine and immune system and improve quality of life (12).

Erythropoietin may protect many organs in our body against ischemia-reperfusion and tissue damage caused by excessive inflammation (13).

Studies revealed that erythropoietin protects the organs to ischemia reperfusion severe tissue damage and dysfunction of the heart (14), kidney (15), brain (16), liver (17), such as organs (18,19).

Ateş E. et al. demonstrated the protective effects of erythropoietin on renal ischemia and reperfusion injury (20).

In our study, although there was less study about septic

shock model with erythropoietin, we have achieved similar results as the data in the available literatures. WBC, fever, urinary volume, urinary creatinin, BUN, blood creatinin values were significantly different between groups sepsis and treated groups (p<0,001; table 1). Significant improvements were observed in histological findings in rats treated with erythropoetin, compared to rats with untreated.(Table 2). Our findings demonstrate that erythropoetin has antioxidant effects of sepsis. In our study we also evaluated the efficiency of erythropoetin by assessing the histopatology of kidney tissue.

CONCLUSION

We constituted an experimental rat model of septic shock, with symptoms similar to those seen in clinical practice, and treated the rats with erythropoetin, which has wide-spectrum efficiency for the treatment of ischemia perfusion injury and inflammation. The treatment was effective, as assessed by clinical and histopathological parametersThese data suggest that erythropoietin is useful for the treatment of septic shock especially the kidney damages. Results of this study should prompt future clinical studies by other investigations. It seems likely that erythropoetin protects the organs against sepsis-induced oxidative organ injury.

Competing interests: The authors declare that they have no competing interest.
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Ethical approval: These animal studies were approved by the Eskişehir Osmangazi University Medical Faculty Ethics Comittee.(access date 19.10.2010-176/2010)

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