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Erythropoietin prevents sepsis -induced clinical severity and haematological changes in mice

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Abstract
We investigated the effect of erythropoietin on sepsis-induced haematological changes in mice. Sepsis was induced by caecal ligation and puncture. In this study, blood was collected after 20 ± 2 hr of surgery from Sham operated (SO), Sham operated (SO)+EPO, sepsis (CLP) and CLP+EPO. Clinical scoring was done. Septic animal showed bloated appearance, mucky eyes, lack of movement and lack of alertness. Erythropoietin treatment reduced the clinical severity in septic mice. Sepsis significantly increased haemoglobin, packed cell volume and decreased red blood cell count, white blood count and platelet count. Erythropoietin significantly reversed the haematological changes to near normal. However erythropoietin post treatment showed no significant change in white blood and platelet count. Erythrocyte indices did not reveal any changes in sepsis except MCH (Mean Corpuscular haemoglobin) which was increased. In septic mice treated with erythropoietin, significant decrease in MCH was observed. Mean platelet volume, red cell distribution width and platelet distribution widths were significantly increased in sepsis. Erythropoietin restored the red cell distribution width compared to control, but no significant change was observed in mean platelet volume and platelet distribution width in septic mice. In conclusion, erythropoietin prevents sepsis-induced haematological changes in mice, but not platelet parameters.

Keywords: Erythropoietin, sepsis, clinical score, haematology, mice

Introduction
Sepsis, the systemic inflammatory response to infection is a highly prevalent disorder whose associated mortality makes it a major public health burden [1] and is considered the major cause of death among critically ill patients in the developed world. Hematologic system plays a major role in oxygen delivery, carbon dioxide disposal, hemostasis, and defense against pathogens. As a result of its widespread distribution and disparate functions, the hematologic system is often unnoticed as an organ in the work-up of the patient with sepsis. Thus, rapid identification and treatment of hematologic dysfunction may lead to improved survival.

There are several animal models which replicate the signs and laboratory findings seen in animal and human sepsis. Caecal ligation and puncture (CLP) model is characterized by increased cardiac output and organ blood flow in early stage (i.e. hyperdynamic phase, 6 h after onset of sepsis [2] and decreased tissue perfusion at the later stage (Hypodynamic phase 18 h after onset of sepsis [3]. This is a model of sepsis due to peritoneal contamination with mixed flora in the presence of devitalized tissue and thus bears an obvious resemblance to clinical problem.

Erythropoietin (EPO), a 31-kDa glycoprotein produced by fetal liver and adult kidneys and its receptor is expressed in the brain, heart, lungs, liver, kidneys, vascular endothelium, and lymphoid tissues, and upregulated by hypoxic stimulation. In a LPS (Lipopolysaccharide) model of sepsis, recombinant human erythropoietin prevented vascular hyporeactivity [4] and improved survival which were associated with inhibition of apoptosis, nitric oxide production and tissue hypoxia [5]. Recent studies demonstrated cardioprotective effect of erythropoietin in a CLP model of sepsis in rats through improvement in left ventricular functions [6]. Similarly EPO reverses sepsis-induced vasoplegia to Noradrenaline through the preservation of α1D adrenoceptor mRNA/protein expression; inhibition of GRK2 mediated desensitization and attenuation of NO overproduction in the mouse aorta [7]. Therefore the present study was undertaken to investigate the effect of erythropoietin post-treatment on sepsis-induced haematological alterations in mice model of sepsis.

Materials and Methods

Animals

Healthy male Swiss Albino mice (30-35 g) were procured from the Laboratory Animal Resource Section, Indian Veterinary Research Institute, Izatnagar, and Uttar Pradesh, India. Mice were housed in different polypropylene cages with free access to feed and water in the Divisional animal house.

Induction of sepsis in mice

Caecal ligation and puncture was produced as described [8]. In short, after seven days of acclimatization period, mice were fasted overnight before the induction of sepsis, but allowed water *ad libitum*. The animals were anesthetized by injection of xylazine (10 µg/g body wt.) and ketamine (80 µg/g body wt. *ip*), a 2-cm ventral midline incision was performed. Then the caecum was exposed and ligated with 3/0 silk just distal to the ileocecal valve to avoid intestinal obstruction, punctured twice with a 21 G needle and returned to the abdomen. The abdominal incision was closed in layers. Normal saline (1 mL/mouse) was given subcutaneously to all mice to prevent dehydration. Sham-operated mice had undergone the same surgical procedure except cecal ligation and puncture. All the surgical maneuvers were carried out according to the procedures laid down by the Institutional Animal Ethics Committee.

Drug administration

Erythropoietin was procured from Zuventus Healthcare Ltd, A joint Venture of Emcure®, Mumbai. SO operated / CLP mice were injected subcutaneously with saline (0.1 ml) and SO+EPO/CLP+EPO mice were injected erythropoietin (1000 IU/kg) subcutaneously. Erythropoietin was administered 1 hour after surgery. The dose and time of administration of erythropoietin was based on previous report [5, 9].

Collection of blood

After 20±2 h surgery, the mice (sham operated/sepsis) were anaesthetized with xylazine and ketamine intraperitoneally, and blood was collected in tubes (BD vacutainer® 3.5 cc) coated with clot activator. Haematology of Sham operated (SO), CLP, SO+EPO and CLP+EPO were done using Celltac α haematology automatic analyser, MEK-6450K (M/s Nihon Kohden Corporation, Japan).

Clinical scoring system

To evaluate the severity induced by sepsis, a clinical scoring system for all four groups was done [10].

Statistical analysis

Data have been expressed as mean ±S.E. Data were analyzed by one-way ANOVA followed by Newman Keuls post hoc test. Difference at $p< 0.05$ was considered statistically significant.

Results and Discussion

In the present study we demonstrated the effect of erythropoietin post treatment on clinical severity and haematological alterations in septic mice. A clinical scoring system was used to record the severity of sepsis for each animal [10]. Clinical scores of 0 to 3 implied mild sepsis and ≥4 severe sepsis. After 20±2 hr after surgery, septic mice showed bloated appearance, mucky eyes, lack of movement and lack of alertness. As observed in the present study erythropoietin was able to reduce the severity of sepsis. The scoring in septic mice was 4.16, likewise in erythropoietin treated septic mice, the score was 1.16.

Sepsis significantly ($p<0.01$) increased the haemoglobin concentration to 16.20 ± 1.29 (g/dl, n=6) compared to Sham operated (11.68 ± 0.44 g/dl, n=6) mice. However, erythropoietin post treatment significantly ($p<0.05$) decreased the haemoglobin concentration to 13.42 ± 0.62 g/dl (n=6) (Table 1). Haemo concentration observed in the present study suggested increased capillary leakage in septic mice [10]. As observed in the present study, sepsis significantly ($p<0.01$) increased the packed cell volume (53.44 ± 4.78%; n=6) compared to Sham operated (36.41±1.62%; n=6) mice. However, erythropoietin post treatment significantly ($p<0.05$) decreased the packed cell volume (44.01±2.93%; n=6). Based on above findings, erythropoietin post treatment was useful and suggestive of beneficial effect in sepsis.

Sepsis significantly decreased the white blood cell count and platelet count (Table 1). Decreased white blood count and platelet count was reported in LPS-induced mice model of sepsis [11]. However, erythropoietin was unable to increase the white blood cell count and platelet count in septic mice.

Table 2 depicts the effect of erythropoietin post treatment on red blood cell count and erythrocyte indices in septic mice. In sham operated mice the red cell count was 9.30±0.42x 10^6/µl, n=6. Red blood cell count was significantly ($p<0.05$) reduced in septic mice (6.96±0.74x 10^6/µl, n=6). Erythropoietin treatment significantly ($p<0.05$) increases the red blood cell count (8.87±0.68x 10^6/µl, n=6) to near normal.

In SO mice, the MCH was 12.71±0.84 pg, (n=6). Sepsis significantly ($p<0.01$) increased the MCH to 24.98±4.00 pg, (n=6). However, erythropoietin post-treatment significantly ($p<0.01$) reduced MCH to 15.51±1.11 pg, (n=6) in septic mice. SO+EPO mice did not cause any significant change in MCH (12.53±0.65 pg, n=6) compared to SO. MCV and MCHC showed no significant difference among all the groups.

Red blood cell distribution width (RDW) is a laboratory index used in the differential diagnosis of anemia. RDW is a simple laboratory test used to evaluate variability in the size and form of red blood cells. Elevated RDW has been associated with increased mortality in intensive care patients [12, 13], patients with community acquired pneumonia [14, 15], gram-negative bacteremia [16] and severe sepsis [17]. As observed in the present study, sepsis increased significantly red cell distribution width, which is in accordance with the increased red cell distribution width observed with septic patient in an earlier study [18]. Erythropoietin post treatment reversed red cell distribution width (16.43 ± 0.83%; n=6) in septic mice (Table 3) Some authors have found an association between RDW and proinflammatory cytokines [19, 20]. It is well known that cytokines are inducers of iNOS. In the present study, an increase in TNF-α and IL-1β was observed (data not shown) and erythropoietin post treatment significantly reduced the increase in TNF-α and IL-1β. This implies the beneficial effect of erythropoietin post treatment in septic mice.

Platelet indices are potentially useful markers for the early diagnosis of thromboembolic diseases. An increase in both mean platelet volume (MPV) and platelet distribution width (PDW) was present due to platelet activation. PDW is a more specific marker of platelet activation, since it does not increase during simple platelet swelling [21]. Sepsis significantly increased platelet distribution width and mean platelet volume compared to sham operated (Table 3).

Erythropoietin did not prevent the increase in platelet distribution width and mean platelet volume in septic mice. In patients with sepsis, increased destruction and consumption of platelets are the main causes of thrombocytopenia [22]. Taken together all the above findings suggest that erythropoietin prevents sepsis-induced haematological changes but not platelet parameters. So, further studies are required to delineate the mechanism behind the effect of erythropoietin post treatment on changes in platelet functions in septic mice.

Table 1: Effect of erythropoietin post treatment on Haemoglobin, Packed cell volume, white blood cell count and platelet count in septic mice

Group	Haemoglobin (g/dL) Mean ± SE	Packed cell Volume (%) Mean ± SE	White blood cell count(x 10^3/µl) Mean ± SE	Platelet count (x 10^3/µl) Mean ± SE
SO+ vehicle	11.68±1.45	36.41±1.62	5.51±0.77	993.5±70.10
CLP+ vehicle	16.20±1.29*	53.44±4.78*	2.31±0.33$	636.5±59.57$
SO + EPO	11.92±0.29	38.06±1.49	5.5±0.60	968.5±60.50
CLP + EPO	13.42±0.62#	44.01±2.93#	4.13±0.85	832.3±141.9

* $p<0.01$ compared to SO + vehicle, # $p<0.05$ compared to CLP+ vehicle $ $p<0.05$ compared to SO + vehicle

Table 2: Effect of erythropoietin post treatment on RBC and erythrocyte indices in septic mice

Group	RBC (x 10^6/µl) Mean ± SE	MCH (pg)Mean ± SE	MCV (fl)Mean ± SE	MCHC (g/dl) Mean ± SE
SO+ vehicle	9.30 ± 0.42	12.71±0.84	43.69 ± 0.85	32.24 ± 1.159
CLP+ vehicle	6.96 ± 0.72*	24.98±4.00*	46.65 ± 4.97	30.90 ± 2.37
SO+EPO	9.61 ± 0.57	12.53±0.65	44.23 ± 3.28	31.68 ± 1.92
CLP+EPO	8.87 ± 0.68#	15.51±1.11$	45.23 ± 4.66	31.19 ± 2.60

* $p<0.01$ compared to SO + vehicle, # $p<0.05$ compared to CLP+ vehicle $ $p<0.01$ compared to CLP + vehicle

Table 3: Effect of erythropoietin post treatment on platelet distribution width (A) and mean platelet volume (B) in septic mice

Group	Red cell distribution width (%)	Platelet distribution width (%) Mean ± SE	Mean platelet volume (fl) Mean ± SE
SO+ vehicle	15.58±0.41	15.80±0.71	4.37±0.18
CLP+ vehicle	19.27±1.45*	18.65±1.10*	6.13±0.60*
SO + EPO	15.22±0.61	15.45±0.29	4.40±0.30
CLP + EPO	16.43±0.83#	16.48±0.74	5.28±0.40

* $p<0.05$ compared to SO + vehicle, # $p<0.05$ compared to CLP +vehicle

References

1. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from. 1979 through. 2000.N. Engl. J Med. 2003; 348(16):1546-1554.
2. Wang P, Ba ZF, Chaudry IH. Endothelium-dependent relaxation is depressed at the macro- and microcirculatory levels during sepsis. Am. J Physiol. 1995; 269:R988-R994.
3. Hubbard WJ, Choudhry M, Schwacha MG, Kerby JD, Rue LW, Bland KI *et al*. Cecal ligation and puncture. Shock. 2005; 24:52-57.
4. Roberta d'Emmanuele di Villa B, Rosalinda S, Emma M, Stefania M, Giuseppina A, Thiemermann C *et al*. Recombinant Human Erythropoietin prevents lipopolysaccharide-induced vascular hyporeactivity in the rat. Shock, 2009; 31(5):529-34.
5. Aoshiba K, Onizawa S, Tsuji T, Nagai A. Therapeutic effects of erythropoietin in murine models of endotoxin shock**.** Crit Care Med. 2009; 37(3):889-898.
6. Qin Y, Zhang X, Yu Y, Bian X, Dong S. Cardioprotective effect of erythropoietin on sepsis-induced myocardial injury in rats. World. J Emerg. Med. 2013; 3:215-222.
7. Kandasamy K, Choudhury S, Singh V, Addison MP, Darzi SA, Kasa JK *et al*. Erythropoietin Reverses Sepsis-Induced Vasoplegia to Norepinephrine Through Preservation of α1D-Adrenoceptor mRNA Expression and Inhibition of GRK2-Mediated Desensitization in Mouse Aorta. J Cardiovasc Pharmacol Ther. 2016; 21(1):100-13.
8. Wichtermann KA, Baue AE, Chaudry IH. Sepsis and septic shock- A review of laboratory models and a proposal. J Surg. Res. 1980; 29:189-201.
9. Areeg I, Sina K, Coldewey M, Nimesh S, Patel A, Rogazzo M *et al*. Erythropoietin attenuates cardiac dysfunction in experimental sepsis in mice via activation of the β-common receptor. Dis Model Mech. 2013; 6(4):1021-30.
10. Zolfaghari PS, Pinto BB, Dyson A, Singer M. The metabolic phenotype of rodent sepsis: cause for concern? Intens. Care Med. Expe. 2013; 1:6.
11. Brendt P, Rehfeld I, Kamphausen A, Kreissig C, Peters J. Lipopolysaccharide interference in erythropoiesis in mice. Anaesthesia, 2012; 67:493-500.
12. Wang F, Pan W, Pan S, Ge J, Wang S, Chen M. Red cell distribution width as a novel predictor of mortality in ICU patients. Ann. Med.2011; 43:40-46.
13. Bazick HS, Chang D, Mahadevappa, K, Gibbons FK, Christopher KB. Red cell distribution width and all-cause mortality in critically ill patients. Cri. Care. Med.2011; 39:1913-1921.
14. Braun E, Domany E, Kenig Y, Mazor Y, Makhoul BF, Azzam ZS *et al*. Elevated red cell distribution width predicts poor outcome in young patients with community acquired pneumonia. Crit. Care. 2011; 15:R194. doi: 10.1186/cc10355.
15. Lee JH, Chung HJ, Kim K, Jo YH, Rhee JE, Kim YJ *et al*. Red cell distribution width as a prognostic marker in patients with community-acquired pneumonia. Am. J Emerg. Med. 2013; 31:72-79.
16. Ku NS, Kim HW, Oh HJ, Kim YC, Kim MH, Song JE *et al*. Red blood cell distribution width is an independent predictor of mortality in patients with gramnegativebacteremia. Shock. 2012; 38:123-127.
17. Jo YH, Kim K, Lee JH, Kang C, Kim T, Park HM *et al*. Red cell distribution width is a prognostic factor in severe

sepsis and septic shock. Am. J Emerg. Med. 2013; 31:545-548.
18. Lorenzo L, Dıaz C, Gonzlez O, Garcıa D, Jimenez A, Borreguero-Leon JM. Red Blood Cell Distribution Width during the First Week Is Associated with Severity and Mortality in Septic Patients PLoS ONE. 2014; 9(8):e105436. doi:10.1371/journal.pone.0105436.
19. Scharte M, Fink MP. Red blood cell physiology in critical illness. Crit Care Med. 2003; 31:S651-657.
20. Pierce CN, Larson DF. Inflammatory cytokine inhibition of erythropoiesis in patients implanted with a mechanical circulatory assist device. Perfusion. 2005; 20:83-90.
21. Vagdatli E, Gounari E, Lazaridou E, Katsibourlia E, Tsikopoulou F, Labrianou I. Platelet distribution width: a simple, practical and specific marker of activation of coagulation. Hippokratia. 2010; 14(1):28-32.
22. Guo YL, Liu DQ, Bian Z, Zhang CY, Zen K. Down-regulation of platelet surface CD47 expression in Escherichia coli O157:H7 infection-induced thrombocytopenia. PLoS One. 2009; 4:e7131.