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Original Article

Recombinant human thrombopoietin in alleviating endothelial cell injury in sepsis

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ABSTRACT

Background: To evaluate the effect of recombinant human thrombopoietin (rhTPO) on clinical prognosis by exploring changes in endothelial cell injury markers and inflammatory factors in patients with sepsis after treatment with rhTPO.

Methods: This retrospective observational study involved patients with sepsis (diagnosed according to Sepsis 3.0) admitted to Shanghai General Hospital intensive care unit from January 1, 2019 to December 31, 2022. Patients were divided into two groups (control and rhTPO) according to whether they received rhTPO. Baseline information, clinical data, prognosis, and survival status of the patients, as well as inflammatory factors and immune function indicators were collected. The main monitoring indicators were endothelial cell-specific molecule (ESM-1), human heparin-binding protein (HBP), and CD31; secondary monitoring indicators were interleukin (IL)-6, tumor necrosis factor (TNF)-α, extravascular lung water index, platelet, antithrombin III, fibrinogen, and international normalized ratio. We used intraperitoneal injection of lipopolysaccharide (LPS) to establish a mouse model of sepsis. Mice were randomly divided into four groups: normal saline, LPS, LPS + rhTPO, and LPS + rhTPO + LY294002. Plasma indicators in mice were measured by enzyme-linked immunosorbent assay.

Results: A total of 84 patients were included in the study. After 7 days of treatment, ESM-1 decreased more significantly in the rhTPO group than in the control group compared with day 1 (median=38.6 [interquartile range, IQR: 7.2 to 67.8] pg/mL *vs.* median=23.0 [IQR: −15.7 to 51.5] pg/mL, P=0.008). HBP and CD31 also decreased significantly in the rhTPO group compared with the control group (median=59.6 [IQR: −1.9 to 91.9] pg/mL *vs.* median=2.4 [IQR: −23.2 to 43.2] pg/mL; median=2.4 [IQR: 0.4 to 3.5] pg/mL *vs.* median=−0.6 [IQR: −2.2 to 0.8] pg/mL, P <0.001). Inflammatory markers IL-6 and TNF-α decreased more significantly in the rhTPO group than in the control group compared with day 1 (median=46.0 [IQR: 15.8 to 99.1] pg/mL *vs.* median=31.2 [IQR: 19.7 to 171.0] pg/mL, P <0.001; median=17.2 [IQR: 6.4 to 23.2] pg/mL *vs.* median=0.0 [IQR: 0.0 to 13.8] pg/mL, P=0.010). LPS + rhTPO-treated mice showed significantly lower vascular von Willebrand factor (P=0.003), vascular endothelial growth factor (P=0.002), IL-6 (P <0.001), and TNF-α (P <0.001) than mice in the LPS group. Endothelial cell damage factors vascular von Willebrand factor (P=0.012), vascular endothelial growth factor (P=0.001), IL-6 (P <0.001), and TNF-α (P=0.001) were significantly elevated by inhibiting the PI3K/Akt pathway.

Conclusion: rhTPO alleviates endothelial injury and inflammatory indices in sepsis, and may regulate septic endothelial cell injury through the PI3K/Akt pathway.

Introduction

Sepsis, the most common critical illness with a high mortality rate, involves tissue and organ dysfunction caused by infection imbalance. In recent years, despite the development of clinical guidelines regulating treatment, coupled with advances in organ function support technology, the case fatality rate for sepsis has remained above 25%.[1]

Loss of vascular endothelial barrier function is the central link in sepsis pathogenesis. Bacterial adhesion to endothelial

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cells is a crucial promoter of host mechanism dysfunction in sepsis and is related to host systemic inflammation and the coagulation response.[2] In sepsis, a series of changes in endothelium-related proteins and molecules occur,[3,4] which affect coagulation, create an inflammatory response caused by leukocyte recruitment and cytokine/chemokine release, and induce a hyperosmotic state.[5] Endothelial barrier dysfunction and microvascular leakage are causes of organ failure and sepsis-related complications (such as acute respiratory distress syndrome) in sepsis.[6] The severity of vascular endothelial cell damage may be an independent and important indicator of the prognosis of sepsis. Current treatment for sepsis involves a cluster strategy of infection source control, timely antibiotic treatment, fluid resuscitation, organ support therapy, etc. However, these methods cannot effectively improve endothelial cell injury, and the therapeutic effect is unsatisfactory. Recombinant human thrombopoietin (rhTPO) is used in the treatment of chemotherapy-induced thrombocytopenia, such as hematologic diseases and tumors, and is recommended for severe thrombocytopenia caused by sepsis.[7] Animal studies have shown that rhTPO can improve endothelial injury,[8] but no clinical study has evaluated rhTPO for endothelial cell injury caused by sepsis.

The phosphatidylinositol 3-kinase (PI3K)/Akt pathway is instrumental in mediating signals in inflammation, apoptosis, and other pathophysiological processes. Activation of downstream signaling molecules regulated by the PI3K/Akt signaling pathway plays a key role in regulating immune responses and inflammatory factor release *in vivo* and *ex vivo*, and is crucial in maintaining balance of the body's internal environment, which is closely related to sepsis development.[9] Therefore, regulating the expression of PI3K/Akt pathway molecules is a research hotspot for the treatment of sepsis. However, whether PI3K/Akt is involved in thrombopoietin (TPO) regulation of endothelial cell injury in sepsis has not been reported.

The purpose of this study was to investigate the effects of rhTPO on endothelial cell injury markers and inflammatory factors in patients with sepsis and to provide a reference for the formulation of targeted treatment for such patients and a preliminary study of the mechanisms.

Methods

Patients

This was a retrospective observational study. We collected patients with sepsis who met the diagnostic criteria of Sepsis 3.0 and were admitted to the intensive care unit of Shanghai General Hospital from January 1, 2019, to December 31, 2022. Inclusion criteria: (1) age ≥18 years old and ≤80 years old; (2) patients with confirmed or clinically diagnosed infection; and (3) acute change in sequential organ failure score (ΔSOFA) ≥2 (the baseline SOFA score for patients with unknown existing organ dysfunction was 0). Exclusion criteria: (1) history of hematopoietic stem cell transplantation or liver, kidney, lung, pancreas, and other solid organ transplantation; (2) history of coronary heart disease, myocardial infarction, or other serious cardiovascular diseases; (3) history of unresectable malignant solid tumors with multiple metastases, hematologic tumors, or autoimmune diseases that were not controlled; (4) survival time <24 h; and (5) pregnant or nursing patients.

Patients were divided into two groups – control (no-rhTPO) and rhTPO – according to whether they were treated with rhTPO. The sepsis treatment regimens of the two groups were consistent, and the criteria were based on the Surviving Sepsis Campaign (SSC) guidelines for severe sepsis/septic shock,[1] including a series of therapeutic measures such as early fluid resuscitation, anti-infection therapy, steroid therapy, coagulation therapy, intravenous nutrition, and blood product infusion. The rhTPO treatment consisted of 15,000 units via subcutaneous injection once daily. The drug was withdrawn when platelet clinical recovery, which is a platelet count (PC) $\geq 100 \times 10^9$/L or an increase in PC of $\geq 50 \times 10^9$/L for 3 days, was achieved.

Sample detection

Expression levels of human endothelial cell-specific molecule (ESM-1, also known as endocan), human heparin-binding protein (HBP), and CD31 were detected in the serum using enzyme-linked immunosorbent assay (ELISA) kits (ab213776, Abcam, Cambridge, UK), (AE93678Hu-96T, AMEKO, Shanghai, China), and (E-EL-H1640c, Elabscience, Wuhan, China), respectively. The levels of interleukin-6 (IL-6) and tumor necrosis factor (TNF)-α inflammatory factors in the serum of the patients were detected in the enrolled patients using the SIEMENS inflammatory factor assay kit (chemiluminescence immunoassay).

Extravascular lung water (EVLW) index detection

All patients underwent an ultrasound examination of the lungs, looking at four quadrants – two anterior and two lateral – on each side of the chest. Three positive quadrants determined an EVLW index >10 mL/kg ("positive" is defined as three or more B-lines in the quadrant).

Outcomes

Primary outcome: the levels of ESM-1, HBP, and CD31 decreased on day 7 after enrollment compared with day 1. Secondary outcomes: the levels of IL-6 and TNF-α decreased on day 7 after enrollment compared with day 1, 28-day mortality, levels of platelets, fibrinogen, antithrombin III (ATIII) and international normalized ratio (INR), length of hospital stay, mechanical ventilation use, need for dialysis, and norepinephrine utilization.

In vivo experiments

Healthy specific pathogen-free (SPF)-grade C57BL/6 male mice, 8 weeks old and weighing approximately 20±2 g, were used. Before the experiment, the mice were fed and watered *ad libitum*, and the ambient temperature was maintained at 20 °C.

The mice were randomly divided into four groups (six mice per group): NS (normal saline), lipopolysaccharide (LPS) (mice were injected intraperitoneally with LPS 50 mg/kg for modeling), LPS + rhTPO (rhTPO was administered transperitoneally, 1.1×10^4 U/kg, 2 h after LPS intraperitoneal injection), and LPS + rhTPO + LY294002 (LY294002 was injected at 1 mg/25 g of body weight at 1 h before LPS administration. LY294002 can permeate cells, specifically inhibit PI3K and inhibit the PI3K/Akt signaling pathway). Diet, body weight, and general

condition of the mice were recorded. Mice were euthanized with mild ether, and blood was collected in a sterile heparinized tube after puncturing the heart. Blood samples were centrifuged at 2500 *g* for 10 min and plasma supernatants were collected. ELISA kits were used to detect vascular Von Willebrand factor (vWF) (E-EL-M1247c, Elabscience), IL-6 (E-EL-M0044c, Elabscience), TNF-α (E-EL-M3063, Elabscience), and vascular endothelial growth factor (VEGF) (PV957, Beyotime, Shanghai, China) in the plasma samples.

Statistical analysis

SPSS 22.0 (SPSS Inc., Chicago, IL,USA) was used for statistical analysis, with mean ± standard deviation used to describe normally distributed data and median (interquartile range [IQR]) used for data with a skewed distribution. An independent sample *t*-test was used to analyze the difference between the two groups for data with a normal distribution, and the Mann–Whitney *U* test was used for comparison of non-normally distributed data. Counting data were tested by chi-squared test or Fisher's test. P <0.05 indicated that the difference was statistically significant. GraphPad Prism 5 (GraphPad Software Inc., San Diego,USA) was used to draw figures.

Results

Demographic and baseline characteristics between the two groups

In this study, 116 patients with sepsis met the diagnostic criteria of Sepsis 3.0 and were admitted to the Shanghai General Hospital intensive care unit from January 1, 2019, to December 31, 2022. According to the exclusion criteria, 32 patients were removed from the study cohort (19 had been previously diagnosed with severe cardiovascular disease, 7 had end-stage tumors, 5 refused all treatment, and 1 had a history of antibiotic or other drug allergies). Thus, 84 patients (72.4%) were included in the study. Among them, 26 patients were treated with rhTPO (rhTPO group), and 58 patients were not treated with rhTPO (control group) (Figure 1).

Patient demographics and baseline characteristics are shown in Table 1. A total of 51 patients (60.7%) were male and 33 (49.3%) were female. The median age was 70 (IQR: 58–70) years, and 60 (71.4%) patients had comorbidities. Hypertension, diabetes, and diabetes mellitus were the most common comorbidities. The median APACHE II score was 14.0 (IQR: 14.0–23.0), and the median SOFA score was 9.0 (IQR: 9.0–15.7). There were no statistically significant differences in the APACHE II scores (P=0.275) and SOFA scores (P=0.245).

The utilization rate of mechanical ventilation, norepinephrine, and dialysis was 63.1%, 53.6%, and 27.4%, respectively. The duration of mechanical ventilation was not significantly different between the rhTPO group and the control group (6.5 days *vs.* 3.0 days, P=0.106). The median utilization time of norepinephrine (1.0 day *vs.* 0.0 days, P=0.018) and utilization rate of dialysis (46.1% *vs.* 19.0%, P=0.010) were significantly greater in patients of the rhTPO group compared with those of the control group.

Mortality at 28 days, 3 months, and 1 year were the same in each group and there were no significant differences between the two groups (rhTPO group 23.1% *vs.* control group 29.3%, P=0.099) (Table 1).

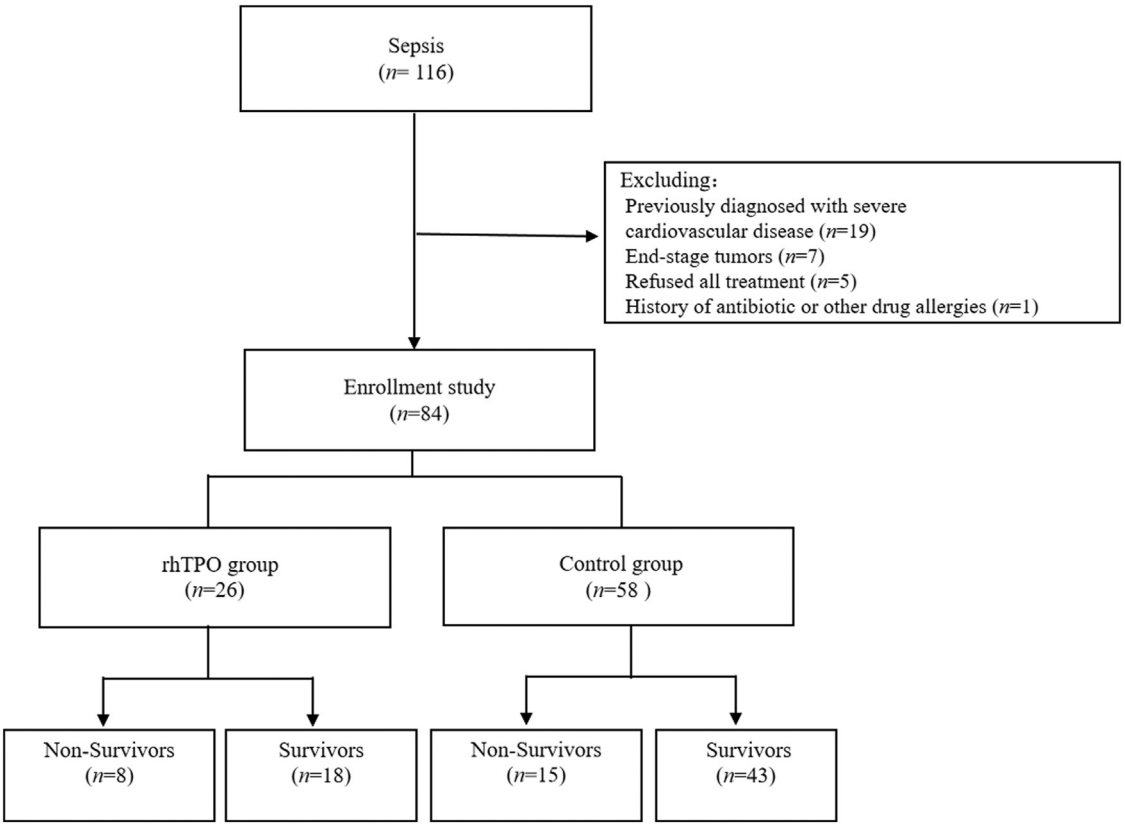

Figure 1. Flowchart of the research. rhTPO: Recombinant human thrombopoietin.

Table 1
Patient's demographics and clinical characteristics.

Characteristics	Total (*n*=84)	rhTPO (*n*=26)	Control (*n*=58)	*P*-value
Sex (male)	51 (60.7)	15 (57.7)	36 (62.1)	0.704
Age (years)	70.0 (58.0 to 70.0)	66.5 (56.2 to 72.0)	61.0 (46.0 to 70.2)	0.063
Comorbidities				
Hypertension	37 (44.0)	9 (34.6)	28 (48.3)	0.244
Diabetes	14 (16.7)	5 (19.2)	9 (15.5)	0.673
Immune diseases	8 (9.5)	3 (11.5)	5 (8.6)	0.698
CKD	10 (11.9)	5 (19.2)	5 (8.6)	0.165
Liver disease	7 (8.3)	4 (15.4)	3 (5.2)	0.195
COPD	11 (13.1)	2 (7.7)	9 (15.5)	0.490
SOFA	9.0 (9.0 to 15.7)	11.5 (7.0 to 13.0)	8.0 (7.0 to 12.0)	0.245
APACHE II	14.0 (14.0 to 23.0)	18.5 (13.7 to 21.2)	16.0 (12.0 to 20.0)	0.275
Mortality (28 days)	23 (27.4)	6 (23.1)	17 (29.3)	0.099
Mortality (3 months)	23 (27.4)	6 (23.1)	17 (29.3)	0.099
Mortality (1 year)	23 (27.4)	6 (23.1)	17 (29.3)	0.099
Mechanical ventilation	53 (63.1)	19 (73.1)	34 (58.6)	0.204
Duration of mechanical ventilation (days)	4.0 (0.0 to 9.0)	6.5 (0.0 to 11.2)	3.0 (0.0 to 8.0)	0.106
Norepinephrine use	45 (53.6)	18 (69.2)	27 (46.6)	0.054
Duration of norepinephrine use (days)	1.0 (0.0 to 3.0)	1.0 (0.0 to 6.5)	0.0 (0.0 to 2.0)	0.018
Need for dialysis	23 (27.4)	12 (46.1)	11 (19.0)	0.010
Time Need for dialysis (days)	0.0 (0.0 to 1.7)	0.0 (0.0 to 4.2)	0.0 (0.0 to 0.0)	0.198

Data are expressed as *n* (%) or median (interquartile range).
APACHE: Acute Physiology and Chronic Health Evaluation; CKD: Chronic kidney disease; COPD: Chronic obstructive pulmonary disease; rhTPO: Recombinant human thrombopoietin; SOFA: Sequential organ failure assessment.

Levels of ESM-1, HBP, and CD31

After 7 days of treatment, the endothelial injury index of ESM-1 decreased more significantly in the rhTPO group than in the control group compared with day 1; the median reduction of ESM-1 was 38.6 (IQR: 7.2 to 67.8) pg/mL *vs.* 23.0 (IQR: −15.7 to 51.5) pg/mL (P=0.008). HBP decreased significantly in patients in the rhTPO group compared with that in patients in the control group (P=0.001); the median reduction of HBP was 59.6 (IQR: −1.9 to 91.9) pg/mL *vs.* 2.4 (IQR: −23.2 to 43.2) pg/mL. CD31 also decreased significantly (P <0.001); the median reduction of CD31 was 2.4 (IQR: 0.4 to 3.5) pg/mL *vs.* −0.6 (IQR: −2.2 to 0.8) pg/mL (Figure 2 and Table 2).

Levels of IL-6, TNF-α, and EVLW

After 7 days of rhTPO treatment, the inflammatory index IL-6 decreased more significantly in the rhTPO group than in the control group compared with day 1; the median reduction of IL-6 was 46.0 (IQR: 15.8 to 99.1) pg/mL *vs.* 31.2 (IQR: 19.7 to 171.0) pg/mL (P <0.001). The inflammatory index TNF-α also decreased significantly in the rhTPO group compared with that in the control group; the median reduction of TNF-α was 17.2 (IQR: 6.4 to 23.2) pg/mL *vs.* 0.0 (IQR: 0.0 to 13.8) pg/mL (P=0.010, Figure 2). Although there was no statistical difference between the two groups for EVLW, the improvement rate of EVLW was greater in the rhTPO group (61.5% *vs.* 55.2%, Table 2).

Table 2
Patient's laboratory results (*n*=84).

Laboratory results	Total (*n*=84)	rhTPO (*n*=26)	Control (*n*=58)	*P*-value
Day 1 ESM-1 (pg/mL)	243.5 (225.0 to 275.3)	238.6 (217.5 to 276.4)	257.9 (227.3 to 274.7)	0.089
Day 7 ESM-1 (pg/mL)	209.9 (197.1 to 240.7)	198.6 (171.0 to 223.8)	227.3 (208.1 to 247.7)	<0.001
△ESM-1 (pg/mL)	23.0 (1.1 to 59.0)	38.6 (7.2 to 67.8)	23.0 (−15.7 to 51.5)	0.008
Day 1 HBP (pg/mL)	524.5 (484.6 to 557.8)	519.9 (484.6 to 561.8)	528.5 (487.3 to 543.2)	0.440
Day 7 HBP (pg/mL)	501.2 (446.5 to 529.4)	457.9 (422.2 to 503.8)	526.2 (498.6 to 536.7)	<0.001
△HBP (pg/mL)	27.7 (−11.2 to 68.9)	59.6 (−1.9 to 91.9)	2.4 (−23.2 to 43.2)	0.001
Day 1 IL-6 (pg/mL)	100.0 (52.1 to 319.5)	100.0 (57.8 to 376.6)	100.0 (46.5 to 271.0)	0.050
Day 7 IL-6 (pg/mL)	32.0 (16.3 to 111)	55.0 (14.8 to 99.1)	73.0 (19.9 to 195.2)	0.080
△IL-6 (pg/mL)	27.5 (3.4 to 200.1)	46.0 (15.8 to 99.1)	31.2 (19.7 to 171.0)	<0.001
Day 1 TNF-α (pg/mL)	20.0 (13.9 to 33.6)	20.5 (17.7 to 39.5)	17.5 (12.6 to 30.7)	0.184
Day 7 TNF-α (pg/mL)	15.6 (12.1 to 24.4)	14.35 (10.4 to 19.5)	16.4 (12.3 to 25.2)	0.566
△TNF-α (pg/mL)	5.8 (0.0 to 17.9)	17.2 (6.4 to 23.2)	0.0 (0.0 to 13.8)	0.010
Day 1 CD31 (pg/mL)	10.5 (8.5 to 12.7)	11.4 (9.7 to 13.2)	10.0 (8.2 to 12.1)	0.031
Day 7 CD31 (pg/mL)	10.5 (8.7 to 12.7)	9.4 (7.5 to 10.7)	11.6 (9.1 to 13.3)	<0.001
△CD31 (pg/mL)	0.1 (−2.1 to 1.8)	2.4 (0.4 to 3.5)	−0.6 (−2.2 to 0.8)	<0.001
Day 1 EVLW >10	37 (44.0)	14 (53.8)	23 (39.7)	0.226
Day 7 EVLW >10	23 (27.4)	6 (23.1)	17 (29.3)	0.554
EVLW improve	48 (57.1)	16 (61.5)	32 (55.2)	0.586

Data are expressed as *n* (%) or median (interquartile range).
△: The expression level difference between day 7 and day 1.
ESM-1: Endothelial cell-specific molecule 1; EVLW: Extravascular lung water; HBP: Heparin-binding protein; IL-6: Interleukin 6; rhTPO: Recombinant human thrombopoietin; TNF-α: Tumor necrosis factor-α.

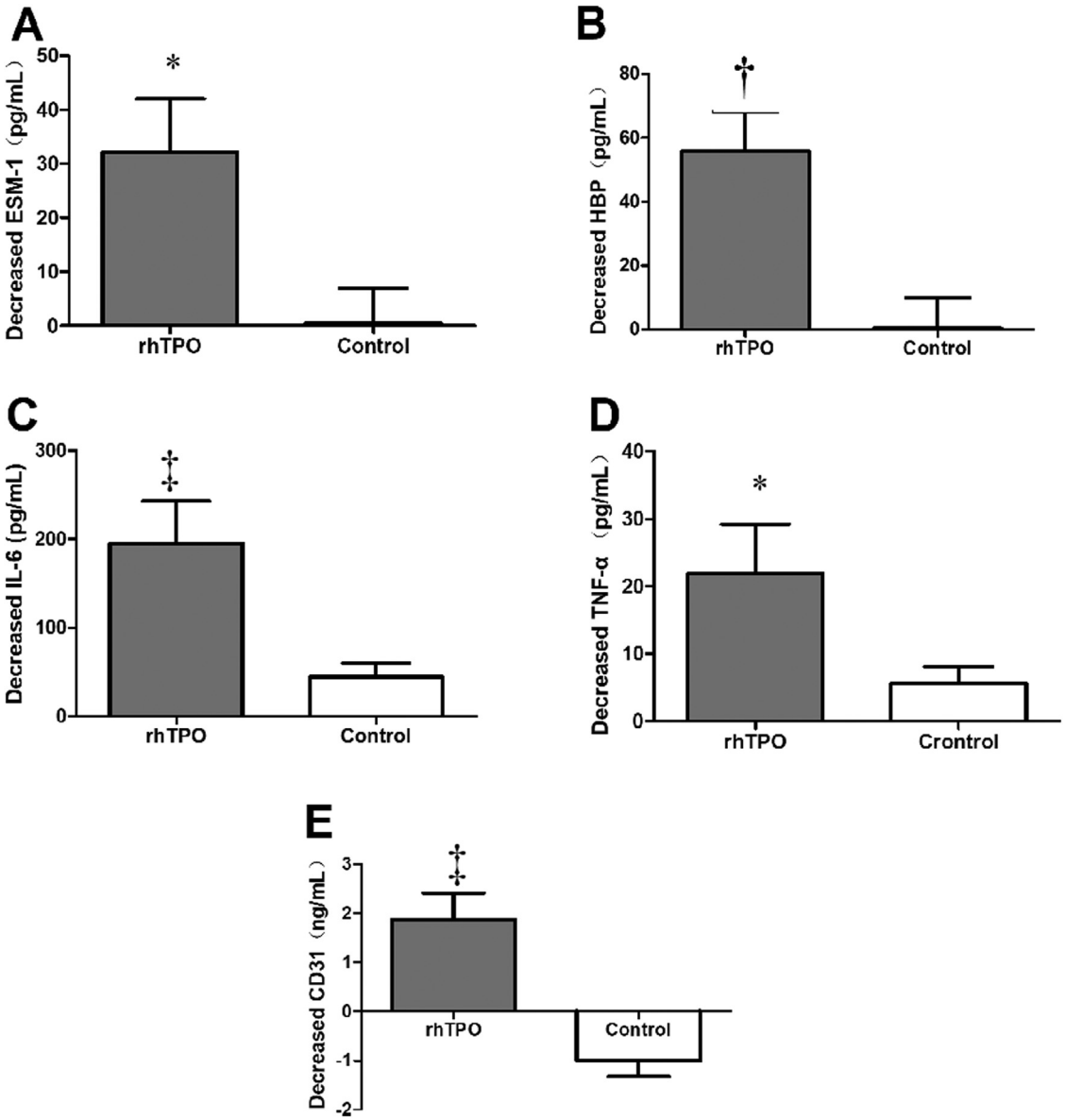

Figure 2. Endothelial injury and inflammatory markers in sepsis. A: The levels of ESM-1 decreased on day 7 after enrolment compared with day 1. B: The levels of HBP decreased on day 7 after enrolment compared with day 1. C: The levels of IL-6 decreased on day 7 after enrolment compared with day 1. D: The levels of TNF-α decreased on day 7 after enrolment compared with day 1. E: The levels of CD31 decreased on day 7 after enrollment compared with day 1.
$^{*}P$ <0.05; $^{\dagger}P$ <0.01; $^{\ddagger}P$ <0.001.
ESM-1: Endothelial cell-specific molecule 1; HBP: Heparin-binding protein; IL-6: Interleukin 6; rhTPO: Recombinant human thrombopoietin; TNF-α: Tumor necrosis factor-α.

Levels of platelets, fibrinogen, ATIII, and INR

The increase in numbers of platelets in the rhTPO group was significantly higher than that in the control group; the median increment of platelet numbers was 61.5 × 10^9/L (IQR: 12.0 to 115.0) × 10^9/L *vs.* 27.0 × 10^9/L (IQR: −18.0 to 87.0) × 10^9/L (P=0.004). Fibrinogen and INR also decreased significantly, the median increment of fibrinogen was 0.6 (IQR: 0.3 to 1.1) g/L *vs.* 1.1 (IQR: 0.2 to 2.4) g/L (P=0.008) and the median increment of INR was 0.2 (IQR: 0.1 to 0.3) *vs.* 0.1 (IQR: 0.0 to 0.2) (P=0.037), in the rhTPO group compared with the control group, but the change of ATIII was not significant, the median increment of ATIII was 5.20 (IQR: 6.00 to 16.20)% *vs.* 0.05 (IQR:−13.90 to 15.50)% (P=0.787) (Figure 3).

In vivo experiments

LPS + rhTPO-treated mice had significantly lower vWF (P=0.003), VEGF (P=0.002), IL-6 (P <0.001), and TNF-α (P <0.001) compared with mice in the LPS group. The endothelial cell damage factors vWF (P=0.012), VEGF (P=0.001), IL-6 (P <0.001), and TNF-α (P=0.001) were significantly elevated by inhibiting the PI3K/Akt pathway, as detected by ELISA (Figure 4 and Table 3).

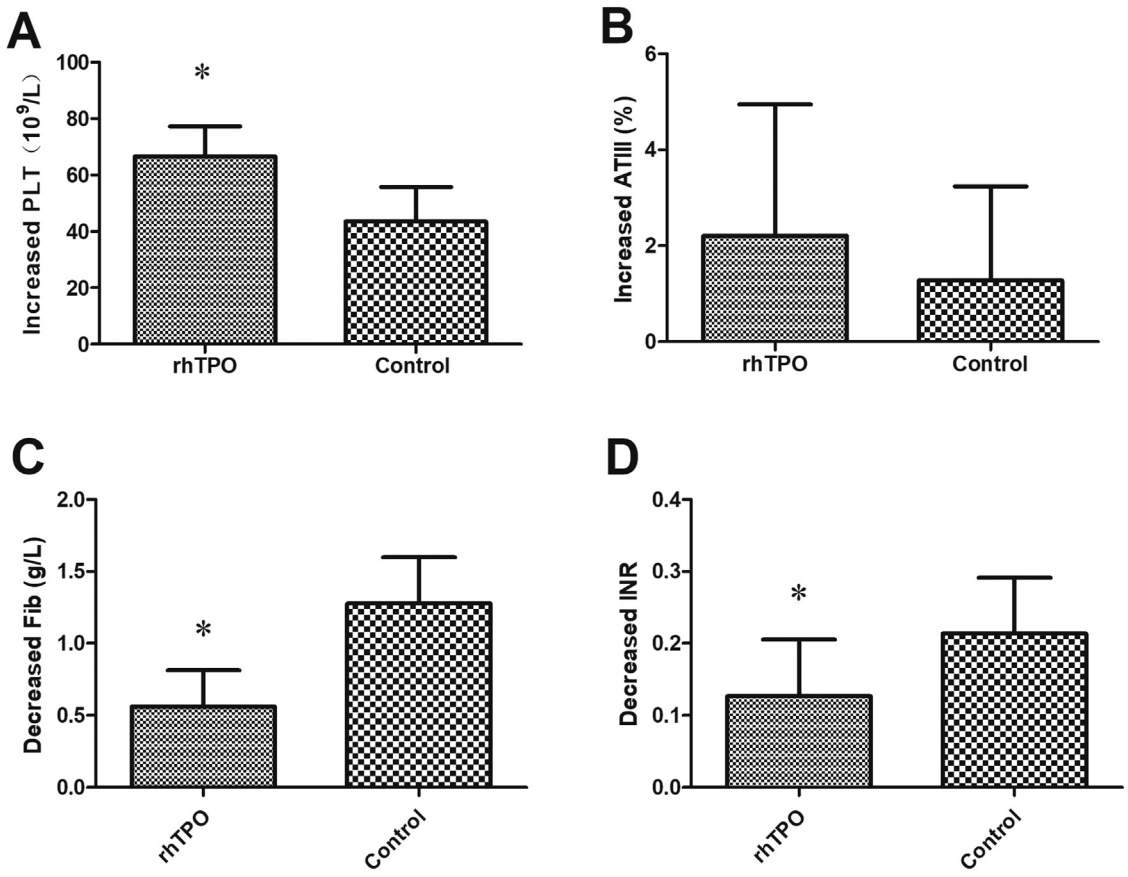

Figure 3. Coagulation function in sepsis. A: PLT numbers increased on day 7 after enrolment compared with those on day 1. B: ATIII levels increased on day 7 after enrolment compared with those on day 1. C: Fib levels increased on day 7 after enrolment compared with those on day 1. D: INR levels increased on day 7 after enrollment compared with those on day 1.
*P <0.01.
ATIII: Antithrombin III; Fib: Fibrinogen; INR: International normalized ratio; PLT: Platelet; rhTPO: Recombinant human thrombopoietin.

Table 3
ELISA results detecting indicators of endothelial damage in sepsis model mice.

ELISA Results	NS (n=6)	LPS (n=6)	LPS + rhTPO (n=6)	LPS + rhTPO + LY294002 (n=6)	P-value (LPS *vs.* LPS + rhTPO)	P-value (LPS + rhTPO *vs.* LPS + rhTPO + LY294002)
vWF (ng/mL)	41.2 ± 6.4	199.9 ± 8.2	141.6 ± 13.7	184.0 ± 9.5	0.003	0.012
VEGF (pg/mL)	138.7 ± 22.0	550.1 ± 49.3	331.3 ± 11.2	546.2 ± 41.8	0.002	0.001
IL-6 (pg/mL)	268.0 ± 24.7	891.8 ± 32.7	473.4 ± 16.4	854.5 ± 35.3	<0.001	<0.001
TNF-α (pg/mL)	69.5 ± 5.7	142.3 ± 7.4	67.3 ± 6.4	125.4 ± 10.5	<0.001	0.001

Values are expressed as the mean ± standard deviation.
ELISA: Enzyme-linked immunosorbent assay; IL-6: Interleukin-6; LPS: Lipopolysaccharide; NS: Normal saline; rhTPO: Recombinant human thrombopoietin; TNF-α: Tumor necrosis factor-α; VEGF: Vascular endothelial growth factor; vWF: Vascular Von Willebrand factor.

Discussion

The purpose of this study was to show that rhTPO can improve endothelial injury and inflammatory indices in sepsis patients. Simultaneously, the study validated that rhTPO can improve coagulation function in patients with sepsis.

Sepsis, the most common critical illness with a high mortality rate, involves tissue and organ dysfunction caused by infection imbalance. In recent years, despite the development of relevant clinical guidelines regulating treatment, coupled with advances in organ function support technology, the case fatality rate of sepsis has remained above 25%.[1] In this study, the mortality of sepsis was 27.4%. Loss of vascular endothelial barrier function is a central link in the pathogenesis of sepsis, and bacterial adhesion to endothelial cells is a crucial promoter of host mechanism dysfunction in sepsis and is related to host systemic inflammation and the coagulation response.[2] In sepsis, a series of changes in endothelium-related proteins and molecules occur,[3,4] which affect coagulation and the inflammatory response caused by leukocyte recruitment and cytokine/chemokine release and induce a hyperosmotic state.[5] We found disruption in coagulation function and endothelial function in the sepsis patients in our study. Endothelial barrier dysfunction and microvascular leakage cause organ failure and sepsis-related complications (such as acute respiratory distress syndrome) in sepsis.[6] The severity of vascular endothe-

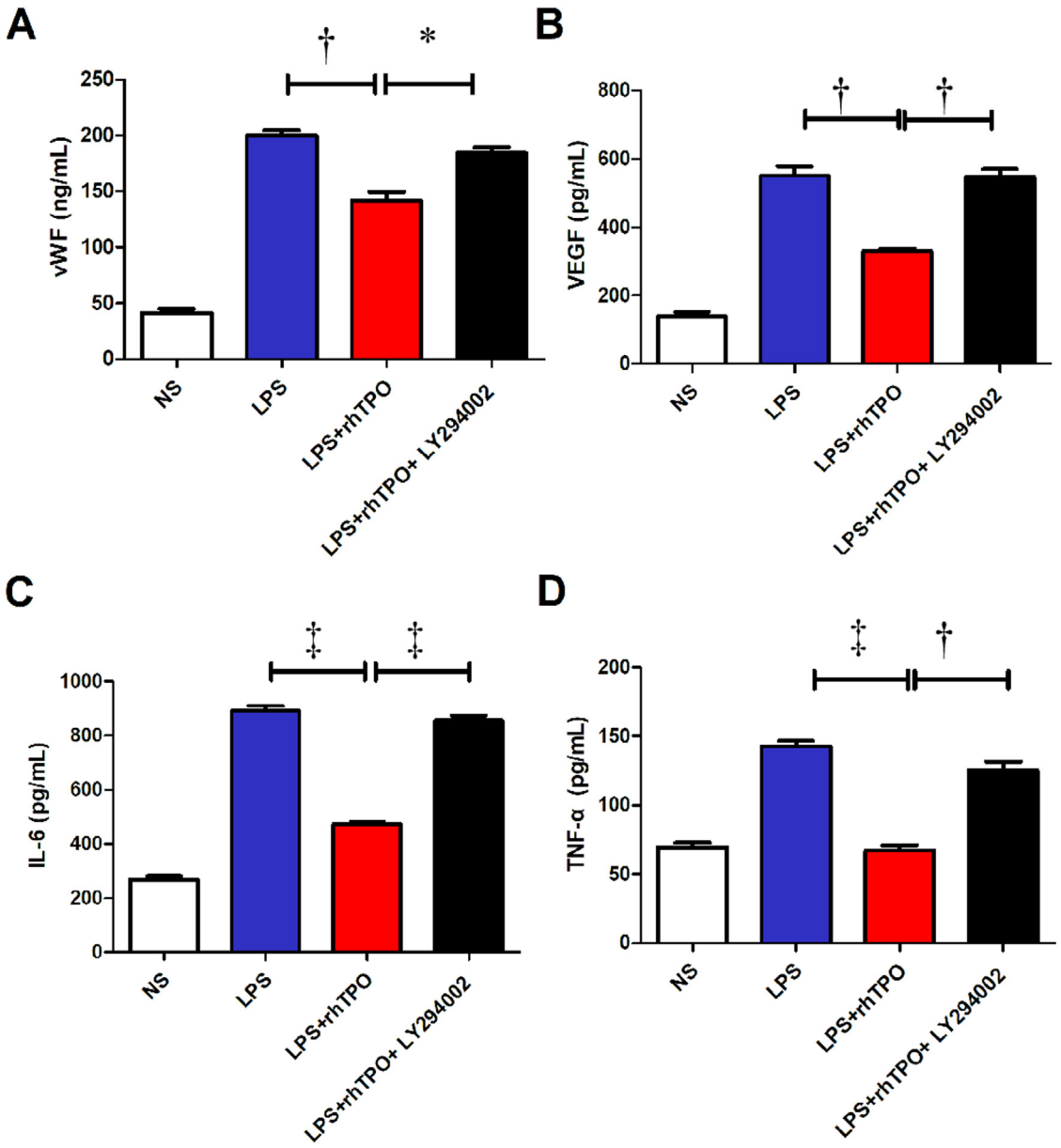

Figure 4. *In vivo* study of endothelial cell injury after rhTPO treatment. ELISA detection of various endothelial cell injury factors in mice plasma. A: vWF; B: VEGF; C: IL-6; D: TNF-α. $^{*}P$ <0.05; $^{\dagger}P$ <0.01; $^{\ddagger}P$ <0.001.
ELISA: Enzyme-linked immunosorbent assay; IL-6: Interleukin-6; LPS: Lipopolysaccharide; NS: Normal saline; rhTPO: Recombinant human thrombopoietin; TNF-α: Tumor necrosis factor-α; VEGF: Vascular endothelial growth factor; vWF: Vascular Von Willebrand factor.

lial cell damage may be an independent and important indicator of sepsis prognosis. rhTPO is a full-length glycosylated TPO expressed by Chinese hamster ovarian cells that have been purified and found to have similar effects to endogenous TPO. The 2018 edition of the Chinese Expert Consensus on the Diagnosis and Treatment of Infection-induced Senile Multiorgan Dysfunction Syndrome[7] highlights that Wu's[10] prospective single-center study recommends rhTPO for the treatment of thrombocytopenia secondary to infection, with the study showing that for septic patients with thrombocytopenia after surgical abdominal infection, rhTPO could restore PC to normal within 5 days in 76.32% of patients. However, this restoration was seen in only 17.5% of the patients in the control group. Moreover, rhTPO can significantly reduce the mortality of sepsis patients (16.9% *vs.* 39.2%), and no rhTPO-related serious adverse reactions have been observed.[10] Among adult patients in an intensive care unit, the TPO levels in patients with septic shock were significantly greater than those in patients without severe sepsis.[11] The plasma TPO concentration of patients with secondary systemic inflammatory response syndrome (SIRS) infection was significantly higher than that of patients with non-septic SIRS.[12] In a burn study, the plasma TPO levels in patients with sepsis were higher than those in patients without sepsis.[13] These studies suggest that TPO is closely related to sepsis.

Our clinical studies found that rhTPO can rapidly increase the number of platelets in patients with severe thrombocytopenia associated with sepsis, shorten the time of platelet recovery, increase the proportion of patients with platelet recovery within 7 days, and improve shock.[14] In this study, we further determined the levels of ESM-1, HBP, IL-6, and TNF-α and found that

ESM-1, HBP, IL-6, and TNF-α were significantly reduced in patients using rhTPO compared with those without rhTPO on day 7. ESM-1 is a cell adhesion molecule that is routinely expressed on the surface of endothelial cells and directly reflects the degree of endothelial injury. HBP can regulate the function of vascular endothelial cells and affect permeability and the inflammatory response. IL-6 levels have been reported to reflect endothelial cell dysfunction,[15] and TNF-α can directly cause endothelial injury and is significantly correlated with the severity of endothelial injury.[16] Therefore, we speculated that rhTPO may also play a role in improving endothelial cell injury. rhTPO can act on multiple links in the platelet generation process,[17] regulate the release of inflammatory mediators, and reduce the inflammatory response.[8,18,19] In addition, rhTPO has the advantages of a rapid effect and high safety in clinical treatment, and has shown good clinical application value in the treatment of severe thrombocytopenia accompanied by sepsis.[10,20] However, the specific mechanism of TPO in sepsis has yet to be elucidated.

Wang et al.[9] conducted an *in vitro* study using human umbilical vein endothelial cells (HUVECs) pre-treated with CoCl2 and TPO. A Cell Counting Kit-8(CCK-8) assay was used to measure cell viability, flow cytometry was employed to determine apoptosis rate, expression of the apoptosis protein caspase-3 and changes in mitochondrial membrane potential, and western blotting was used to assess the effect of TPO on phosphorylation of the Akt pathway. TPO had a protective effect against apoptosis induced by chemical hypoxia, and this protection may be mediated by activation of the PI3K/Akt pathway, which reduces activation and expression of caspase-3 and stabilizes mitochondrial membrane potential. Cheng et al.[21] used an indirect transwell co-culture system to study the interaction between HUVECs and human lung adenocarcinoma CL1–5 cells. Morphological and molecular changes in the HUVECs were investigated, and the PI3K/Akt signaling pathway was found to be involved in endothelial tube formation when stimulated by lung cancer cells. Inhibition of PI3K could reverse the increased angiogenesis and induce apoptosis in HUVECs. In addition, Ramella et al.[22] investigated the effect of TPO on coronary blood flow and the expression of c-Mpl and endothelial nitric oxide synthase (eNOS) in isolated rat hearts. They also examined the expression of eNOS in human coronary endothelial cells. TPO was found to play a crucial role in the proliferation and differentiation of precursor endothelial cells and induced PI3K/Akt-dependent phosphorylation of eNOS and release of nitric oxide, leading to dose-dependent dilation of the coronary artery and regulation of coronary blood flow. Our study found that the endothelial cell injury factors vWF, VEGF, IL-6, and TNF-α were significantly elevated if the PI3K/Akt pathway was inhibited, even with rhTPO treatment. In endothelial cell injury in sepsis, TPO may protect endothelial cells by activating the PI3K/Akt pathway through binding to Mpl.

Our study does have some limitations. The study is an observational study, not a randomized controlled study, which will have an influence on the results of the study. However, the study was based on genuine patients and reflects real clinical treatment results. The study is a single-center study and may not necessarily represent the results of other research centers. However, the findings of this study can guide clinical treatment at this center or region and provide a reference for the treatment of sepsis in other regions. The sample size of this study is small, which may affect the statistics and results. Therefore, expansion of the sample size is needed to verify the results. In addition, only univariate analysis was conducted for this observational study, and confounding bias could exist. Further mechanistic studies also need to be explored.

Conclusions

We found that rhTPO in the treatment of sepsis can regulate the release of inflammatory mediators, reduce the inflammatory response, reduce the levels of inflammatory factors IL-6 and TNF-α reflecting endothelial injury, and reduce sepsis mortality. Our findings provide a theoretical basis for the search for new targets for the treatment of sepsis, whereby rhTPO may regulate septic endothelial cell injury through the PI3K/Akt pathway.

Author Contributions

Yun Xie: Writing – review & editing, Writing – original draft, Conceptualization. **Hui Lv:** Data curation. **Daonan Chen:** Methodology, Investigation. **Peijie Huang:** Project administration, Data curation. **Shaohong Wu:** Validation. **Hongchao Shi:** Project administration. **Qi Zhao:** Formal analysis. **Ruilan Wang:** Project administration.

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Ethics Statement

This study met the medical ethical standards and was approved by the Ethics Review Committee of Shanghai General Hospital (Approval No. [2021]KY037). All patients received informed consent. We took care to protect the privacy of the enrolled patients. During the study, we deleted the names of the patients and only registered their hospital numbers for data verification.

Conflict of Interest

All authors have completed the ICMJE uniform disclosure form. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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